***C. elegans* Development, Cell Biology and Gene Expression Meeting**

2016 Ciliate Molecular Biology Conference

57th Annual Drosophila Research Conference

Mouse Genetics Conference

Population, Evolutionary, and Quantitative Genetics Meeting

Yeast Genetics Meeting

12th International Conference on Zebrafish Development and Genetics

FULL ABSTRACTS BOOK

Orlando World Center Marriott
July 13-17, 2016

Genetics Society of America, 9650 Rockville Pike, Bethesda, MD 20814
www.genetics2016.org, 301-634-7039

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C. elegans DEVELOPMENT, CELL BIOLOGY AND GENE EXPRESSION MEETING

Plenary and Platform Session Abstracts

C. elegans Development, Cell Biology And Gene Expression Meeting

SCHEDULE AT-A-GLANCE

Wednesday, July 13		
2:00pm-9:30pm	Speaker Ready Room Open	Hall of Cities - Anaheim
7:00pm-9:00pm	Scientific Session: Plenary Session 1: Germline Dynamics	Grand Ballroom 8A
9:00pm-11:00pm	Opening Mixer with Exhibits	Cypress Ballroom
Thursday, July 14		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities -Anaheim
7:45am-10:00am	Genetics and Determinants of Health Joint Plenary Session	Palms Ballroom
8:00am-4:00pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:30am-12:30pm	Scientific Session: Genomics, Gene Regulation and Technology	Grand Ballroom 8A
12:30pm-1:30pm	Mentoring Roundtables #1	North Tower - Harbor Beach
12:30pm-1:30pm	Speaking Up for Genetics and Model Organism Research	Crystal Ballroom H
1:30pm-3:30pm	Poster Presentations 1:30pm-2:30pm: Even-numbered posters 2:30pm-3:30pm: Odd-numbered posters t	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center and Job Fair	Cypress Ballroom 1C
4:00pm-6:00pm	Scientific Session: Intracellular Organelles, Trafficking, and the Cytoskeleton	Grand Ballroom 8A
4:00pm-6:00pm	Plenary Session and Workshop for Undergraduate Researchers	North Tower - Sawgrass
7:45pm-9:45pm	Scientific Session: Plenary Session 2: Systems Biology	Grand Ballroom 8A
10:00pm-11:30pm	*Science Cafe Event	Palms Ballroom Sabal
Friday, July 15		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-9:30am	Scientific Session: Aging and Cell Death	Grand Ballroom 8A
8:00am-4:30pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:00am-11:00am	Scientific Session: Cell Cycle, Cell Division, Cytokinesis	Grand Ballroom 8A
11:00am-12:00pm	Scientific Session: Cell Polarity and Cell Fate	Grand Ballroom 8A
12:00pm-1:30pm	*Editor's Panel Discussion and Roundtable	North Tower - Harbor Beach
1:30pm-3:30pm	Poster Presentations 1:30pm-2:10pm: "A" poster authors present 2:10pm-2:50pm: "B" poster authors present 2:50pm-3:30pm: "C" poster authors present	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center	Cypress Ballroom 1C

** Ticketed Event*

#TAGC16

Friday, July 15 (continued)		
2:00pm-2:45pm	GeneticsCareers Workshop - Nailing the Job Talk	Cypress Ballroom 1B
4:00pm-6:00pm	Scientific Session: Cell Patterning and Morphogenesis	Grand Ballroom 8A
6:00pm-7:30pm	*Women in Genetics Panel and Networking	North Tower - Harbor Beach
7:30pm-9:30pm	Development and Evolution Joint Plenary Session	Palms Ballroom
Saturday, July 16		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-10:00am	Workshops: See topics and descriptions under the Workshop Section	Multiple locations
8:00am-12:00pm	Exhibits Open	Cypress Ballroom
8:00am-9:00am	Trainee Bootcamp Workshops: Session 1	North Tower
9:00am-10:00am	Trainee Bootcamp Workshops: Session 2	North Tower
10:00am-12:00pm	Poster Presentations 10:00am-11:00am Odd-numbered posters 11:00am-12:00pm Even-numbered posters	Cypress Ballroom *(Posters must be removed by 1pm)*
10:00am-12:00pm	GeneticsCareers Center	Cypress Ballroom 1C
10:30am-11:15am	GeneticsCareers Workshop	Cypress Ballroom 1B
12:15pm-1:45pm	*Mentoring Roundtables #2	North Tower - Harbor Beach
1:45pm-3:45pm	Scientific Session: Meiosis, Germ Line Development, and Sex Determination	Grand Ballroom 8A
4:00pm-6:00pm	Scientific Session: RNAi, microRNAs, and Developmental Timing	Grand Ballroom 8A
4:00pm-6:00pm	Workshop: You Can Publish That, Too - Publishing Education Resources	North Tower Bahamas
7:30pm-9:30pm	Scientific Session: Plenary Session 3: Development and Disease	Grand Ballroom 8A
Sunday, July 17		
10:30am-12:30pm	Technology and its Application Joint Plenary Session	Palms Ballroom

** Ticketed Event*

W397 Domestication of *C. elegans* Sperm. *Michael Miller*. Univ Alabama at Birmingham, Birmingham, AL.
no abstract submitted

W398 Visualization and quantification of the transcriptional response to GLP-1/Notch signaling in the germline stem cell niche. *Judith Kimble*[1,2], ChangHwan Lee[1,2], Erika Sorensen[1,2]. 1) Univ. Wisconsin, Madison, WI; 2) HHMI.

Notch signaling regulates stem cells, proliferation and differentiation throughout the animal kingdom. In the *C. elegans* gonad, the somatic distal tip cell (DTC) niche uses GLP-1/Notch signaling to maintain germline stem cells (GSCs), which generate, maintain and regenerate the germline tissue. We recently identified the *sygl-1* gene as a direct downstream target of GLP-1/Notch transcriptional activation and key GSC regulator (1). Here we use single molecule fluorescence *in situ* hybridization (2) to visualize *sygl-1* transcripts in germ cells of the progenitor zone, the region harboring GSCs. Using exon- and intron-specific probes, we distinguish active transcription sites (ATS) in nuclei and mature transcripts in the cytoplasm. With a custom MATLAB code, we record number, intensity and spatial coordinates of both ATS and mRNAs. This approach provides a direct and quantitative readout of the endogenous transcriptional response to GLP-1/Notch. In wild-type germlines, *sygl-1* ATS occur stochastically, with little correlation between intensities in a nucleus or with cell cycle stage. Yet the response is patterned: ATS are restricted to the distal ~1/3 of the progenitor zone, and within that region, the percent nuclei with *sygl-1* ATS is graded, from 65% at the distal end to <5% 30 µm or 7 germ cell diameters from the distal end. The *sygl-1* transcriptional response is gone in *glp-1* null mutants, expanded in *glp-1* gain of function mutants, abolished within 30 minutes after shifting *glp-1(ts)* to restrictive temperature and less robust than wild-type in *glp-1(ts)* mutants at permissive temperature. Comparison of readouts in wild-type and *glp-1(ts)* at permissive temperature reveals how the response varies as a function of signaling strength: weaker signaling leads to fewer ATS-positive nuclei, lower ATS signal intensity and shorter ATS extent from the DTC. To learn how the graded ATS spatial pattern is generated, we used *glp-1(ts)* to turn off GLP-1/Notch and abolish ATS and then turn it back on to monitor how fast the ATS signals reform. Their pattern was fully regenerated within an hour, too fast for the main patterning mechanism to be signal decay as germ cells move proximally within the niche. We suggest instead that the strength of GLP-1/Notch signaling is graded in the distal progenitor zone. The major conclusions are that the transcriptional response to Notch signaling is stochastic and that signaling strength determines probability and intensity of transcriptional firing. This high resolution view of the Notch transcriptional response in stem cells will serve as a paradigm for analyzing the readout of signaling more generally.

(1) Kershner et al. (2014) *PNAS* 111, 3739-3744; (2) Raj and van Oudenaarden (2008) *Cell* 135, 216-226.

W399 Dynein subunit DLC-1 promotes localization and function of stem cell regulator FBF-2 in *C. elegans*. *X. Wang*[1], M. Ellenbecker[1], J. Olson[1], J. Bailey[1], D. Rasoloson[2], E. Voronina[1]. 1) University of Montana, Missoula, MT; 2) Johns Hopkins University School of Medicine/HHMI, Baltimore, MD.

PUF family RNA-binding proteins are conserved eukaryotic mRNA regulators. FBF-1 and FBF-2, two PUF family proteins in *C. elegans*, are translational repressors required for maintenance of germline stem cells (Crittenden et al., 2002). FBF-1 and FBF-2 are very similar to each other but employ different mechanisms to silence target mRNAs. FBF-2 function depends on its localization to P granules, RNA granules of germ cells (Voronina et al., 2012). In this study, we found that dynein light chain DLC-1 is required for FBF-2 recruitment to P granules. To understand the role of cofactors in FBF-2 function, we characterized FBF-2 interactome by mass-spectroscopy. By genetic interaction assay, *dlc-1* was identified as a component of FBF-2 RNP specifically contributing to FBF-2-mediated regulation. One known role of DLC-1 is aiding assembly of the dynein motor complex involved in trafficking of organelles, proteins and mRNAs. Knock-down of *dlc-1* by RNAi leads to dispersion of FBF-2 from P granules and decreased FBF-2 regulatory activity. However, FBF-2 localization and activity are not affected by depletion of dynein motor subunit, suggesting DLC-1 cooperates with FBF-2 in dynein-independent manner. In the in vitro pulldown assay, DLC-1 interacts with FBF-2, but not FBF-1. Typically, LC8 associates with its partner proteins through the KXTQT or GIQVD recognition sequence. However, FBF-2 does not contain canonical LC8 recognition sites. To investigate how FBF-2 interacts with DLC-1, we are determining the structure of DLC-1 bound to one of the DLC-1-binding regions of FBF-2. Preliminary data suggests that FBF-2 peptide binds the same site on DLC-1 as DLC-1's conventional partners. To test whether FBF-2 localization depends on direct interaction with DLC-1 in vivo, we generated FBF-2 mutant transgene with mutations of DLC-1 binding sites. The FBF-2 mutant dispersed away from P granules, suggesting that direct interaction with DLC-1 is required for FBF-2 perinuclear localization in vivo. In vitro RNA binding assay suggested that FBF-2 mutant binds target RNA with same affinity as wildtype FBF-2.

LC8 light chains are now recognized as regulatory hub proteins essential for diverse protein networks. Our finding of cooperation between FBF-2 and DLC-1 introduces the idea that DLC-1 homologs directly impact regulatory networks affecting germline stem cell maintenance. This insight will advance understanding of regulatory mechanisms of germline stem cell maintenance in other organisms, including humans.

W400 After extrusion, the second polar body is internalized via receptor-mediated phagocytosis in *C. elegans* embryos. *A. M. Wehman*, G. Fazeli, B. Karmann, E. Schruf. Uni Würzburg, Würzburg, Bayern, DE.

Polar bodies are commonly used for preimplantation genetic diagnosis in human embryos. During egg development, polar bodies are extruded to expel the extra DNA made during Meiosis and to form a haploid oocyte. In contrast to the well-studied mechanisms of polar body extrusion, their fate after extrusion is unknown in any system.

We studied the fate of polar bodies using time-lapse imaging of fluorescent reporter strains and a panel of *C. elegans* mutants. We found that the first polar body becomes trapped between eggshell layers and persists until hatching. In contrast, the second polar body is internalized in a stereotyped manner by one of the anterior AB daughter cells around the 4-cell stage. Prior to internalization, actin and the phagocytic receptor CED-1 are enriched around the second polar body, consistent with formation of a phagocytic cup. CED-1 persists around the phagosome after internalization and is required for polar body internalization. Other CED-10-dependent engulfment pathway proteins, such as CED-2, are also

required, indicating that the polar body is internalized via receptor-mediated phagocytosis.

As part of a candidate screen, we also discovered that the second polar body was rarely internalized in PI3K-deficient *vps-34* mutants or in *rab-5* RNAi-treated embryos. The phagocytic receptor CED-1 is known to be localized to the plasma membrane via retromer-mediated recycling, which has been shown to require PI3K function. Furthermore, PI3K localization is known to depend on the small GTPase Rab5. Therefore, we examined CED-1 localization and discovered that CED-1 is no longer found around the second polar body in *vps-34* mutants and in *rab-5*-depleted embryos, explaining the observed defects in polar body internalization.

Thus, embryos internalize the second polar body via receptor-mediated phagocytosis, showing that the polar body signals to neighboring cells. This observation raises the possibility that polar bodies have a function after extrusion, which could indicate that polar body removal for genetic testing may not be non-invasive.

W401 X-specific targeting of the *C. elegans* dosage compensation complex. *Sevinc Ercan*, Sarah Albritton, Anna-Lena Kranz, Lara Winterkorn. New York University, New York, NY.

Sequence-specific transcription factors (TFs) bind to a small fraction of their sequence motifs in the genome. TF cooperativity and chromatin structure influence TF specificity. Here, we studied the factors that lead to X-chromosome specific targeting of a specialized condensin complex that constitutes the core of the *C. elegans* dosage compensation complex (DCC). Based on the ChIP-seq analysis of DCC recruiters, genomic distribution of the DCC recruitment DNA sequence motif, histone modifications and histone occupancy, we found that the DCC recruiter SDC-2 is required to open chromatin to allow DCC binding to clusters of motifs on the X chromosome. Thus, DNA sequence motif clustering and chromatin explain part of the specificity of DCC binding. However, motif and chromatin do not explain why some DCC recruitment sites with fewer motifs on the X are bound, and why some strong motif clusters on autosomes are not bound. To address this, we inserted different recruitment sites on the autosomes and tested their ability to recruit. Our results suggest a model in which long-distance cooperation between recruitment sites increase X-specificity of DCC recruitment. Extending this observation to other systems would imply a function for three-dimensional conformation of chromosomes in regulating specificity of protein targeting.

W402 Properties and activities of enhancers and promoters. *Chiara Cerrato*, Carson Woodbury, Eva Zeiser, Julie Ahringer. The Gurdon Institute, University of Cambridge, Cambridge, UK.

Gene expression is controlled through regulatory elements such as promoters and enhancers. Enhancers and other regulatory regions are usually bound by sequence-specific transcription factors that activate or repress transcription from promoters. The interactions, activities, and regulatory events that occur at and between enhancers and promoters are poorly understood.

We previously mapped the genome-wide landscape of RNA Polymerase II transcription initiation and elongation in *C. elegans* (Chen et al 2013). We identified ~75,000 sites of transcription initiation across the genome, most of which are bidirectional. In addition to transcription initiation sites at promoters, we also observed widespread bidirectional transcription initiation at enhancers. This pattern, which is also seen at mammalian enhancer regions, indicates a surprising similarity between enhancers and promoters.

We have been conducting transgenic assays to investigate and compare the activities of enhancers and promoters. We will present the results of these experiments, including findings showing that most tested core promoters contain extensive spatial and temporal information and that ~50% of tested enhancers can act as protein coding promoters, usually driving a subset of the associated gene's expression pattern.

W403 Quantitative analysis of context-dependent regulation by the Wnt pathway at single cell resolution. *J. I. Murray*, A. L. Zacharias, T. D. Lavon, E. Preston. University of Pennsylvania, Philadelphia, PA.

Signaling pathways achieve specificity in target gene activation through the dependence on additional inputs, commonly referred to as "context", such cell-specific transcription factors and appropriate chromatin states. To evaluate whether quantitative differences in activity of the Wnt pathway could also contribute to context-specific regulation, we measured the nuclear localization of the Wnt signaling pathway effectors, TCF and β-catenin, in all cells throughout development in *C. elegans* embryos. We found that the effect of Wnt compounds over successive exposures: we observed significantly increased nuclear localization of TCF and β-catenin in Wnt-signaled cells whose parents had also received a Wnt signal. This trans-generational "memory" of Wnt signaling influences target gene regulation suggesting that the level of signaling pathway activity can act an additional form of context.

To better understand how context information is encoded in Wnt target enhancers, we are investigating the dependence of enhancer activity on the organization and affinity of binding sites for TCF and other co-regulatory, cell-specific transcription factors. We identified 20 novel targets of the Wnt signaling pathway in *C. elegans* embryonic development, each expressed in a different subset of Wnt-signaled cells. We used modENCODE data and sequence conservation to identify 88 putative Wnt target enhancers and created a pipeline to test their activity in embryos. We image transgenic worms carrying fluorescent enhancer reporter constructs and a ubiquitously expressed GFP-histone at ~1 minute temporal resolution. Image analysis software identifies the nuclei and measures the reporter intensity, generating quantitative expression data for each individual cell at each timepoint from a single-celled zygote to a 600-cell elongating embryo, which we then compare with the expression of reporters driven by the full locus. Combining motif analysis of these enhancers with expression of candidate regulators from the EPIC database predicts specific regulators of each context-dependent enhancer, which we are currently testing experimentally.

W404 ShootingStar: Real-Time Tracking and Optical Manipulation of Single Cells in Development. *P. Shah*, A. Santella, Z. Bao. Memorial Sloan Kettering Cancer Center, New York, NY.

While *in toto* imaging and image analysis methods have advanced the study of multicellular phenomena in development at single-cell resolution, little progress has been made in the design of tools to perturb complex tissues with comparable spatial and temporal resolution.

Mutagenesis and RNAi are powerful tools for dissecting the genetic basis of developmental phenomena, yet neither offers the specificity or the temporal control required for dissecting the roles played by individual cells in coordinating developmental processes. Classical single cell perturbation techniques such as laser cell ablation and newer technologies built around photoactivatable reagents offer significant promise in filling this need. Their use to-date, however, remains limited by the challenge of reliably identifying target cells.

We have developed a platform for the real-time segmentation and tracking of single cells in 3D tissues to enable reproducible single cell perturbations at high throughput and without a need for cell-specific markers. ShootingStar can currently maintain real-time throughput on desktop hardware for C. *elegans* datasets until the onset of twitching and for D. *rerio* and D. *melanogaster* embryos containing on the order of thousands of cells. We have benchmarked this platform by the successive and parallel ablations of neural progenitors and neuronal support cells in the embryo of C. *elegans*. Specifically, we have performed parallel ablations of all 6 GLR cells to probe their role in nerve ring positioning. Ablated embryos exhibit no gross defects in nerve ring morphology or positioning but hatch late with the nerve ring exhibiting a strong anterior shift and evidence of defasciculation (N=3). While anatomical evidence has suggested that the GLR's play a role in nerve ring assembly, our observations instead suggest a role in maintaining nerve ring positioning during elongation and pharyngeal morphogenesis. While our applications to-date have focused on laser cell ablation, the architecture of ShootingStar allows for a diverse set of perturbations such as single-cell heatshock or the use of photoconvertible fluorophores to selectively label single cells on-demand. Current efforts are directed towards demonstrating these applications as well as the parallelization of cell tracking to enable real-time lineage tracing later in development for larger model organisms. The long-term goal of our work is to establish ShootingStar as a generalized platform for the study of single cell behaviors in complex multicellular environments *in vitro* and *in vivo*.

W405 Tissue-specific analysis of nuclear organization through development of a novel FLP/Frt-based toolkit for spatiotemporal control of gene expression. *P. Askjaer*, C. Muñoz-Jiménez, C. Ayuso, A. Dobrzynska, G. Gómez-Saldivar, L. Riquelme. CSIC-Uni Pablo de Olavide, Seville, ES.

The nuclear envelope (NE) plays critical roles in gene expression through controlling access to the nucleus, anchoring of chromosomes at the nuclear periphery and serving as platform for chromatin-interacting proteins, such as histone modifiers and transcription factors. Several human diseases are attributed to alterations in NE structure, including the laminopathies, whose name refers to underlying mutations in components of the nuclear lamina and lamina-associated proteins. One example is Emery-Dreifuss muscular dystrophy, which is caused by mutations in the inner nuclear membrane protein emerin or in lamin.

We recently found that emerin/EMR-1 is required for correct neuromuscular junction activity in *C. elegans*. Firstly, EMR-1 associates with genes involved in muscle and neuronal function and deletion of *emr-1* causes local changes in nuclear architecture. Secondly, transcriptome analyses revealed that EMR-1 is associated with gene repression, particularly of genes implicated in muscle and nervous system function. Thirdly we demonstrated that *emr-1* mutants are sensitive to the cholinesterase inhibitor aldicarb, indicating altered activity at neuromuscular junctions.

Although many NE proteins are ubiquitously expressed, laminopathies often affect a single tissue. This has led to the hypothesis that tissue-specific alterations in nuclear organization are responsible for particular clinical manifestations of laminopathies. Specifically, changes in interactions between NE proteins and chromatin are thought to be relevant, but has not been explored in intact organisms due to technical limitations. This triggered us to develop novel tools to dissect the function of EMR-1 in tissue-specific nuclear organization. Using an optimized FLP recombinase, we show specific and efficient activation of dual color reporters is selected tissues. Importantly, our system is based on single-copy FLP and Frt transgenes integrated into the genome by MosSCI to ensure reproducible expression and to facilitate crosses. Until now, the repertoire of cell types amenable to analysis includes striated and nonstriated muscles, intestine, M cell lineage, seam cell lineage, hypodermis, and neurons (pan-neuronal and specific neuronal subtypes). We have implemented our FLP/Frt system to perform tissue-specific DamID, which has provided EMR-1/chromatin interaction profiles for different cell types, but the tool kit can easily be adapted for spatiotemporal control of other transgenes, including fluorescent reporters, dominant alleles or suicide genes.

W406 Systematic engineering of a temperature-optimized Gal4/UAS system for transcriptional control of gene expression in *Caenorhabditis elegans*. *Jonathan Liu*[1,2], Han Wang[1,2], Shahla Gharib[1,2], Navin Pokala[3], Paul W. Sternberg[1,2]. 1) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA; 2) Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA; 3) Department of Life Sciences, New York Institute of Technology, Old Westbury, NY.

A critical tool for studying gene function is the ability to control gene expression in desired temporal and spatial patterns. The bipartite Gal4/UAS system from yeast has become a powerful tool for manipulating gene expression in many model organisms since its discovery. However, this system has not been shown to work in *C. elegans*. Here, we systematically compare the transcriptional efficacy of three major components of this system- the activation domain, UAS copy number, and DNA-binding domain, all in order to develop an optimized Gal4/UAS system for gene expression in *C. elegans*. Most importantly, we find that the performance of Gal4 is heavily dependent on temperature and performs poorly at 20°C or below. To combat this, we use evolutionary analysis to identify a well conserved Gal4 from the yeast *Saccharomyces kudriavzevii* whose optimal growth temperature is 23-24°C. This new Gal4 is stronger, capable of recapitulating endogenous promoter strength, and also displays temperature robustness across the 15-25°C range. Our optimized Gal4/UAS system is capable of driving expression in a variety of tissues; we go on to demonstrate tissue-specific rescue, as well as gain-of-function channelrhodopsin experiments in neurons. All together, this constitutes a fully functioning bipartite gene expression system to increase the rate and rigor of research in *C. elegans* biology.

W407 Longevity and its transgenerational inheritance is enabled by H3K9 methylation. *T. W. Lee*, A. E. Engstrom, D. J. Katz. Emory University, Atlanta, GA.

Longevity is a complex trait influenced by environmental, genetic, and epigenetic factors. WDR-5, a member of the COMPASS complex, methylates histone 3 at lysine 4 (H3K4). Previously, the Brunet lab has shown that *wdr-5* mutants are long-lived, and this longevity is inherited by wild-type descendants. We demonstrate that longevity in this background is a transgenerational phenotype that takes several generations to manifest after the loss of WDR-5. Consistent with the gradual appearance of longevity in *wdr-5* mutant populations, we see that lifespan correlates with levels of dimethylation of histone 3 at lysine 9 (H3K9me2), a mark associated with repressive chromatin. This result suggests that H3K9me2 may be inherited transgenerationally and confer longevity in *wdr-5* mutants. To examine this possibility, we mutated *met-2*, the methyltransferase required for all germline H3K9me2, in *wdr-5* mutants. We show that the extended lifespan of *wdr-5* mutants is dependent on *met-2*, further implicating H3K9me2 in the mechanism of longevity. Moreover, our finding that H3K9me2 is heritable indicates that it may also be involved in the inheritance of longevity in *wdr-5* mutants. To test this possibility, we deleted *met-2* in descendants of *wdr-5* mutants, and find that the loss of *met-2* abolishes the inheritance of longevity. Taken together, these data support a model in which H3K9me2 facilitates longevity and its epigenetic inheritance.

W408 CRISPR-mediated synthetic genetic analysis reveals genetic interactions among RNA binding proteins affecting fitness and lifespan. *Adam Norris*, Xicotencatl Gracida, John Calarco. Harvard University, Cambridge, MA.

Experiments in unicellular organisms such as yeast have revealed widespread synthetic genetic interactions between genes and demonstrated that systematic analysis of genetic interactions can be utilized to assign shared biological functions to genes. As such, the ability to broadly survey genetic interactions with knockout alleles in metazoans would be highly desirable. We recently developed such a system in *C. elegans* to perform CRISPR/Cas9-based synthetic genetic analysis (CRISPR-SGA) using null mutations in a multicellular organism for the first time. Homologous recombination-mediated repair of double-strand breaks triggered by CRISPR/Cas9 replaces a target gene with one of two heterologous GFP transgenes, allowing reporter fluorescence to be followed as a proxy for the gene deletion. This enables generation of large numbers of double mutants by simply crossing the compatible single mutant animals and identifying double mutants by fluorescence microscopy. We applied CRISPR-SGA to genetic interactions between RNA binding proteins. RNA binding proteins are critical mediators of post-transcriptional regulation, but the majority of metazoan RNA binding proteins have no known molecular function or phenotype. Moreover, RNA binding proteins often act combinatorially to control post-transcriptional regulation, suggesting that single RNA binding protein mutations may lead to subtle or no phenotypes due to genetic redundancy. We deleted 11 non-essential evolutionarily-conserved RNA binding proteins and generated all 55 possible double mutants. Mutants were tested with a quantitative assay that measures competitive fitness compared to wild-type over multiple generations. We identified a number of double mutants with substantially lower fitness than would be predicted based on the additive defects of the two single mutant fitness scores. Follow-up on one such synthetic fitness defect revealed severe lifespan reduction in *exc-7;mbl-1* strains, though neither single mutant has a substantial effect on lifespan. The defect appears to be a specific aging phenotype, because developmental viability is not affected and additional markers of organismal health do not begin to decline until adulthood. Current follow-up experiments include RNA-Seq on the mutants as they age to shed light on the targets of the two RNA binding proteins, and tissue-specific rescue to determine the relevant tissue for the phenotype. These experiments demonstrate a substantial degree of synthetic fitness defects among RNA binding proteins and reveal pairs of genes important for maintaining organismal physiology. Moreover, our CRISPR-SGA system should be broadly applicable to anyone interested in metazoan synthetic genetics.

W409 Investigating the role of microtubule minus-end proteins in noncentrosomal microtubule organization during epithelial development. *Taylor Skokan*, Claire Baumer, Jessica Feldman. Stanford University, Stanford, CA.

The microtubule cytoskeleton is spatially reorganized from the centrosomes to new subcellular sites during cell differentiation, a process accomplished by a reassignment of microtubule organizing center (MTOC) proteins and function. In the developing *C. elegans* intestine, all MTOC function is reassigned from the centrosomes to the future apical cell membrane during cell polarization; microtubules grow from and are anchored at the apical membrane. This MTOC reassignment occurs relatively rapidly and synchronously in all polarizing intestinal cells and so affords us a system in which to probe the mechanisms controlling the formation of these new non-centrosomal MTOCs at the onset of cell differentiation. We find that microtubule minus-end binding proteins NOCA-1, PTRN-1, Ninein-like protein (T04F8.6), and γ-tubulin small complex (γ-TuSC) proteins GIP-1/GCP3, GIP-2/GCP2, and TBG-1/γ-tubulin localize to the apical MTOC. To determine the requirement of these proteins in membrane MTOC formation, we are taking advantage of a tissue-specific protein degradation system (Armenti et al. 2014) to deplete endogenous GIP-1 exclusively in the developing intestine around the time of MTOC reassignment. Using this method, we have found that GIP-1 is not required for the apical localization of the apical polarity protein PAR-3; however, GIP-1 is required for normal apical localization of GIP-2 and TBG-1. Surprisingly, apical microtubules are present in GIP-1-depleted embryos, suggesting an alternative method for nucleation and/or localization of microtubules at these sites. In other tissues, it has previously been reported that ptrn-1 functions in parallel to γ-TuSC to generate non-centrosomal microtubules; however, we find apically localized microtubules after depletion of both GIP-1 and PTRN-1 in developing intestines, suggesting the existence of a third pathway. GIP-1 appears to be essential for normal mitosis in the developing intestine as has been reported in the early embryo; embryos depleted of intestinal GIP-1 have fewer intestinal nuclei as compared to control embryos, and these nuclei appear consistently enlarged. Together, these results demonstrate that apical GIP-1 localization is required for the polarization of γ-TuSCs in the developing intestine, but that additional pathways exist to establish an apical MTOC. Furthermore, centrosomal GIP-1 localization is required for normal spindle formation and mitosis. Further investigation is necessary to determine how γ-TuSC-independent microtubules are generated at the apical membrane, and whether this population of microtubules differs from those of control embryos in quantity and dynamics.

W410 Cytoskeletal elements function together to move larval P-cell nuclei through constricted spaces. *Courtney Bone*, Yu-Tai Chang, Natalie Cain, Shaun Murphy, Daniel Starr. University of California Davis, Davis, CA.

Nuclear migration is a critical aspect of many developmental and cellular processes including fertilization, cell polarization, and differentiation. Disruptions of the nuclear envelope migration machinery lead to a variety of diseases including Hutchinson-Gilford Progeria Syndrome, Emery-Dreifuss Muscular Dystrophy, cancer and autism. For nuclear migration to occur, a bridge termed the LINC complex (for linker of nucleoskeleton and cytoskeleton), forms across the nuclear envelope to connect nuclear lamins to microtubule motors. In *C. elegans*, the LINC complex utilized for moving nuclei consists of the KASH protein, UNC-83 in the outer nuclear membrane and the SUN protein UNC-84 in the inner nuclear membrane. We have previously shown that UNC-83 recruits microtubule motors to the surface of the nucleus and UNC-84 interacts with the lamin LMN-1 to disperse forces throughout the nucleus. P-cell nuclear migration occurs during mid L1. The P-cell nucleus is approximately 3-4 μm in diameter, but must migrate through a 150 nm space between the body wall muscle and the cuticle of the worm. We hypothesize that dramatic cytoskeletal and nucleoskeletal rearrangements are necessary for nuclear migration through constricted spaces. We are investigating the roles of the cytoskeleton and nucleoskeleton during this nuclear migration event by visualizing these components *in vivo*. We aim to characterize the interacting roles of microtubule and actin pathways, as well as identify the role of the nuclear lamina in P-cell nuclear migration. Our data suggest a role for dynein as the primary microtubule motor functioning to move nuclei during P cell nuclear migration, while kinesin plays a more minor role. This is opposite to the roles of kinesin and dynein in embryonic hypodermal nuclear migrations. Additionally, a genetic screen for enhancers of *unc-84* implicates the actin cytoskeleton in P-cell nuclear migration. We are therefore investigating the dynamics of the actin network in P-cell development by live cell imaging of tagged actin binding proteins. We hypothesize nuclear lamina breakdown is necessary for nuclear reorganization to squeeze into the constricted space and used CRISPR/Cas9 to make a functional, GFP-tagged LMN-1. Our working model postulates that the nuclear lamina is remodeled to allow nuclei to squeeze through tight spaces, dynein provides the major force for P-cell nuclear migration, and kinesin and actin cables within the cell assist in nuclear migration.

W411 Mitochondria localize to injured axons to support regeneration. *S. Han*, H. Baig, M. Hammarlund. Yale Univ, New Haven, CT.

Axon regeneration after nerve injury requires generation of injury signals, elaboration of a growth cone, and target-directed growth. Little is known about the role of mitochondria during axon regeneration. Here we use *in vivo* single-neuron analysis to investigate the relationship between nerve injury, mitochondrial localization, and axon regeneration. Mitochondria accumulate in injured axons, and axons that fail to increase mitochondria have poor regeneration. Experimental alterations to mitochondrial distribution or mitochondrial respiratory chain function result in corresponding changes to regeneration outcomes. Axonal mitochondria are specifically required for growth cone migration, identifying a key energy challenge for injured neurons. Finally, mitochondrial localization to the axon after injury is regulated in part by dual-leucine zipper kinase-1 (DLK-1), a conserved regulator of axon regeneration. These data identify regulation of axonal mitochondria as a new cell biological mechanism that helps determine the regenerative response of injured neurons.

W412 *C.elegans* as a model to study extracellular vesicle biology, dynamics and function. *Jyothi Shilpa Akella*[1*], Juan Wang[1], Malan Silva[1], Julie Maguire[1], David Hall[2], Maureen Barr[1]. 1) Dept. of Genetics, Rutgers University, Piscataway, NJ; 2) Center for C.elegans Anatomy, Albert Einstein College of Medicine, Bronx, NY.

Extracellular vesicles (EVs) are nano-sized packages released by cells that function in intercellular communication. Cilia are hair-like projections that play important roles in development and signaling. The cilium both releases and binds to EVs. EVs play a role in cell signaling in health and pathologies, and may carry beneficial or toxic cargo. A fundamental understanding of the biogenesis, release, uptake, and signaling of ciliary EVs is lacking. Here, we demonstrate the utility of the nematode *C.elegans* as a model to identify the molecules and mechanisms that play a role in EV biology and function.

A subset of the ciliated neurons of *C.elegans* release EVs containing select cargo into the environment. These cargo include the polycystins LOV-1 and PKD-2. *C.elegans* EVs regulate the mating behaviors in a cargo-dependent manner. Using a candidate gene approach combined with live imaging of mutants that express GFP-tagged EV cargo, we are determining mechanisms driving biogenesis and release. Defects in the release of GFP-tagged EV cargo from the tips of EV releasing neurons (EVNs) and/or the accumulation of GFP-tagged EV cargo at the base of the EVNs indicates defects in EV biogenesis and/or release. We then use Transmission Electron Microscopy to determine whether the gene is required for EV biogenesis or EV release. Using these approaches, we have uncovered a requirement for cilia, the intraflagellar transport (IFT) machinery, a ciliary kinesin KLP-6, a stress-activated p38 MAPK PMK-1, and a novel myristoylated protein CIL-7. We are now determining whether a RAB protein and a phospholipid flippase play a role in ciliary EV biology. Thus, *C.elegans* can be used to study the relationship between cilia and EVs, and also identify the factors that regulate EV composition and function in health and disease.

Future studies are aimed at the identification of EV cargo, environmental conditions, and the components that regulate the biogenesis and release of EVs containing distinct cargoes.

W413 The TspanC8 tetraspanins TSP-12 and TSP-14 function through the ADAM10 protease SUP-17 to promote BMP signaling in *C. elegans*. *Lin Wang*, Zhiyu Liu, Herong Shi, Jun Liu. Cornell University, Ithaca, NY.

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor β (TGFβ) superfamily of ligands. Malfunction of the pathway in humans can cause various developmental or physiological disorders. Thus it is critical that the BMP pathway is tightly regulated. Our lab has developed a sensitive and specific genetic screen in *C. elegans* to identify novel components that function to modulate BMP signaling (Liu et al., 2015). Using this screen, we have recently identified two evolutionarily conserved tetraspanins, TSP-12 and TSP-14, and an ADAM (a disintegrin

and metalloprotease) protease SUP-17, that function in promoting BMP signaling. TSP-12 and TSP-14 are paralogs that belong to the TspanC8 subfamily of tetraspanins, while SUP-17 is the ortholog of ADAM10. We have found that TSP-12 and TSP-14 function redundantly through SUP-17 to promote BMP signaling. All three proteins are expressed, and function, in the BMP receptor-expressing cells to modulate pathway activity. Both TSP-12 and TSP-14 can bind SUP-17 in a membrane-based yeast two-hybrid system, and are required for the cell surface localization of SUP-17. SUP-17/ADAM10 is known to cleave cell surface proteins through a process called 'ectodomain shedding'. One of the best known substrates of SUP-17/ADAM10 is Notch. We have found that the function of TSP-12, TSP-14 and SUP-17 in BMP signaling is independent of Notch signaling. We further provide genetic evidence that the neogenin homolog UNC-40, another positive modulator of BMP signaling (Tian et al., 2013), is one substrate of SUP-17/ADAM10. TSP-12, TSP-14, SUP-17 and UNC-40 are all conserved in mammals. We are currently determining whether their roles in BMP signaling are also conserved in mammals.

*The first two authors contributed equally to this work.

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W414 Conserved NEKL/MLT protein network controls molting in *C. elegans*. *V. Lazetic*, J. Yochem, E. Karina, D. Fay. University of Wyoming, Laramie, WY.

Molting in *C. elegans* is essential for larval growth and development and involves remodeling of the apical extracellular matrix of the epidermis (cuticle) through cycles of degradation and re-synthesis. Using a genetic approach, we identified two NIMA family serine/threonine kinases, NEKL-2 and NEKL-3, and three ankyrin-repeat proteins (MLT-2, MLT-3, and MLT-4), as essential for the completion of molting. Furthermore, genetic analyses indicate that *nekl-2* and *nekl-3* carry out partially redundant functions. NEKL-3 is highly conserved and very similar to mammalian Nek6 and Nek7, which regulate several cell cycle processes involving microtubules. Co-expression of mammalian Nek6 and Nek7 rescue molting defects in *nekl-3* mutants, suggesting that these proteins share a conserved molecular function. Surprisingly, *nekl-3* is not required for cell cycle regulation in *C. elegans*, but functions specifically in the epidermis to promote cuticle shedding, possibly through the regulation of cytoskeleton architecture, vesicular trafficking, or both. NEKL-2 is the most similar to mammalian Nek8, which may control microtubule organization during the formation of primary cilia. NEKL-2 functions specifically in the epidermis, a tissue lacking cilia, where it promotes the endocytosis of LRP-1/megalin, an essential molting factor and receptor for low-density lipoproteins. Furthermore, NEKL-2 functions as global regulator of clathrin-mediated endocytosis in the epidermis. Our data, together with studies of their mammalian orthologs, indicate that NEKL-2 and NEKL-3 associate with ankyrin-repeat proteins, which may function as scaffolds for NEKL-2 and NEKL-3 signaling. Loss of several conserved ankyrin-rich proteins (MLT-2/ANKS6, MLT-3/ANKS3 and MLT-4/INVS) leads to specific defects in molting similar to *nekl-2* and *nekl-3* mutants, and also show defects in LRP-1 trafficking. CRISRP-generated fluorescently labeled NEKL-2, MLT-2 and MLT-4 largely colocalize with each other in the main epidermal tissue of *C. elegans*. Correspondingly, NEKL-3 and MLT-3 show similar expression patterns and Y2H analysis indicates that NEKL-3 and MLT-3 physically interact. This interaction is important for the proper localization of NEKL-3 in the cytosol, as *mlt-3* depletion leads to abnormal nuclear accumulation of NEKL-3. Interestingly, *nekl-3*, *nekl-2* and *mlt-4* defects are partially suppressed by *cdc-42* depletion, suggesting that CDC-42 could be a target for negative regulation by the NEKL–MLT network, which may regulate trafficking and/or cytoskeletal organization during molting cycles. Taken together, our studies have identified several unique roles for NIMA family kinases that we expect are conserved in other eukaryotes, including mammals, but which have been largely overlooked.

W415 The catalytic activity of twitchin's kinase domain inhibits muscle activity. *Guy Benian*[1], Yohei Matsunaga[1], Hyundoo Hwang[2], Rhys Williams[3], McKenna Penley[1], Hiroshi Qadota[1], Levi Morran[1], Hang Lu[2], Olga Mayans[3]. 1) Emory Univ, Atlanta, GA; 2) Georgia Institute of Technology, Atlanta, GA; 3) University of Konstanz, Germany.

Sarcomeres, the fundamental unit of muscle contraction, contain giant polypeptides (>700,000 Da) consisting of multiple immunoglobulin and fibronectin type 3 domains, one or two protein kinase domains, and in some cases, highly elastic regions. *C. elegans* has 3 such proteins; twitchin (encoded by *unc-22*), UNC-89 and TTN-1. The function, substrates and mechanism of activation of the kinase domains of these giant proteins are unknown. *unc-22* mutants display "twitching" of the animal's surface, ~1-2 times/s, which originates from the underlying muscle, and also, to varying extents, reduced locomotion and disorganized sarcomeres. We wondered how the kinase domain of twitchin contributes to this phenotype. We converted a highly conserved lysine (K) involved in ATP binding to alanine (A) and showed that this abolishes the kinase activity of recombinant twitchin kinase in vitro towards an artificial substrate. The behavior of the KtoA mutated protein is very similar to that of the wild type protein; crystals have been grown and we plan to obtain a structure and compare it to the published wildtype structure. We used CRISPR/Cas9 to introduce the same mutation in the endogenous *unc-22* gene, resulting in expression of a kinase-dead twitchin. This mutant does not twitch, and shows normal sarcomeric structure by immunostaining using a battery of antibodies to sarcomeric proteins. Remarkably, however, a thrashing assay in liquid reveals that *unc-22(KtoA)* moves ~25% faster than wild type. While crawling on an agar surface *unc-22(KtoA)* has a ~60% greater velocity than wildtype. Using an optogenetic method, the KtoA mutant shows an abnormal pattern with more overall contraction, and faster rates of contraction and of relaxation. Thus, twitchin kinase activity is required to inhibit the contraction/relaxation cycle. The closest homolog of twitchin in vertebrates is titin, but titin's kinase domain is an inactive pseudokinase. Twitchin kinase from Aplysia is also active, as is the kinase domain from the twitchin homolog in Drosophila called projectin. We wondered if there was a selective advantage in maintaining kinase activity. To test this hypothesis in *C. elegans*, we conducted a competitive fitness assay; competing either wild type individuals or *unc-22(KtoA)* mutants against a GFP-marked tester strain. After 4 generations, the wildtype strain

exhibited significantly greater fitness than the *unc-22(KtoA)* strain. Specifically the wildtype strain increased in frequency ~17% relative to the tester strain, while the *unc-22(KtoA)* strain decreased ~20% in frequency over 4 generations. Thus, there is a substantial selective advantage in maintaining the kinase activity of twitchin.

W416 Modeling cerebral cavernous malformations in *C. elegans*. *Brent Derry*, Swati Pal. Hospital for Sick Children, Toronto, ON, CA.

Cerebral Cavernous Malformations (CCM) are vascular disorders of central nervous system that arise from weakened blood capillaries causing blood leakage. Three genes have been shown to cause familial CCM in humans, *CCM1*, *CCM2* and *CCM3*, and two are conserved in the worm (*kri-1*/*CCM1* and *ccm-3*/*CCM3*). Patients with mutations in *CCM3* have a earlier disease onset and a more severe prognosis than *CCM1* or *CCM2* patients. CCM1-3 form a ternary complex but CCM-3 protein resides predominantly in the STriatin Interacting Phosphatase and Kinase (STRIPAK) complex in cells. We previously showed that *kri-1* is required in the soma to promote DNA damage-induced germline apoptosis (Ito *et al*., 2010) whereas *ccm-3* is required for excretory canal extension (Lant *et al*., 2015), demonstrating distinct cellular functions for these genes. Using the germline as a model we show that CCM-3/STRIPAK is also required for rachis development by regulating both endocytic recycling and the anctinomysin cytoskeleton.

During development germ cells undergo incomplete cytokinesis to create openings into a common lumen (rachis), which requires the coordinated activity of cytoskeletal proteins and receptor-mediated signalling pathways, such as Notch and Ras/MAPK. *ccm-3* mutants have fewer mitotically proliferating germ cells and reduced GLP-1/Notch. Since we previously showed that *ccm-3* mutants are defective in endocytic recycling we asked if mitotic cell proliferation could be restored by inhibiting *sel-9,* which negatively regulates transport of GLP-1 to the membrane surface. Indeed, *sel-9(RNAi)* restored mitotic cell proliferation in *ccm-3* mutants to wild type levels. We also observed diminished Ras/MAPK signalling and failure to present the vitellogenin receptor RME-2 on oocytes, suggesting a general defect in endocytic recycling. Consistent with this, we found that CCM-3 colocalizes with the endosome recycling protein RAB-11.

Loss of anillin proteins results in germline defects that are strikingly similar to *ccm-3* mutants, suggesting potential interactions between these scaffolding proteins in promoting rachis development. CCM-3 and its binding partner GCK-1 are required for cortical localization of ANI-1 and the non-muscle myosin NMY-2. Knockdown of *ccm-3* also results in defective mitotic division and loss of polarity proteins in the germline and embryo. Based on these results we propose that CCM-3 functions to couple vesicle trafficking and acto-myosin organization in order to fine-tune membrane patterning and polarity in the germline and embryo. Using *C. elegans* to understand the CCM-3 regulatory network should help identify non-surgical therapies for treating CCM patients, which we are pursuing also through small molecule screens.

W417 A regulatory map of the C.elegans nervous system. *Oliver Hobert*. Columbia University, New York, NY.

no abstract submitted

W418 Single-cell *C. elegans* transcriptomics: Deciphering the expression of all genes in all cells throughout development. *I. Yanai*[1,2], T. Hashimshony[2], A. Cole[2]. 1) New York University, New York City, NY; 2) Faculty of Biology, Technion, Israel.

To understand embryogenesis, it is crucial to elucidate the expression state of each gene in every cell of the organism. This is not feasible at present for most organisms, yet it is conceivable for the nematode *C. elegans* given that only 1,304 cells occur by the first larval stage. Over thirty years ago, the full cell lineage of *C. elegans* was deciphered through the pioneering work of John Sulston. While this work defined the ancestry and fate of each cell, the overall underlying gene regulatory networks remain unknown. To assist in this challenge, we previously pioneered a multiplexed single-cell RNA-Seq method called CEL-Seq. Here, we invoke CEL-Seq to define the transcriptome of each cell throughout *C. elegans* embryogenesis. We collected and processed over two thousand cells from fifty embryos spanning the fertilized egg to the penultimate cell division. We found that cells generally cluster according to their stage and lineage. To decipher the exact identity of each cell we made use of the wealth of previously defined tissue-specific gene expression. The resulting database of gene expression in virtually all cells is a tremendous resource that will be important for developmental biologists, systems biologists, and computational biologists alike. We used the data to explore the general questions of how a cell produces progeny of distinct fates, and how different cells reach the same fate. For this we characterized cell-fate specification at single-cell resolution and report the patterns of similarity in differences for muscle and pharyngeal cells produced by different founder-cell lineages. Our work renders *C. elegans* the first organism for which the states of all genes are known throughout embryogenesis. While this has been a major undertaking for us, we expect our approach to be routinely adopted for the study of different species and genetic perturbations to further illuminate embryogenesis.

W419 A combined binary interaction and phenotypic map of *C. elegans* cell polarity proteins. *Mike Boxem*[1*], Diana Klompstra[2,3], Monique van der Voet[1], Irma Lemmens[4,5], João Ramalho[1], Susan Nieuwenhuize[1], Sander van den Heuvel[1], Jan Tavernier[4,5], Jeremy Nance[2,3], Thijs Koorman[1]. 1) Division of Developmental Biology, Department of Biology, Faculty of Science, Utrecht University, 3584 CH, Utrecht, The Netherlands; 2) Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, New York 10016, USA; 3) Department of Cell Biology, NYU School of Medicine, New York, New York 10016, USA; 4) 4Department of Medical Protein Research, VIB, 9000 Ghent, Belgium; 5) 5Department of Biochemistry, Faculty of Medicine and Health Sciences, Ghent University, 9000 Ghent, Belgium.

The ability to polarize is a fundamental cellular property, required for processes such as cell migration and asymmetric cell division, and for the specification of functionally distinct domains. Several key determinants of cell polarity have been identified, including the Par, Crumbs, and Scribble groups of cortical polarity regulators. However, we know comparatively little of the mechanisms through which cortical polarity is integrated with cellular events such as cytoskeletal rearrangement, organization of a polarized trafficking machinery, and functional specialization of membrane domains. A full understanding of polarity establishment will require a comprehensive knowledge of the proteins

involved in this process and the molecular interactions between them.

Here, we study the network of physical interactions that underlies polarity establishment in the nematode *Caenorhabditis elegans* using a combination of large-scale yeast two-hybrid screens and phenotypic profiling. Using a fragment-based yeast two-hybrid strategy, we identified 439 interactions between 296 proteins, as well as the protein regions that mediate these interactions. Phenotypic profiling of the network resulted in the identification of 100 physically interacting protein pairs for which RNAi-mediated depletion caused a defect in the same polarity-related process. We demonstrate the predictive capabilities of the network by showing that the physical interaction between the RhoGAP PAC-1 and PAR-6 is required for radial polarization of the *C. elegans* embryo. Our network represents a valuable resource of candidate interactions that can be used to further our insight into cell polarization.

W420 Caenorhabditis Genetics Center (CGC). *Aric Daul*, Theresa Stiernagle, Julie Knott, Kemi Awoyinka, Ann E. Rougvie. CGC, Univ of Minnesota, Minneapolis, MN.

The Caenorhabditis Genetics Center (CGC) is supported by the National Institutes of Health - Office of Research Infrastructure Programs (NIH-ORIP) and housed at the University of Minnesota. Our primary aim is to acquire, curate, and maintain Caenorhabditis strains and distribute them upon request to researchers throughout the world. There are now nearly 20,000 different strains in the collection, including more than two thousand whole-genome sequenced strains obtained from the Million Mutation Project and other sources. We strive to have at least one allele of every published gene and all chromosome rearrangements, duplications and deficiencies. Selected multiple-mutant stocks and transgenic strains are also available, such as strains that express various fluorescent protein reporter fusions. The CGC has initiated a small research component aimed at enhancing the genetic tool kit available to *C. elegans* researchers, including labeling balancer chromosomes with fluorescent markers (some of which are now available). The CGC also distributes stocks of nematode species closely related to *C. elegans* and bacterial strains necessary for nematode growth. A searchable list of strains, including information about CGC stocks, is accessible through the CGC website (http://cbs.umn.edu/cgc/home) and WormBase (http://www.wormbase.org). Requests for strains should be made on-line through our website. Follow us on Twitter **@cgc_worms** for updates on new strains and other news.

As mandated by NIH-ORIP, a small yearly user fee and charge per strain is assessed with each order. The CGC strongly encourages use of credit cards for payments. We provide yearly reports to the NIH with statistics that reflect our services to the worm community. A key tracked parameter is the number of published papers that acknowledge the CGC for providing strains. **Please remember to acknowledge the CGC in your publications!**

W421 Genetic Background and Experimental Reproducibility Play Critical Roles in Identifying Chemical Compounds with Robust Positive Effects on Longevity. *Mark Lucanic*[1], Max Guo[2], Gordon Lithgow[1], Monica Driscoll[3], Patrick Phillips[4], Caenorhabditis Intervention Testing Program. 1) Buck Institute for Research on Aging, Novato, CA; 2) Division of Aging Biology, National Institute on Aging, 7201 Wisconsin Avenue, Bethesda, MD; 3) Rutgers University, Dept. of Molecular Biology and Biochemistry, Nelson Biological Laboratories, Piscataway, NJ; 4) Institute of Ecology and Evolution, University of Oregon, Eugene, OR.

Defining approaches that limit the debilitating consequences of aging is a major challenge of our time. One approach toward improving mid- and later life health focuses on pharmacological interventions that optimize healthy aging across a diverse population. The *Caenorhabditis elegans* Intervention Testing Program (CITP) formed to study the effects of promising chemicals across diverse genetic backgrounds as a means to identify robust candidates likely to target conserved processes. To test the reproducibility of these lifespan studies, we assessed longevity in 22 *Caenorhabditis* nematode natural isolates spanning 3 species with multiple biological replicates across 3 laboratories. Although our analyses attributed virtually no variation among the locations, at each site ~10% of observed variation was associated with individual trials, with *C. briggsae* isolates in particular displaying large variations from trial-to-trial. We next tested 10 chemicals previously reported to affect longevity across a genetically diverse subset of these strains. We found that the reported dietary restriction mimetics in our set of chemicals promoted longevity in the *C. elegans* strains, but as a group exhibited inconsistent effects across the other *Caenorhabditis* strains. In contrast, the common laboratory dye ThioflavinT showed generally potent and robust positive effects on lifespan across the *Caenorhabditis* genus. Our survival analysis results indicated that assessment of genetic and experimental sources of variation is important for the identification of compounds with robust effects on longevity. Our results further highlight specific chemicals that warrant further exploration as potential leads for pharmacological interventions that can improve health in aging animals.

W422 The neuroendocrine peptide DAF-7/TGF-β is a key regulator of dietary restriction in *C. elegans*. *M. Fletcher*, D. Kim. MIT, Cambridge, MA.

The neuromodulator DAF-7/TGF-β is a key regulator of *C. elegans* physiology and behavior. DAF-7 is expressed in and secreted from chemosensory neurons and expression levels have been shown to be sensitive to environmental factors such as food availability and pheromone concentration. Signaling through DAF-7 regulates dauer formation and fat metabolism, as well as a number of diverse behaviors. Additionally, *daf-7* mutations have been observed to extend lifespan in a *daf-16* dependent manner. Here, we show that response to dietary restriction (DR), a treatment that extends lifespan, is also dependent on *daf-7*. We further investigate the dynamics of *daf-7* expression throughout the aging process of *C. elegans* to characterize how this signaling pathway acts to modulate lifespan and allow *C. elegans* to respond to changes in nutritional availability to promote longevity under dietary restriction. Understanding how limited nutritional resources elicit a response in this particular neuroendocrine pathway to confer lifespan benefits may allow us to gain insight into how DR may be acting to delay the aging process across species.

W423 *dbl-1*/TGF-β and *daf-12*/NHR signaling mediate cell-nonautonomous effects of *daf-16*/FOXO on starvation-induced developmental arrest. *R. E. W. Kaplan*, Y. Chen, B. T. Moore, J. M. Jordan, C. S. Maxwell, A. J. Schindler, L. R. Baugh. Duke University, Durham, NC.

Nutrient availability has profound influence on development. In the nematode *C. elegans,* nutrient availability governs post-embryonic development. L1-stage larvae remain in a state of developmental arrest after hatching until they feed. This “L1 arrest” (or "L1 diapause") is associated with increased stress resistance, supporting starvation survival. Loss of the transcription factor *daf-16*/FOXO, an effector of insulin/IGF signaling, results in arrest-defective and starvation-sensitive phenotypes. We show that *daf-16*/FOXO regulates L1 arrest cell-nonautonomously, suggesting that insulin/IGF signaling regulates at least one additional signaling pathway. We used mRNA-seq to identify candidate signaling molecules affected by *daf-16*/FOXO during L1 arrest. *dbl-1*/TGF-β, a ligand for the Sma/Mab pathway, *daf-12*/NHR and *daf-36*/oxygenase, an upstream component of the *daf-12* steroid hormone signaling pathway, were up-regulated during L1 arrest in a *daf-16*/FOXO mutant. Using genetic epistasis analysis, we show that *dbl-1*/TGF-β and *daf-12*/NHR steroid hormone signaling pathways are required for the *daf-16*/FOXO arrest-defective phenotype, suggesting that *daf-16*/FOXO represses *dbl-1*/TGF-β, *daf-12*/NHR and *daf-36*/oxygenase. The *dbl-1*/TGF-β and *daf-12*/NHR pathways have not previously been shown to affect L1 development, but we found that disruption of these pathways delayed L1 development in fed larvae, consistent with these pathways promoting development in starved *daf-16*/FOXO mutants. Though the *dbl-1*/TGF-β and *daf-12*/NHR pathways are epistatic to *daf-16*/FOXO for the arrest-defective phenotype, disruption of these pathways does not suppress starvation sensitivity of *daf-16*/FOXO mutants. This observation uncouples starvation survival from developmental arrest, indicating that DAF-16/FOXO targets distinct effectors for each phenotype and revealing that inappropriate development during starvation does not cause the early demise of *daf-16*/FOXO mutants. Overall, this study shows that *daf-16*/FOXO promotes developmental arrest cell-nonautonomously by repressing pathways that promote larval development.

W424 Fasting protects against proteostasis defects induced by hypoxia. *N. Iranon*, D. Miller. University of Washington, Seattle, WA.

Oxygen plays a critical role in aerobic metabolism. Accordingly, low oxygen conditions (hypoxia) can impair essential physiological processes and cause cellular damage and death, such as is observed as a result of stroke and cardiovascular disease. However, hypoxic preconditioning, in which a non-injurious hypoxic exposure precedes the damaging hypoxic insult, has been shown to be effective at attenuating the cellular damage caused by hypoxia, suggesting that there are cellular mechanisms that can protect against hypoxic damage. We have found that specific concentrations of hypoxia cause a disruption of protein homeostasis in *C. elegans*, as measured by increased aggregation of polyglutamine proteins in the body wall muscles. Here, we show that nutritional cues regulate the effect of hypoxia on proteostasis. Animals that are fasted develop dramatically fewer protein aggregates compared to their fed counterparts when exposed to hypoxia. Polyglutamine and amyloid-beta protein aggregation in the body wall muscles is thought to be cytotoxic, resulting in age-dependent uncoordination and eventual paralysis. In support of this, fed animals exposed to hypoxia have an accelerated rate of paralysis compared to controls maintained in room air. However, animals that are fasted prior to hypoxia are partially rescued from this expedited paralysis rate. We also demonstrate that fasted animals are resistant to the long-term effects of hypoxia on proteostasis. When animals are exposed to hypoxia for a short period of time, they do not display increased polyglutamine aggregation. Yet, as the animals continue to grow and develop in room air, the appearance of aggregates is accelerated in fed animals exposed to hypoxia, whereas fasted animals accumulate aggregates at a rate identical to room air controls. Taken together, our results underscore the influential role of nutritional state on both the immediate and long-term effects of hypoxia on proteostasis.

W425 Omega-3 and -6 fatty acids allocate somatic and germline lipids to ensure fitness during nutrient and oxidative stress in *Caenorhabditis elegans*. *Sean Curran*, Dana Lynn. University of Southern California, Los Angeles, CA.

Animals in nature are continually challenged by periods of feast and famine as resources inevitably fluctuate, and must allocate somatic reserves for reproduction to abate evolutionary pressures. We identify an age-dependent lipid homeostasis pathway in *Caenorhabditis elegans* that regulates the mobilization of lipids from the soma to the germline, which supports fecundity but at the cost of survival in nutrient-poor and oxidative stress environments. This trade-off is responsive to the levels of dietary carbohydrates and organismal oleic acid and is coupled to activation of the cytoprotective transcription factor SKN-1 in both laboratory-derived and natural isolates of *C. elegans*. The homeostatic balance of lipid stores between the somatic and germ cells is mediated by arachidonic acid (omega-6) and eicosapentaenoic acid (omega-3) precursors of eicosanoid signaling molecules. Our results describe a mechanism for resource reallocation within intact animals that influences reproductive fitness at the cost of somatic resilience.

W426 RAB-35 coordinates the engulfment and degradation of apoptotic cell corpses. *R. C. Haley*, Ying Wang, Zheng Zhou. Baylor College of Medicine, Houston, TX.

In metazoan organisms, apoptotic cells are swiftly engulfed by phagocytes and degraded inside phagosomes through phagosome maturation. Defects in this process can cause inflammatory responses and autoimmune diseases. In the nematode *C. elegans*, a number of proteins that control the recognition, engulfment, and degradation of apoptotic cells have been identified. In particular, small GTPases known as Rabs, well known for their role in vesicle trafficking, have been implicated in the maturation of developing phagosomes and the degradation of apoptotic cell corpses. To systematically identify all the RAB proteins that play roles in apoptotic cell clearance, we performed an RNAi screen targeting 29 RAB-encoding genes in the *C. elegans* genome, searching for the presence of extra cell corpses in worm embryos. In addition to *rab-2*, *rab-5*, and *rab-7*, which were previously known to function in phagosome maturation, we discovered that inactivation of *rab-35* also results in the *ced* (cell death abnormal) phenotype indicative of defects in apoptotic cell clearance. RAB-35 is an oncogene that has been implicated in a number of cellular processes such as the termination of cytokinesis and the control of the PI3K/AKT growth pathway. We have observed that RAB-35 localizes to the nascent phagosome in a biphasic manner around the time of phagosomal sealing. Subsequent

experiments revealed that a *rab-35* loss-of-function causes defects in both the engulfment and subsequent degradation of cell corpses. Moreover, genetic tests indicated that *rab-35* acts upstream of *rab-7* and may represent a novel cell-corpse removal pathway. We are investigating the molecular function of RAB-35 in this pathway, in particular the functional relationship between RAB-35 and the lipid second messengers PtdIns(4,5)P_2 and PtdIns(3)P.

W427 Hemicentin regulates Anillin to promote cytokinesis in *Caenorhabditis elegans* germ cells. *YC. Tse*, J. Wu, L. Zeng. Southern University of Science and Technology, Shenzhen, CN.

The success of cell division to equally segregate genetic materials into two daughter cells depends on the constriction of actomyosin-based contractile ring. The spatial and temporal regulation of contractile ring at the division plane primarily depends on two parallel intracellular signals, centralspindlin-dependent and astral relaxation pathways, in which both of them are studied extensively. However, the extracellular signal that regulates cytokinetic processes is poorly investigated. Up to now, only hemicentin (HIM-4), an extracellular matrix protein, has been showed may regulate cytokinesis in mouse embryos and *C. elegans* germline. Depletion of HIM-4 in both model organisms leads to multinucleated cells. However, the molecular mechanism of HIM-4 in cytokinesis regulation is still not clear. In this study, we demonstrate that HIM-4 is recruited to the site of cleavage furrow and promotes proper cytokinesis in *C. elegans* germ cell. The timing of contractile ring formation and cleavage furrow closure is greatly delayed in HIM-4-depleted germ cells. In addition, the recruitment and dynamic of ANI-1 is reduced in HIM-4-depleted gonad, while non-muscle myosin (NMY-2) and ANI-2 remain unchanged. Collectively, our data suggest that HIM-4 may regulate cytokinesis by promoting and stabilizing ANI-1 to the site of division in *C. elegans* germ cells.

W428 ATX-2, The *C. elegans* Ortholog of Human Ataxin-2, Regulates Centrosome Size and Microtubule Dynamics. *Michael Stubenvoll*, Jeff Medley, Mi Hye Song. Oakland University, Rochester, MI.

Centrosomes are critical sites for orchestrating microtubule behavior and exhibit dynamic changes in size during the cell cycle. As cell cycle progresses to mitosis, centrosomes recruit more microtubules to form bipolar spindles that ensure accurate chromosome segregation. The *szy-20* gene encodes a conserved RNA-binding protein that negatively regulates centrosome duplication by opposing ZYG-1, a key centrosome duplication factor. Centrosomes in *szy-20* mutants exhibit elevated levels of centrosomal proteins, resulting in defective microtubule dynamics and embryonic lethality. SZY-20 contains putative RNA-binding domains (SUZ, SUZ-C): mutating these domains has been shown to perturb *in vitro* RNA-binding of SZY-20 and its capacity to regulate centrosome size *in vivo*. Despite the finding that SZY-20 negatively regulates ZYG-1, no direct interaction between the two proteins has been found. Thus, identifying additional factors that function between SZY-20 and ZYG-1 should provide further insights into the molecular mechanism by which the RNA-binding protein SZY-20 influences centrosome assembly.

Toward this end, we identified an RNA-binding protein, ATX-2, that physically associates with SZY-20. ATX-2 is the *C. elegans* ortholog of human Ataxin-2 that is implicated in human neurodegenerative disease. We show a new role for ATX-2 in regulating centrosome size and microtubule dynamics. Depletion of ATX-2 results in embryonic lethality and cytokinesis failure in wild type embryos, and restores centrosome duplication to *zyg-1*mutants. ATX-2 forms a complex with SZY-20 in an RNA-independent fashion. In this pathway, SZY-20 promotes ATX-2 abundance, which inversely correlates with centrosome size. Centrosomes in *atx-2* mutants exhibit elevated levels of centrosome factors (ZYG-1, SPD-5, γ-Tubulin), increasing microtubule nucleating activity but hindering microtubule growth. We show that ATX-2 influences microtubule behavior by regulating the microtubule nucleating factor γ-Tubulin and microtubule destabilizer KLP-7. Our data suggest that RNA-binding proteins play an active role in controlling microtubule dynamics and provide insight into the control of proper centrosome size and microtubule behavior.

W429 Developing quantitative resource for computational analysis from images of *C. elegans* embryogenesis in a public database Phenobank. *Y. Tohsato*, H. Okada, J. Takayama, K. Kyoda, S. Onami. RIKEN Quantitative Biology Center, Kobe, Japan.

Along with the recent popularization of live-imaging technologies, various types of microscopy images are becoming available in public databases. Quantitative data such as positions and shapes of nuclei and cells, and their temporal changes obtained from these images are important resources for understanding biological phenomena as dynamical systems. Such resources can be used in various kinds of computational analysis including phenotype screening and mathematical modeling. Here, we develop a new resource of quantitative data on nuclear division dynamics in *C. elegans* RNAi-treated embryos. Quantitative data were extracted from two-dimensional time-lapse differential interference contrast microscopy images in a public database, Phenobank, using our newly developed automated image processing system. They consist of 1,579 datasets of quantitative data for RNAi-treated embryos. These datasets include three sets of data for each of the genes (i.e. 518/549 of all genes) that exerted defects in embryogenesis without inducing F0 sterility when depleted individually by RNAi. Each data contains the outlines of nuclear regions and their temporal changes. All data were verified through manual error correction, and annotated with cell name and anterior-posterior axis. To demonstrate the usage of such resource, we applied it to computational phenotype screening on female pronuclear migration. In this screening, fifteen candidate genes were selected for faster or slower migration by calculating the maximum migration speed. Seven out of the fifteen genes were confirmed to reproduce each of the phenotype in our independent experiments. Among them, *sds-22* and *F44B9.8* exhibited significantly faster and slower migration respectively than wild-type. *sds-22*(RNAi) and *F44B9.8*(RNAi) embryos expressing GFP::tubulin exhibited markedly larger and smaller asters respectively, consistent with the nuclear tracking along microtubules mechanism. This resource will be openly available in SSBD database (http://ssbd.qbic.riken.jp).

W430 PAR polarity proteins promote enhanced spindle assembly checkpoint activity in germline blastomeres. *A. R. Gerhold*[1], P. S. Maddox[2], J.-C. Labbé[1]. 1) University of Montréal, Montréal, Québec, CA; 2) University of North Carolina, Chapel Hill Chapel Hill, NC.

The spindle assembly checkpoint (SAC) is a fundamental mitotic regulator that maintains genome stability by ensuring the fidelity of

chromosome segregation. Defects in SAC surveillance can lead to aneuploidy, while several major chemotherapeutics (e.g. Taxol) rely on SAC-mediated mitotic arrest. The strength of the SAC varies between cell types and organisms, yet the reason for these differences is poorly understood. We have recently characterized SAC activity in C. elegans germline stem and progenitor cells (GSPCs) using *in situ* live-imaging of GSPC mitosis in whole-mount animals. Our work indicates that the SAC is stronger in GSPCs than in early embryonic blastomeres, providing a platform to dissect how SAC activity can vary between cell types and to uncover stem cell-specific adaptations. GSPCs are derived from an embryonic germline founder cell that is specified by a series of asymmetric cell divisions that are regulated by the highly conserved PAR polarity proteins. We used a fast-acting temperature-sensitive mutation that triggers a SAC response by inducing monopolar spindles, combined with live imaging, to determine whether the strength of the SAC varies between different cell lineages during embryogenesis. In agreement with a recent report, we found that the strength of the SAC scales with cell size, with smaller cells showing a stronger SAC response. Interestingly, however, cells in the germline lineage have a stronger SAC than would be predicted based solely on cell size. We found that enhanced checkpoint activity at the two-cell stage, in the smaller germline P1 blastomere, relative to its larger somatic sibling (AB), is only partially dependent on cell size, indicating that increased SAC activity in P1 depends on the asymmetric partitioning or control of checkpoint regulators. Indeed, in the absence of PAR proteins, when differences in both cell size and asymmetric segregation of cell fate determinants are disrupted, the SAC response in P1 and AB is identical. Neither MDF-1 nor BUB-3, two core SAC proteins, are enriched in P1 relative to AB, suggesting that enhanced SAC activity in P1 may be achieved via means other than simply increasing the concentration of SAC proteins. We are currently investigating whether recruitment of SAC proteins to unattached or improperly attached chromosomes differs between AB and P1. Altogether, our results suggest that enhanced SAC regulation may be a feature of germline cells throughout development and point to a novel interaction between PAR polarity regulators and the SAC.

W431 The balance of PAR polarity dictates cellular division patterning. *Yenwei Lim*[1], Fumio Motegi[1,2,3]. 1) Temasek Life-science Laboratory, Singapore, SG; 2) Mechanobiology Institute, Singapore, SG; 3) National University of Singapore, Singapore, SG.

A hallmark of living cells is the ability to generate copies of them but also to give rise to differentiated cell types. Every cell has to make a binary choice between symmetric or asymmetric cell divisions for cell fate diversification and tissue homeostasis during development. However, much remains unknown about the mechanism behind the cellular division making. To assess the mechanisms underlying the regulatory switch of division patterns, we have developed a system with *C. elegans* two-celled zygotes, which maintain robust developmental program, dictating the anterior and posterior blastomeres to commit symmetric and asymmetric division, respectively. By artificially manipulating spatial pattern of PAR polarity and segregation of fate determinants, we found that tipping the balance of antagonizing PAR proteins, PAR-2 and PAR-6, is sufficient to reprogram cell division patterns, resulting in all combinations of asymmetric and/or symmetric divisions at two-celled zygotes. The choice of division patterns does not rely on segregation of fate determinants, cellular size asymmetry, and cell cycle asynchrony. Our findings support the model, where balance between antagonizing PAR levels within a cell is the critical determinant for its choice in division patterns.

W432 Unraveling cell polarity dynamics with single-cell biochemistry. *Daniel J. Dickinson*, Bob Goldstein. Department of Biology and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.

Cell polarity is a fundamental feature of eukaryotic cells and plays a major role in many developmental processes. The conserved PAR protein system mediates polarization in a wide variety of animal cell types including epithelia, neurons and stem cells. PAR proteins localize asymmetrically within a cell and direct the polarization of downstream cellular processes. Although some in vitro binding interactions between PAR proteins are known, a fundamental unanswered question is how PAR proteins are organized biochemically into a signaling network that can mediate cell polarization. To address this question, we have developed a method to quantify protein-protein interactions at the single-molecule level, and with temporal resolution, in single cells. We are applying this approach to the *C. elegans* zygote, a single cell that polarizes in a defined manner with known and reproducible timing. We have identified PAR protein complexes that are specifically up- or down-regulated as the zygote polarizes. In particular, our data identify PAR-3 oligomerization as a key regulatory node for controlling cell polarization: PAR-3 forms large oligomers during polarity establishment, but these oligomers are absent in both stably polarized and non-polarized cells. Quantitative live imaging experiments suggest that PAR-3 oligomerization may contribute to polarity establishment by facilitating redistribution of the PAR-3/PAR-6/aPKC complex to the anterior half of the zygote. Ongoing experiments that combine single-cell biochemistry, quantitative live cell imaging, CRISPR/Cas9-induced targeted mutations and computational modeling in this simple system will reveal how regulated interactions between PAR proteins contribute to the establishment and maintenance of cell polarity.

W433 A Critical Role for Lipid Synthesis and Polyunsaturated Fatty Acids in *C. elegans* Early Embryonic Development. *J. S. Watts*[1], Diane Morton[2], Kenneth Kemphues[2], Jennifer Watts[1]. 1) Washington State University, Pullman, Wa; 2) Cornell University, Ithaca, NY.

The transition from oocyte-to-embryo is a dramatic process in which the previously quiescent oocyte undergoes a series of highly coordinated events that lead to maturation, fertilization, and initiation of mitotic divisions. In the nematode *Caenorhabditis elegans*, this process occurs rapidly and continuously when reproductive maturity is reached. In the short time between fertilization and the first mitotic division, the *C. elegans* embryo completes meiosis, establishes a polarity axis critical for development, and forms a complex multilayered eggshell. A growing body of evidence indicates that *de novo* lipid synthesis and lipid modification are critical for these early embryonic events. Here, we demonstrate the central role that the *C. elegans* holocarboxylase synthetase, BPL-1 performs in fatty acid *de novo* synthesis and in the early embryo. Mutations in the *bpl-1* gene are maternal-effect lethal, and cause defects in PAR polarity, meiosis, and synthesis of the eggshell permeability barrier. We found that a major consequence of disrupting BPL-1 and other *de novo* synthesis genes was a change in fatty acid composition, specifically, a large decrease in polyunsaturated fatty acids (PUFAs). The decrease in PUFAs was much greater in embryos than in

the parent, suggesting that maintenance of embryonic fatty acid composition is more dependent on the lipid biosynthesis machinery than the soma. We provide evidence that dietary malonyl-CoA and biotin support *de novo* synthesis in the whole worm, but not in the embryo, which helps to explain the greater need for *de novo* lipid synthesis machinery in embryos. Further, analysis of fatty acid desaturase mutants demonstrates that PUFAs contribute to synthesis of the embryonic permeability barrier and completion of meiosis. Our evidence supports a model in which dietary and *de novo* synthesized malonyl-CoA converge to support *de novo* synthesis of fatty acids and maintenance of lipid composition in *C. elegans*, but in the sequestered environment of the early embryo, dietary support is unavailable, increasing the importance of the fatty acid *de novo* synthesis machinery to maintain fatty acid composition and to synthesize the specific lipids required in the embryonic permeability barrier.

W434 The forkhead transcription factor UNC-130 integrates both BMP and Notch signaling to regulate dorsoventral patterning of the *C. elegans* postembryonic mesoderm. *Qinfang Shen*, Herong Shi, Leila Toulabi, Erin Nicklow, Jun Liu. Cornell University, Ithaca, NY.

The *C. elegans* postembryonic mesoderm, the M lineage, exhibits distinct dorsoventral asymmetry: the dorsal side produces two non-muscle coelomocytes (CCs), while the ventral side produces two sex myoblasts (SMs). We have previously shown that LIN-12/Notch signaling and the antagonism of BMP signaling by the Schnurri protein SMA-9 function independently to promote ventral and dorsal M lineage fates, respectively (Foehr and Liu, 2008). How these distinct signaling inputs are integrated is not known. We have found that the forkhead transcription factor UNC-130 is one of the key factors that function to integrate these distinct signaling inputs, and that this function of UNC-130 is independent of UNC-129, a TGF-beta factor whose expression in ventral bodywall muscles is known to be regulated by UNC-130 (Nash et al., 2000). A functional UNC-130::GFP is exclusively expressed in the ventral M lineage starting at the 8-M stage. We have found that the ventral M lineage expression of UNC-130::GFP requires LIN-12/Notch signaling, while the lack of UNC-130::GFP expression in the early M lineage and the dorsal M lineage is due to the antagonism of BMP signaling by SMA-9. We further showed that *unc-130(0),* but not *unc-129(0),* mutation can suppress the M lineage dorsal-to-ventral fate transformation defect of *sma-9(0)* mutants. These findings provide critical molecular evidence for the role of SMA-9/Schnurri in antagonizing BMP signaling in *C. elegans*. *unc-130(0)* mutants have low penetrance of dorsoventral patterning defects in the M lineage, suggesting that additional factors function together with UNC-130 to integrate the two signaling inputs. Current work focuses on identifying these additional factors.

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W435 VPR-1 MSP domains coordinate reproductive development with striated muscle metabolism. *Timothy Cole*[1], Jessica Schultz[1], Jack Vibbert[1], Pauline Cottee[1], Hieu Hoang[1], Se-Jin Lee[1], Sung Min Han[1], Jin Chen[2], Michael Miller[1]. 1) University of Alabama Birmingham, Birmingham, AL; 2) Vanderbilt University, Nashville, AL.

The major sperm protein domain (MSP) is an evolutionarily conserved immunoglobulin-like structure with extracellular signaling and intracellular functions. The *C. elegans* genome encodes numerous proteins containing MSPs, including about 28 isoforms expressed specifically in sperm. *vpr-1* encodes a type II endoplasmic reticulum protein with an N-terminal MSP, coiled coil motif, and C-terminal transmembrane domain. This broadly expressed, ancestral form has homologs in most animal species called VAPs. Human VAPB/ALS8 is associated with amyotrophic lateral sclerosis (ALS), a muscle disease caused by motor neuron degeneration. We have shown that the VAP MSP is proteolytically liberated from the transmembrane domain in the cytosol and secreted by an unconventional mechanism. In *C. elegans*, the secreted MSP interacts with the CLR-1 Lar-like receptor, thereby promoting mitochondrial localization to body wall muscle I-bands (Han et al. 2012 and 2013). Here we show that muscle mitochondrial abnormalities emerge during larval development. In addition to muscle defects, *vpr-1* null mutants are sterile, due to arrested gonad development at the L2-L3 stage. Genetic mosaic and cell type specific expression studies suggest that the germ line, nervous system, and intestine are cellular sites of VPR-1 MSP secretion. Using Cas9 genome-editing technology, we show that endogenous CLR-1 is expressed throughout the muscle plasma membrane in larval and adult worms. MSP to CLR-1 signaling is sufficient to remodel muscle mitochondria during the L4 stage and adulthood, concurrent with gamete development. We used the binary Q system to temporally control VPR-1 expression. The results indicate that VPR-1 is specifically required for gonad development in a short time window around late embryogenesis or early L1. In mammals, VAP MSPs are found in blood and cerebrospinal fluid (Tsuda et al. 2008; Deidda et al. 2014). Similarly, our studies in *C. elegans* are consistent with VPR-1 MSP being secreted into the pseudocoelom. We are also characterizing mouse Vapb mutants, which have muscle mitochondrial abnormalities in fast-twitch skeletal muscle fibers. Taken together, our data support the model that secreted VAP MSP domains coordinate gonad development with energy metabolism in the neuromuscular system. These results have provocative implications for ALS and other motor neuron degeneration diseases, such as spinal muscular atrophy and Kennedy's disease.

W436 Sensory Activity Maintains Proper Neural Connectivity in *C. elegans*. *Joy Li*, Kristine Andersen, Benjamin Barsi-Rhyne, Jackie Pyle, Kristine Miller, Christopher Vargas, Aruna Varshney, Bryan Tsujimoto, Alan Tran, Alex Duong, Joori Park, Emma Holdrich, Miri VanHoven. San Jose State University, San Jose, CA.

Neuronal activity has been implicated in the establishment and maintenance of appropriate synaptic connections in vertebrate and invertebrate systems. However, the molecular mechanisms by which neuronal activity affects connectivity are poorly understood. To

understand this process, we have focused our studies on the PHB phasmid sensory neurons. The PHB neurons mediate an aversive response to low concentrations of sodium dodecyl sulfate (SDS) (Hilliard et al., *Current Biology*, 2002). In response to SDS, PHB neurons terminate backward movement via their connections with AVA interneurons. Our preliminary results using the WincG.2 cGMP biomarker (a kind gift from N. L'Etoile) indicate that exposure to behaviorally relevant concentrations of SDS results in an increase in cGMP in PHB sensory neurons. We are currently taking a genetic approach to elucidating the pathway by which SDS is sensed by the phasmid neurons using a high throughput assay adapted from the method developed by Hilliard and colleagues (*Current Biology*, 2002). Using this assay, we find that *odr-3*/*Gαolf* and *tax-2*/*CNG-channel β subunit*, in addition to previously discovered *tax-4*/*CNG-channel α subunit* (Hilliard et al., *Current Biology*, 2002), are required for SDS chemosensation. To determine if defects in sensory signaling affect sensory synapses, we utilized the split-GFP-based trans-synaptic marker NLG-1 GRASP (Neuroligin-1 GFP Reconstitution Across Synaptic Partners) to visualize synapses between PHBs and AVA interneurons in live animals. Interestingly, we find that odr-3/*Gαolf* mutants have synapses that are normal at the L1 stage but significantly reduced in later larval stages, while phasmid neuron morphology appears normal. Cell-specific rescue experiments indicate that odr-3/*Gαolf* functions in PHB neurons. Subcellular localization of odr-3/*Gαolf* shows localization to the PHB cilia, consistent with a role in sensory signaling being required to maintain synaptic integrity. These results indicate that *C. elegans* may be a powerful model organism for elucidating the molecular mechanisms by which sensory activity affects synaptic connectivity. Our future goals are to identify the remainder of the chemosensory signaling pathway and to further characterize the mechanism by which sensory activity maintains synapses using molecular genetic and physiological approaches. Funded by NIH (1R01NS087544 to MV at SJSU and NL at UCSF, 5T34GM008253 MARC undergraduate fellowship to CV, 2R25GM071381 RISE undergraduate fellowships to CV and JP), HHMI (SCRIBE 52006312 undergraduate fellowship to BB and KM), and NSF (RUMBA REU 1004350 fellowship to KA and EH).

W437 A transient, pre-cuticular apical extracellular matrix defines tiny tube diameter. *J. D. Cohen*, H. K. Gill (co first author), R. Forman-Rubinsky, M. V. Sundaram. University of Pennsylvania SOM, Philadelphia, PA.

Apical extracellular matrix (aECM) shapes apical domains of many epithelia, but the mechanism by which it does so is still poorly understood. The aECM contains glycosaminoglycans and various glycoproteins, including mucins and zona pellucida domain (ZPD) proteins. Many ZPD proteins form fibrils via the ZPD, but how fibril formation is controlled and how fibrils influence cell shape is not clear. Loss of ZPD proteins causes a number of human diseases, including chronic kidney disease, vascular disease, and deafness. We are interested in the role of the aECM and ZPD proteins in stabilizing seamless tubes, a specialized cell type found in capillary beds of the mammalian vascular system in which a single cell encloses a lumen without junction along its length. By forward genetic screening, we identified 85 alleles required for maintaining the duct cell, a seamless tube of the *C. elegans* excretory system. Two of these alleles disrupt *let-653*, which encodes a secreted ZPD protein that also contains mucin-like and PAN-Apple domains (putative carbohydrate or protein interaction domains also found in plasminogen and other blood coagulation proteins). LET-653 is present within the developing duct lumen as the duct elongates from a simple cylinder to a highly elongated and asymmetric shape, but then disappears prior to the bulk of cuticle secretion. In *let-653* mutants, duct lumen diameter becomes irregular during elongation and the lumen and cell eventually fragment, showing that LET-653 is essential for tube integrity. The LET-653 ZPD, but not the N-terminal PAN domains, is sufficient to rescue these duct cell defects. To address the mechanism by which LET-653 defines lumen diameter, we turned to the much larger vulva lumen, whose shape LET-653 subtly influences. Tagged LET-653 was found in two distinct pools within this lumen: at the apical membrane, where it was highly stable as assessed by FRAP, and in the center of the vulva, where it was mobile. Structure-function analyses revealed that LET-653's ZPD was necessary and sufficient for the apical localization, while the PAN domains localize the protein to the center of the vulva lumen. Ectopic expression of full-length LET-653, but not the PAN domains alone, could expand lumen diameter in the developing gut tube, where LET-653 is not normally expressed. Together, these data support a model in which the PAN domains regulate the ability of the LET-653 ZPD to interact with other factors at the apical membrane, and this ZPD interaction promotes expansion of lumen diameter. We are currently testing if other genes identified in our screen might encode the apical factor(s) that interact with LET-653 for this mechanism.

W438 Rotating and elongating embryos: SPIM microscopy reveals how planar polarity could be established during morphogenesis. *X. Yang*[1*], T. Ferraro[1], J. Pontabry[2], N. Maghelli[3], L. Royer[3], S. Grill[3], G. Myers[3], M. Labouesse[1]. 1) Institut de Biologie Paris-Seine, Paris, FR; 2) Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, FR; 3) Max Planck Institute of Molecular cell Biology and Genetics, Dresden, Germany.

The process of morphogenesis in *C. elegans* embryos is largely driven by epidermal cells. Unlike in *Drosophila* and zebrafish embryonic development, no cell division or cell rearrangement is involved in *C. elegans* morphogenesis. Cell shape changes within the epidermis, which is characterized by junction lengthening along the anterior/posterior direction, play a key role in this process. The nature and the mechanical forces stimulating junction lengthening, as well as the cellular mechanisms involved in their lengthening are not understood.

Our lab observed that junction elongation along anterior/posterior (A/P) direction increases after muscle becomes active, and fails in muscle defective embryos. To better understand which role muscles play in driving epidermal cell junction lengthening during embryo elongation, we examined the global and local movement patterns using Single Plane Illumination Microcopy (SPIM), focusing on epidermal adherens junctions and muscle nuclei. We analyzed so far four wild-type embryos, five muscle defective embryos and twelve Rho-kinase mutant embryos. We found that wild-type embryos rotated strongly soon after muscle became active, and equally frequently to an outward or inward direction. However, muscle defective and Rho-kinase mutant embryos, which stop elongation at the 2-fold stage, scarcely rotated, suggesting that rotations are important for embryo elongation. Using cross-correlation analysis to compare the perimeter and area changes of each seam cell, we observed that the head, body and tail mechanically behaved as partially independent entities. This local pattern was lost in muscle defective and Rho-kinase mutant embryos. We next sought to understand how such movements could account for the polarized extension of

junctions along the A/P direction, keeping in mind that *C. elegans* embryos are radially symmetric. By measuring the distance between two dorsal or ventral muscle nuclei, respectively, we found that muscles on both sides generally contract alternatively, accounting for the rotation movement. Analysis of seam cell roughness and aspect ratio showed that junctions were stretched along the A/P direction when seam cells were positioned outwards during rotation. This asymmetric muscle activity defines the source of polarity in *C. elegans* embryo and provides the local driving force of epidermis stretching. Furthermore, we propose that *C. elegans* embryos extend in a ratchet mode due to the alternate pattern of muscle contractions.

W439 UNC-33/CRMP inhibits growth cone protrusion in axon repulsion from UNC-6/netrin. *Mahekta Gujar*, Erik Lundquist. University of Kansas, Lawrence, KS.

Previous studies showed that UNC-6/Netrin and its receptors UNC-40/DCC and UNC-5 control axon guidance by regulating protrusion of growth cone lamellipodia and filopodia (Norris et al., 2011; Norris et al., 2014). UNC-6 stimulates protrusion in growth cones attracted to UNC-6 via the UNC-40 homodimeric receptor, and inhibits protrusion in growth cones repelled from UNC-6 via the UNC-5/UNC-40 heterodimeric receptor. Protrusion via UNC-40 requires CDC-42, the Rac GTPases MIG-2 and CED-10, the Rac GEF TIAM-1, and the actin modulating molecules Arp2/3 and UNC-115/abLIM. We delineated a pathway downstream of UNC-5/UNC-40 required to inhibit protrusion that also involves the Rac GTPases and a distinct GEF, UNC-73/Trio, as well as the microtubule-interacting protein UNC-33, similar to Collapsin response mediating protein (CRMP). In repelled VD growth cones, it is likely that both of these complexes are active, as *unc-40* mutants suppress the excess growth cone protrusion of *unc-5* mutants (Norris et al., 2011). UNC-5 and UNC-6 restrict F-actin accumulation to the dorsal, protrusive region of the VD growth cone (Norris et al., 2011).

unc-33 mutant VD growth cones resembled *unc-5*, with excess lamellipodial and filopodial protrusion. We used EBP-2::GFP expression in VD neurons to monitor microtubule + ends in VD growth cones. We found that *unc-33* mutants displayed greatly increased numbers of EBP-2::GFP puncta in VD growth cones and protrusions, suggesting that UNC-33 normally prevents MT + end accumulation in growth cones. *unc-44/*ankyrin mutants showed the same phenotype. Importantly, *unc-5* mutants also showed increased numbers of EBP-2::GFP puncta in growth cones, consistent with UNC-33 acting in the UNC-5 pathway. Interestingly, *unc-73(rh40)*, which specifically affects the Rac GEF domain, did not cause increased EBP-2::GFP puncta despite having excessively protrusive growth cones similar to UNC-33, indicating that increased numbers of EBP-2::GFP puncta were not a secondary consequence of an enlarged growth cone. *unc-73(e936)*, which affects both the Rac and Rho GEF domains, did show increased EBP-2::GFP puncta similar to *unc-33.* These experiments suggest MT-dependent and independent mechanisms, and possibly that the MT-dependent mechanism involves Rho and the independent mechanism involves Rac and possibly actin. In sum, these studies indicate that growth cone protrusion can be inhibited by restricting MT + ends from growth cones, and that UNC-33 is involved in this process. Asymmetric regulation of UNC-33 across the growth cone might result in directed migration away from UNC-6 in axon repulsion.

W440 WAVE/SCAR promotes alpha-catenin accumulation and junctional maturation in developing *C. elegans* epithelia. *Martha Soto*, Sofya Borinskaya, Falshruti Patel, Yelena Bernadskaya, Sailaja Mandalapu, Maria Agapito, Maryam Honarbakhsh. Rutgers - RWJMS, Piscataway, NJ.

Actin is an integral component of the apical junction of epithelial cells. However, the function of the branched actin network at apical junctions is still not clear. We have conducted genetic screens for the essential regulators of cell migrations during *C. elegan*s embryonic morphogenesis and identified a pathway that includes the GTPase Rac1/CED-10, the WAVE/SCAR/GEX complex and the Arp2/3 complex. Consequently we have shown that these branched actin regulators promote enrichment of membrane proteins required for apical junction development in *C. elegans* epithelia. We therefore investigated how Arp2/3 and its WAVE/SCAR nucleation promoting factor help build and maintain apical junctions. TEM studies showed that depletion of WAVE/SCAR components post-embryonically results in reduced size of adherens junctions. This is similar to the effects of depleting either of two *C. elegans* apical junction complexes, the Cadherin Complex or the DLG-1/AJM-1 Complex. Live imaging in embryos showed that WAVE/SCAR and both of these *C. elegans* apical junction complexes promote F-actin accumulation at the apical junction. Accumulation of alpha-catenin/HMP-1, the Cadherin Complex component that binds F-actin, is greatly altered in embryos depleted of WAVE components. Further, WAVE can immunoprecipitate alpha-catenin/HMP-1 and DLG-1/Discs Large, components of two *C. elegans* apical junction complexes. Our studies suggest that distinct *C. elegans* apical junction complexes help recruit WAVE/SCAR, which in turn leads to the enrichment and retention of alpha-catenin at the apical junction, resulting in robust apical actin accumulation.

W441 Mechanical forces drive neuroblast morphogenesis and are required for epidermal enclosure. *A. J. Piekny*, K. Mastronardi, D. Wernike, K. Larocque. Concordia University, Montreal, Quebec, CA.

We recently found that the neuroblasts (neural precursor cells) undergo morphogenetic changes concomitant with the overlying epidermal cells during *C. elegans* ventral enclosure. These events occur during mid-embryogenesis, and may help prepare the embryo to subsequently elongate into the long, thin worm. Subsets of neuroblasts are initially distributed as columns across the embryo, and subsequently organize into ring-like patterns, followed by their redistribution into rows that follow the anterior-posterior axis of the embryo. The formation of similar patterns called rosettes is required for the reorganization and elongation of epithelial tissue in other metazoans, which is mediated by myosin activity and changes in adhesion junctions that tether neighbouring cells. In the neuroblasts, we observed the accumulation of myosin foci and changes in the localization of the adhesion junction components E-cadherin and α-catenin during their reorganization. Further, myosin is required in the neuroblasts for ventral enclosure, and we propose that mechanical forces in the neuroblasts influence constriction of the overlying epidermal cells. The formation of at least one of the rosettes coincides with migration of the overlying ventral epidermal cells, and disrupting neuroblast cell division alters epidermal cell migration as well as the distribution of myosin in these cells. Thus, neuroblast

morphogenesis could 1) decrease the surface area of the neuroblast substrate, making it easier for the overlying epidermal cells to migrate and/or 2) generate tension that is sensed by the epidermal cells and influences active myosin localization. We are currently testing these models by further analyzing the precise movements and shape changes of the neuroblasts that form the rosette(s), and by monitoring how their disruption influences the overlying epidermal cells. In addition, we are using AFM (atomic force microscopy) to determine if there are changes in tension/elasticity of the neuroblasts during ventral enclosure, and how myosin disruption alters this tension. The coordination of myosin-dependent events and forces between cells in different tissues could be a common theme for coordinating morphogenetic events during metazoan development.

W442 Morphogenic movements and cell signalling events during gland cell and pharyngeal organ development in *Caenorhabditis elegans*. *J. Kormish*[1], S. Tkachuk[1], S. R. Kim[1], M. Burg[1], O. Atta[1], M. Singh[1], E. Bennici Clendinnen[1], W. H. Raharjo[2]. 1) University of Manitoba, Winnipeg, Manitoba, CANADA; 2) University of Calgary, Calgary, Alberta, CANADA.

An important question in developmental genetics is how distinct cell types migrate, change shape and interact in a coordinated manner to form an organ. *Caenorhabditis elegans,* with its powerful genetics, invariant cell lineage and optical transparency that allows for live-imaging microscopy during embryo development, is an excellent tool to study cellular migration and coordination of morphogenesis during organ development. The *C. elegans* pharynx is a muscular feeding organ of the upper digestive tract. The pharynx is relatively simple organ, it consists of about 95 cells, but contains diverse cell types including muscle, neuronal, structural, epithelial, and secretory gland cells. During pharynx morphogenesis, these diverse cell types coordinate their movements to rearrange themselves from a "ball of cells" into an extended tubular structure. In my lab, we have focused our studies on the development of the pharyngeal gland cells. Live imaging studies have been used to determine that these cells, in particular the dorsal gland cell, create a unicellular extension through a process called retrograde extension. During this morphogenic process, the cell is born in the anterior aspect of the pharyngeal primordium and migrates to the posterior pharynx leaving a unicellular projection in its migratory wake.

Progress in understanding the molecular underpinnings to retrograde extension will be discussed. A forward genetic screen isolating defects in gland cell migration has been completed and 60 alleles have been isolated that display gland cell under-migration or over-migration defects. Previous work has determined that the FGFR pathway*, egl-15*, and the integrin receptor pathway, *ina-1*, provide a migratory stop cue during gland cell migration. Despite extensive efforts using candidate approaches, identification of downstream components to these receptors during gland migration have been unsuccessful. Loss of function mutations in a gene called *cam-1* display a gland cell under-migration phenotype. The CAM-1 protein is a ROR tyrosine kinase receptor that binds the Wnt ligand and induces a kinase-dependent response to provide a positive cue for gland migration. The forward genetic screen has identified several strains with Cam-1-like gland cell under-migration phenotypes as well as Egl-15 and Ina-1-like over-migration defects. Preliminary mapping of two strains that display penetrant under-migration defects have isolated two alleles to chromosome locations distinct from known *cam-1* pathway components. Further mapping studies, whole genome sequencing and genetic epistasis will be used to define the genetic pathways regulating gland cell migration during pharynx development.

W443 DAF-7/TGFβ signaling in the *C. elegans* germline stem cell niche. *Olga Pekar*[1], Maria C. Ow[2], Kailyn Y. Hui[3], Marcus B. Noyes[3], Sarah E. Hall[2], E. Jane Albert Hubbard[1]. 1) Skirball Institute of Biomolecular Medicine and Department of Cell Biology, NYU School of Medicine, New York, NY; 2) Department of Biology, Syracuse University, Syracuse, NY; 3) Institute for Systems Genetics, NYU School of Medicine, New York, NY.

An appropriate molecular environment is essential for stem and progenitor cells to decide whether to remain undifferentiated or to undergo differentiation. We are using the *C. elegans* germ line as a model for understanding how this decision is influenced by changes in an animal's environmental conditions.

During larval development in *C. elegans,* a pool of proliferative germline stem/progenitor cells accumulates, from which gametes are produced. Previously, our lab showed that the DAF-7/TGFβ-related signaling pathway modulates the accumulation of these cells in response to sensory cues, independent of previously defined roles for this pathway in the dauer decision and lifespan regulation. The expression of *daf-7* in ASI chemosensory neurons provides a link between environmental changes perceived by the animal and the number of proliferative germ cells. The TGFβ receptor (TGFβR) complex and its downstream transcriptional regulatory effectors act in the distal tip cell (DTC), the germline stem cell niche.

We sought the molecular link between TGFβR signaling in the DTC and the response in the germ line. GLP-1/Notch signaling maintains the proliferative pool of germ cells in response to DSL-family ligands LAG-2 and APX-1 produced by the DTC. However, previously, we found that TGFβR signaling can influence germ cell accumulation in a *glp-1*-independent manner, suggesting that it acts in parallel to GLP-1/Notch.

More recently, we found that TGFβR signaling can also promote the expression of *lag-2* in the DTC. This conclusion is based on results obtained using new transcriptional reporters and fluorescence *in situ* hybridization analysis. Expression of *lag-2* is reduced upon disruption of TGFβ signaling, but is restored in the absence of *daf-3* or *daf-5*, the negatively regulated downstream effectors of DAF-7/TGFβ signaling. By both yeast and bacterial one-hybrid assays, we found evidence for direct interaction between the DNA-binding domain of DAF-3 (a homolog of Smad4, that in DAF-7 pathway acts as a transcriptional repressor) and the *lag-2* promoter. By ChIP, we identified potential DAF-3 binding motifs in the same region of the promoter. Currently, we are mutating these sites *in vivo* by CRISPR/Cas9. Initial results from reporter assays indicate that eliminating these motifs abrogates the response to TGFβR signaling, suggesting that they directly mediate the response of *lag-2* to TGFβ.

W444 The combined activity of CPB-1^{CPEB} and GLD-3$^{Bic\text{-}C}$ opposes FBF^{Pum} to prevent the sperm-to-oocyte switch in *C. elegans* males. *C. R. Eckmann*[1,2], C. Hirsch[1], R. Minasaki[1,2]. 1) MPI-CBG, Dresden, DE; 2) Martin-Luther-University Halle-Wittenberg, Halle (Saale), DE.

Gamete formation requires genetic programs that coordinate germ cell differentiation and meiosis with sexual fate choice and maintenance. At the posttranscriptional level these programs are integrated via a dense network of RNA-binding and RNA-modifying proteins. Especially

translational regulators, such as PUF, Nanos and cytoplasmic polyadenylation element-binding (CPEB) family proteins are identified in many organisms as molecular nodes of the germ cell fate decision machinery. *Caenorhabditis elegans* germ cells undergo a programed sperm-to-oocyte switch in the hermaphrodite to produce -in essence- a self-fertile female animal. This is governed by the sequential action of the RNA-binding protein FOG-1^{CPEB}, which initiates male fate specification, and the FBF^{PUF}/NOS-3^{Nanos} RNA regulatory complex that blocks several male fate-promoting (‘*fem’* and ‘*fog’*) genes by preventing their efficient translation, thus limiting sperm production to the final larval stage prior to adulthood. Previously, we showed that in males the Bicaudal-C protein GLD-3 antagonizes FBF-mediated translational repression: while *gld-3* mutant germ cells initiate spermatogenesis during larval stages, adults often fail to maintain their male fate and switch to oogenesis in an *fbf*-dependent manner, suggesting that GLD-3^{Bic-C} is an important player but not the only one in males to keep the female fate-promoting machinery in check.

In search of additional regulators, we identified CPB-1^{CPEB} as a molecular opponent of FBF in sexual fate maintenance. Although *cpb-1* hermaphrodites produce -compared to wild type- a similar number of sperm, indicating no crucial role in the sperm-to-oocyte switch fate, we confirmed a previously suggested role in sperm differentiation; all animals are sterile. Importantly, we found that a large fraction of adult males produce oogenic cells after an initial period of spermatogenesis. Our genetic analysis places *cpb-1* in the core sex determination pathway upstream of *fbf* and in parallel to *gld-3*. Intriguingly, all *gld-3; cpb-1* double mutant male gonads produce exclusively oocytes suggesting that CPB-1 works redundantly with GLD-3 to antagonize FBF, which is further corroborated by their expression patterns. To our surprise, detailed structure-function analysis of transgenic rescuing experiments suggested that CPB-1’s RNA recognition motifs are essential for male fate maintenance, rather than a previously mapped FBF-binding region in its amino terminus. Therefore, CPB-1 likely functions in its capacity as an RNA-binding protein to target mRNAs required for maintenance of male germ cell fate. Our findings reveal a novel role for CPEBs and contrast to work in mammals where a transcriptional network is a key regulator of sexual fate maintenance.

W445 *top-2* is required for proper chromosome segregation during male meiosis in *C. elegans*. *A. N. Jaramillo-Lambert*, H. Smith, A. Golden. NIDDK/NIH, Bethesda, MD.

Type II DNA topoisomerases play a critical role in chromosome fidelity by alleviating topological stresses that arise within chromosomes. Topo II is a large ATP-dependent homodimeric enzyme. Each subunit breaks one DNA strand, passes a second unbroken strand through the break, and then reseals the break. Thus, during mitotic divisions, Topo II enzymes solve topological problems that arise during replication, transcription, sister chromatid segregation, and recombination. In contrast to the single round of DNA replication followed by a single nuclear division during mitosis, meiosis has one round of DNA replication followed by two nuclear divisions. In meiosis II sister chromatids segregate from each other similar to mitosis. Meiosis I, on the other hand, segregates homologs, which requires pairing, synapsis, and recombination. Studies in mammals, yeast, and *Drosophila* have demonstrated that Topo II plays a role in homologous chromosome segregation at meiosis I. However, the exact role Topo II plays during meiosis is unknown. In a screen re-examining temperature sensitive legacy mutants identified thirty years ago, we identified a *C. elegans* allele of Topo II, *top-2(it7ts)*. Initial analysis found that *top-2(it7ts)* mutant males produce dead embryos, even when fertilizing wild-type oocytes. Characterization of early embryonic events indicates fertilization is successful and that sperm components (e.g. centrosomes) are transmitted to the embryo. However, sperm chromatin is not detected in these fertilized embryos. Examination of *top-2(it7ts)* male germ lines revealed that the sperm DNA fails to properly segregate during the meiotic divisions. Chromatin bridges form during anaphase I and the chromatin becomes trapped in the residual body. Failure to segregate DNA into the spermatids results in anucleate sperm that are capable of fertilization; early embryonic events appear normal but embryos arrest around the 200-cell stage. *top-2 (it7ts)* chromosome segregation defects observed during anaphase I are not due to residual entanglements incurred during meiotic DNA replication and are not a consequence of recombination defects. We are currently evaluating the role of *top-2* in chromosome structure.

W446 The t-SNARE *syx-7* promotes cytokinesis during sperm meiosis. *K. Fenker*, G. Stanfield. University of Utah, Salt Lake City, UT.

Mature sperm are specialized cells that are both motile and capable of fertilizing oocytes. To reach this stage, sperm undergo an intricate process of differentiation, and there are many interesting questions regarding the molecular mechanisms guiding their development. We are using *C. elegans* sperm to investigate some of these questions, and have recently identified the t-SNARE syntaxin 7 (*syx-7)* as a new member of the *spe* (spermatogenesis defective) class of genes, as it plays an important role in sperm development.

syx-7 is an ortholog of mammalian STX12, an endosomal t-SNARE. Using CRISPR/Cas9, we generated several deletion alleles of *syx-7*, and found that loss of the gene significantly reduces fertility of both male and hermaphrodite worms. This reduction in fertility is not due to embryonic lethality or female germline defects, but is caused by defective sperm. In animals lacking *syx-7*, the sperm arrest as large, abnormal cells, often containing four nuclei within a common cytoplasm. Preliminary data suggests *syx-7* mutant sperm have defects in their ability to bud off from residual bodies and form haploid spermatids. To further characterize the defects in *syx-7* mutants, we are using time-lapse microscopy to generate movies of *in vitro* sperm development. So far, our data suggest *syx-7* may function in the membrane dynamics that take place during cytokinesis, possibly through delivery of new membrane components or maintenance of a boundary between the budding spermatid and the residual body. We are working to test these models. We have demonstrated *syx-7* functions in sperm to promote fertility, and we are using strains with a CRISPR-generated *gfp::syx-7* to visualize the protein’s localization. So far, we have shown that GFP::SYX-7 localizes to the sperm as well as other tissues, and we are currently determining the subcellular localization of the protein within sperm during spermatogenesis, particularly during the meiotic cell divisions.

W447 Signaling sperm to stop or go: the seminal fluid protease inhibitor SWM-1 regulates *C. elegans* sperm motility. *Daniela Chavez*, Gillian Stanfield. University of Utah, Salt Lake City, UT.

In the race to fertilize oocytes, sperm cells encounter extracellular environments that affect their success. These environments include male and female reproductive tracts as well as seminal fluid. In many species, proteolysis regulators are abundant in seminal fluid and contribute to various aspects of fertility. In *C. elegans*, protease signals regulate the onset of sperm motility, a process called activation that occurs after mating. Spatial and temporal regulation of motility is critical, as either premature or delayed activation results in significantly reduced fertility. During activation, sperm undergo morphogenesis and develop a pseudopod, which they use to crawl toward oocytes. The secreted protease TRY-5 is present in the male gonad and in seminal fluid, where it is required to activate sperm. Upstream of TRY-5, the secreted protease inhibitor SWM-1 is required to inhibit activation, yet how the protease signal is regulated spatially and temporally to control activation is unknown. To understand how SWM-1 regulates activation, we used CRISPR to tag SWM-1 at its endogenous locus. We find that *swm-1* is expressed in both the gonad and extra-gonadal cells and that SWM-1 protein surrounds spermatids. In vas deferens cells, SWM-1 localizes to vesicles closely associated with the apical membrane. Surprisingly, it is transferred with TRY-5 during mating. How seminal fluid is secreted from these vesicles is unknown and we are using our SWM-1 reporter to analyze vesicle dynamics. Tissue-specific expression shows that vas deferens-derived SWM-1 is at least partially sufficient to regulate activation. Our findings lead to a model where SWM-1 is secreted from the vas deferens to inhibit TRY-5 from activating sperm until mating. Intriguingly, the presence of SWM-1 in seminal fluid suggests an additional post-mating role in the hermaphrodite. In support of this hypothesis, SWM-1 has two trypsin-inhibitor-like (TIL) domains, which have distinct reactive sites and appear to play non-equivalent roles in inhibiting activation. To further investigate the role of SWM-1 in fertility, we are using CRISPR to generate reactive site mutants and we are testing SWM-1 for post-mating roles within the hermaphrodite reproductive tract.

W448 The sperm TRP family channel TRP-3 induces a calcium wave in the fertilized oocyte of *C. elegans*. *J. Takayama*, S. Onami. RIKEN QBiC, Kobe, JP.

Calcium waves during fertilization promote the transition from oocyte to embryo. How the fertilizing sperm induces the calcium waves has been explained either by the action of a soluble sperm-delivered factor or by the ligand-receptor interaction between the sperm and the egg. Calcium influx from the extracellular space through a sperm-derived calcium channel was predicted by the calcium conduit model; however, molecular underpinnings for this model have been elusive. Here we show that the sperm-specific plasma membrane calcium-permeable channel TRP-3 (also known as SPE-41) induces a calcium rise in the oocyte upon fertilization in *C. elegans*. Spinning-disk confocal microscopy and computational image analysis revealed that sperm induce an immediate local calcium rise followed by a propagating calcium wave. This biphasic waveform was recapitulated by a simulation that assumes that the oocyte has calcium-induced calcium release (CICR) machinery. Oocytes fertilized by *trp-3* mutant sperm showed a lack of the local calcium rise and a delay in the onset of the propagating wave. Expression of TRP-3 in sperm by sperm-specific expression drivers could rescue the defects partially. Moreover, the stronger the driver activity was, the larger the local calcium rise and the earlier the onset of the propagating calcium wave became. The correlation between the amplitude of the local calcium rise and the delay in the wave onset was again recapitulated by the simulation based on the CICR model. High-speed calcium imaging of the fertilizing sperm cytoplasm revealed that the sperm calcium concentration increases from a resting level, arguing against the bolus introduction of stored calcium from sperm. Moreover, by observing the fertilization between the oocyte whose plasma membrane was labeled with GFP-fused PH domain and the sperm that expresses TRP-3 fused C-terminally with TagRFP-T, we found that fertilization takes place as a single-round direct plasma membrane fusion. These results suggest that sperm-derived TRP-3 channel induces a local calcium rise in the fertilized oocyte. The local calcium rise would then induce a propagating calcium wave probably by the CICR mechanism.

W449 Maternal MEMI specifies the female meiosis II program in *C. elegans*. *Martin Srayko*, Justus Tegha-Dunghu, Maryam Ataeian, Jens Herzog, Ellen Sykes, Caitlin Slomp, Megha Bajaj. University of Alberta, Edmonton, AB, CA.

In most animals, female meiosis completes only after fertilization. Sperm entry has been implicated in providing a signal for the initiation of the final meiotic processes, however, a maternal component required for this has not been previously identified. We report the characterization of a novel family of three highly similar paralogs (*memi-1*, *memi-2*, *memi-3*) that encode oocyte-specific proteins. A temperature-sensitive dominant mutation, *memi-1(sb41),* was originally identified in a screen for maternal-effect lethal mutations in the Mains laboratory (formerly termed *mel-43*)[1]. *memi-1(sb41)* behaves as a hypermorphic mutation, and embryos from mutant mothers fail to exit female meiosis II properly. A *memi-1(deletion)* exhibits no obvious phenotype, however, loss of all three *memi* paralogs via RNAi causes fertilized oocytes to abort meiosis I during anaphase, "skip" meiosis II, and proceed directly into mitosis. The McNally lab showed that a similar phenotype occurs when sperm-activated oocytes are not fertilized[2], suggesting that the MEMI proteins represent a maternal component of a post-fertilization signal that specifies the meiosis II program. The MEMI proteins are degraded before mitosis and sensitive to ZYG-11, a substrate-specific adapter for cullin-based ubiquitin ligase activity[3,4]. Interestingly, the *memi-1(sb41)* hypermorphic mutation results in inappropriate persistence of MEMI-1, but not MEMI-2 or MEMI-3, protein into mitosis. In order to identify potential activators of the *memi* pathway, we performed an RNAi screen for suppressors of *memi-1(sb41)*. This approach identified a sperm-specific PP1 phosphatase. Because reducing the funciton of this PP1 phosphatase suppresses *memi-1(sb41)*, the PP1 might also be required for normal *memi* activity upon fertilization. We are currently testing the hypothesis that sperm-delivered PP1 activates MEMI to specify meiosis II after fertilization.

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The letter preceding the number is the community.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

W450 ELLI-1, a novel germline protein, modulates RNAi activity and P-granule accumulation in *C. elegans*. *D. Updike*, K. Andralojc, A. Campbell, A. Kelly, M. Terrey, P. Tanner, I. Gans, M. Senter-Zapata, E. Khokhar. Mount Desert Island Biological Laboratory, Bar Harbor, ME.

Germ cells contain non-membrane bound cytoplasmic organelles that help maintain germline integrity. In *C. elegans* they are called P granules; without them, germ cells undergo partial masculinization or aberrant differentiation. Many key P-granule components play roles in both exogenous and endogenous small RNA pathways. CSR-1 represents a small-RNA binding P-granule protein that antagonizes the accumulation of sperm-specific transcripts in developing oocytes. Loss of CSR-1 and its cofactors cause a very specific, enlarged P-granule phenotype. To better understand the function of CSR-1 in P granules, PGL-1::GFP expressing worms were mutated and screened for enlarged P granules. Ten mutants were isolated, including multiple alleles of *csr-1* and its cofactors *ego-1*, *ekl-1*, and *drh-3*. Two alleles are in a novel gene now called *elli-1* (**e**n**l**arged germ**li**ne granules). ELLI-1 becomes expressed in primordial germ cells during mid-embryogenesis and continues to be expressed in the adult germline. ELLI-1 forms cytoplasmic aggregates that do not co-localize with P granules, but instead accumulate in the syncytial cytoplasm of the adult germline. Genes encoding P-granule components, including those in the *csr-1* pathway, are up-regulated in *elli-1* mutants, as are several genes that promote RNAi. This overexpression of RNAi genes is reflected in the enhanced RNAi phenotype of *elli-1* worms. Our results suggest that ELLI-1 is acting to modulate the accumulation of key transcripts required for exogenous and endogenous small RNA pathways.

W451 Cell-cycle quiescence maintains *C. elegans* germline stem cells independent of GLP-1/Notch. *Hannah Seidel*[1], Judith Kimble[1,2]. 1) University of Wisconsin - Madison, Madison, WI; 2) HHMI.

Many types of adult stem cells exist in a state of cell-cycle quiescence, yet it has remained unclear whether quiescence plays a role in maintaining the stem cell fate. Here we establish the adult germline of *C. elegans* as model for facultative stem cell quiescence. We find that mitotically dividing germ cells, including germline stem cells, become quiescent in the absence of food. This quiescence is characterized by a slowing of S phase, a block to M-phase entry, and the ability to re-enter M phase rapidly in response to re-feeding. We also demonstrate that cell-cycle quiescence can maintain the stem cell state. The signaling pathway required for stem cell maintenance under conditions of active proliferation (GLP-1/Notch signaling) becomes dispensable under conditions of quiescence. We find that quiescence stabilizes Notch effector proteins, suggesting that changes in protein stability may underlie the effect of quiescence on stem cell maintenance. We are currently investigating the food signals required for quiescence exit, as well as the role of cell-cycle regulators in maintaining quiescence.

W452 Beyond Cell Death: Systematic Analyses of Non-apoptotic CED-3 Caspase Functions in *C. elegans*. *B. P. Weaver*, Y. M. Weaver, M. Han. HHMI & CU Boulder, Boulder, USA.

Recent findings from several labs, including ours, have implicated important non-apoptotic functions for canonical apoptotic regulators across diverse metazoans including nematodes, flies, and mammals. We recently reported a genome-wide RNAi screen in *C. elegans* that identified numerous genes cooperating with the miRISC to ensure robust development (Weaver, et al. 2014). We showed that one interactor, the CED-3 caspase, previously known for its essential role in apoptosis, had a critical non-apoptotic activity negatively regulating the expression of LIN-28, and likely also LIN-14 and DISL-2 (Dis3l2 ribonuclease) in the LIN-28 pluripotency/ developmental timing pathway in late larval development thereby limiting supernumerary seam cell divisions (Weaver, et al. 2014). In order to systematically investigate the extent of possible other non-apoptotic functions for the CED-3 caspase in *C. elegans* development, we performed a *ced-3(lf)* enhancer screen in the double blind using a genome-wide RNAi approach. Following a secondary RNAi confirmation screen, we identified more than 100 interactors from diverse functional categories including protein stability regulators, signal transduction factors, RNA-binding proteins, and transcriptional regulators, thus implicating the involvement of CED-3 caspase in a wide-range of non-apoptotic developmental regulatory pathways. We sought to identify a downstream target for one of these interactions that resulted in a dramatic larval developmental stall. Using a combination of biochemical methods, genetic methods, and tissue-specific reporters, we found that CED-3 negatively regulated a MAPK pathway in the hypodermis (epidermis), in the absence of stress. We found that aberrant activity of the MAPK pathway was responsible for larval delay and that CED-3 caspase contributed to robust late larval development by downregulating this pathway and thereby ensured proper post-embryonic growth rate during normal development. Altogether, our data support a model wherein CED-3 caspase acts as a pro-differentiation factor in later larval stages that is important to ensure both proper developmental timing (previous findings) while also ensuring timely progress through post-embryonic development (current findings).

W453 Two new genes regulate LIN-28 in the juvenile-to-adult transition. *K. Kiontke*[1], R. A. Herrera[1], E. Vuong[2], E. M. Schwarz[3], D. S. Portman[2], D. H. A. Fitch[1,4]. 1) New York University, New York, NY; 2) University of Rochester, New York, NY; 3) Cornell University, Ithaca, NY; 4) New York University, Shanghai, China.

During the juvenile-to-adult transition at the L4 stage, the tail tip of *C. elegans* males changes shape from long and pointed to short and round. The timing of this tail tip morphogenesis (TTM) is under the control of the heterochronic pathway, best known for scheduling seam cell development. Previous work showed that *let-7* and *lin-41* play a role in the timing of TTM: in loss-of-function mutants of *let-7* and gain-of-function mutants of *lin-41*, TTM is delayed into adulthood. We identified two new genes involved in the timing of TTM, *lep-2* and *lep-5* (H36L18.2). Males mutant in either gene have fully un-retracted tail tips that undergo TTM as adults. Also, mutant adults undergo an additional molt and mutant *lep-2* males show defective mating behavior, suggesting that the gene products of *lep-2* and *lep-5* act not only in the tail tip but also in the body epidermis and nervous system. Unexpectedly, neither mutant has defects in the development of the lateral seam. *lep-2* encodes a Makorin, a member of a family of conserved proteins with putative nucleic acid binding and E3 ubiquitin ligase activities. *lep-5* is a long noncoding RNA (lncRNA), highly conserved within *Caenorhabditis* but not identifiable outside of this genus. SL1-spliced *lep-5* lncRNA is

predicted to adopt a complex secondary structure with multiple stem-loops, at least two of which are necessary for its function.
By testing genetic interactions, we determined that *lep-2* and *lep-5* repress *lin-28*. It is known that *lin-28* is downregulated transcriptionally (by *lin-14)* and post-transcriptionally (by *daf-12, lin-4, lin-66,* and *sea-2)* after the L2 stage to ensure that mature *let-7* microRNA is present during L3. We found that both LEP-2 and *lep-5* lncRNA also down-regulate LIN-28 protein levels post-transcriptionally without affecting *lin-28* mRNA levels. Using a photo-convertible LIN-28::Dendra2 fusion, we determined that LEP-2 and *lep-5* lncRNA promote degradation of LIN-28 protein. This is consistent with the predicted E3 ubiquitin ligase activity of LEP-2. The mechanism for the activity of *lep-5* lncRNA is still unknown. Interestingly, the human homolog of LEP-2—Makorin3—as well as human LIN28b, are involved in the regulation of pubertal timing. Together with our findings in *C. elegans*, this suggests a conserved role of Makorins and LIN-28 in timing of the juvenile-to-adult transition.
The fact that seam cell development is unaffected by mutations in *lep-2* and *lep-5* suggest that developmental timing is regulated in a tissue-specific manner. Therefore, the male tail tip is a valuable model for studying the heterochronic pathway in all its complexity.

W454 The Argonaute VSRA-1 Regulates Gene Expression through Multiple Small RNA Pathways. *Julie M. Claycomb*[1], Monica Z. Wu[1], Shikui Tu[2], Zhiping Weng[2]. 1) Univ. of Toronto, Toronto, ON, Canada; 2) Univ. of Massachusetts Medical School, Worcester, MA, USA.

At the core of small RNA pathways are Argonaute (AGO) proteins, which interact with small RNAs to regulate gene expression in a sequence-specific manner. The characterization of AGOs across species, including a subset of the 26 AGOs in *C. elegans,* has demonstrated that AGOs generally interact with non-overlapping complements of small RNAs to perform distinct regulatory functions. Our lab's previous studies on the CSR-1 22G-small RNA pathway in *C. elegans* provided mechanistic insights into a paradigm-shifting role for nuclear small RNA pathways in activating germline gene expression, instead of silencing as occurs in the vast majority of small RNA pathways, like RNAi.

We set out to characterize a well-conserved nematode AGO that was most closely related to CSR-1. Remarkably, we found that this AGO interacts with multiple classes of small RNAs, including the essential *mir-51* family of microRNAs (*Hs-mir-100*), as well as specific subsets of piRNAs and CSR-1 class 22G-RNAs. Owing to this versatile repertoire of small RNA partners, we named this AGO VSRA-1 (Versatile Small RNAs Argonaute).

Loss of *vsra-1* leads to slower larval development. Consistent with functions related to multiple small RNA classes, *vsra-1* displays synthetic genetic interactions with the AGOs that have previously been shown to act with each type of small RNA (*alg-1*/miRNAs, *prg-1*/piRNAs, *csr-1*/22G-RNAs). Using a combination of CRISPR-Cas9 GFP knock-in strains and antibody staining against endogenous VSRA-1, we determined that VSRA-1 is broadly expressed across most developmental stages and tissues including the germline. *In vivo* reporter assays and mRNA-seq data implicate VSRA-1 in silencing gene expression, including a large portion of germline-expressed genes. Moreover, sequencing of VSRA-1-small RNA complexes indicates that VSRA-1 associated small RNAs all appear to target CSR-1 pathway genes. Thus, we propose a model in which VSRA-1, through a complex network of small RNAs, functions antagonistically to the CSR-1 pathway to maintain germline homeostasis.

We are currently pursuing structure-function analyses, using CRISPR-Cas9 and biochemical experiments, to dissect the loading and specification of VSRA-1 small RNAs. Due to its unique property of association with multiple classes of small RNAs, characterization of this fascinating AGO will reveal novel mechanisms regulating germline gene expression.

W455 ALG-5 interacts with a subset of miRNAs to affect male gene expression and fecundity in *C. elegans*. *T. Montgomery*. Colorado State University, Fort Collins, CO.

C. elegans contains 25 Argonautes, of which, only ALG-1 and ALG-2 are known to interact with miRNAs. ALG-5 belongs to the AGO subfamily of Argonautes, which includes ALG-1 and ALG-2, but its role in small RNA pathways is unknown. We analyzed by high-throughput sequencing the small RNAs associated with ALG-5, ALG-1, and ALG-2. We show that ALG-5 interacts with a subset of miRNAs that overlaps with those bound by ALG-1 and ALG-2. *alg-5* is expressed in the germline during oogenesis and is required for optimal fecundity. *alg-5* mutants display modest changes in gene expression, including upregulation of genes involved in male development. Through an exhaustive analysis of ALG-5, ALG-1, and ALG-2 small RNA interactors, we identified only three new miRNAs, indicating that miRNA identification is nearly saturated in *C. elegans*. We also identified ALG-1 and ALG-2 associated small RNAs that do not resemble canonical miRNAs. Our results provide a near-comprehensive analysis of miRNA-Argonaute interactions in adult *C. elegans* and reveal distinct and overlapping roles for ALG-5, ALG-1, and ALG-2 in development, fecundity, and lifespan.

W456 Toward an understanding of cooperative miRNA-mediated silencing. *M. N. Flamand*[1*], H. H. Gan[2], K. Gunsalus[2], T. F. Duchaine[1]. 1) McGill University, Montreal, Quebec, CA; 2) New York University, New York, NY.

MicroRNAs (miRNAs) are derived from gene-encoded RNA hairpins and play critical roles in development, homeostasis, disease, and environmental responses. In *C. elegans* mature miRNAs are loaded in the Argonautes ALG-1 and ALG-2 and direct gene silencing from within the miRNA-Induced Silencing Complex (miRISC). In animals, miRNAs bind to 3' untranslated regions (UTR) of messenger RNAs (mRNAs) through imperfect base pairing. Because of this imperfect base pairing, the identification of miRNA targets remains a challenge that can only be fully answered through direct functional validation. While several lines of evidence point to the importance of cooperative interactions between miRNA-binding sites on individual target mRNAs, they are still largely investigated as functionally independent regulatory units.

Using a unique *C. elegans* embryonic cell-free system, we had previously shown that miRNA-instigated deadenylation, an important mechanism of silencing, requires target site cooperation. Now exploiting an array of biochemical, computational, genome editing and genetic approaches, we decipher the mechanistic bases for cooperative miRNA-mediated silencing. Our findings reveal that miRNA-binding site cooperation is required to recruit miRISC to non-canonical sites on mRNAs, and that miRISC association is not sufficient for target deadenylation. We further demonstrate ALG-1/2 homo- or hetero-dimerization *in vitro*, thus providing an explanation for cooperation in target site recruitment. Using computational modeling of miRNA and 3'UTR-bound Argonaute dimers, we identified two putative miRISC-miRISC

interaction interfaces. Turning to CRISPR/Cas-9 to engineer mutant strains, we systematically screened candidate dimerization determinants to identify Argonaute residues that are critical *in vivo* for miRNA function, for miRISC interactions, and for mRNA target deadenylation.

Our findings unveil molecular determinants for miRNA cooperation, and suggest that miRISC-miRISC interactions play a cornerstone role in the mechanisms of miRNA-mediated silencing.

W457 A continuum of mRNP complexes in embryonic miRNA-mediated silencing. *Thomas Duchaine*[1], Edlyn Wu[1], Ajay Vashisht[2], Clément Chapat[1], Mathieu Flamand[1], Emiliano Cohen[3], Mihail Sarov[4], Yuval Tabach[3], Nahum Sonenberg[1], James Wohlschlegel[2]. 1) Department of Biochemistry and Goodman Cancer Research Centre, McGill Univ., Montreal, Quebec, Canada; 2) Department of Biological Chemistry, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; 3) Hebrew Univ., Hadassah Medical School, Jerusalem, Israel; 4) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

MicroRNAs (miRNAs) impinge on the translation and stability of a wide variety of mRNAs, and play key roles in development, homeostasis and disease. The gene regulation mechanisms they instigate are largely effected through the activities and interactions of the CCR4-NOT deadenylase complex, but the molecular events that occur on target mRNAs and lead to silencing are poorly resolved. Using comparative proteomics, we observed a broad convergence of interactions of germ granule and P body mRNP components on AIN-1/GW182 and NTL-1/CNOT1 in the *C. elegans* embryo. We show that the miRISC progressively matures on the target mRNA from a scanning form into an effector mRNP particle by sequentially recruiting the CCR4-NOT complex, and mRNP components such as the decapping and decay, or germ granule proteins. Finally, we provide evidence for a role of intrinsically disordered proteins in embryonic miRNA-mediated silencing. Our findings define dynamic steps of effector mRNP assembly in embryonic miRNA-mediated silencing, and identify a functional continuum between germ granules and P bodies in the *C. elegans* embryo.

W458 Germline- and soma-specific mechanisms of heritable epigenetic silencing at an endogenous locus. *Olga Minkina*, Craig P. Hunter. Harvard University, Cambridge, MA.

Double stranded RNA (dsRNA) introduced into *C. elegans* can move throughout the worm to initiate RNAi-mediated silencing in recipient cells. DsRNA movement, both within a worm and between generations, requires the conserved dsRNA-transporting protein SID-1. I have found that a transgenic multi-copy array of the *sid-1* upstream intergenic region (promoter and 5' UTR) silences *sid-1* and upstream genes in the germline and soma. This is surprising for two reasons. First, arrays containing multiple copies of promoters have never been shown to stably silence endogenous genes. Second, heritable epigenetic gene silencing has not previously been described in the soma in *C. elegans.* Once silenced, the *sid-1* locus can remain silenced for up to 13 generations in the absence of the array. Reminiscent of paramutation, a silenced *sid-1* locus can silence a naive locus introduced by mating in the germline. Surprisingly, transmission of silencing does not require transmission of chromatin. Instead, small interfering RNAs (siRNAs) mediate *sid-1* silencing in the germline; worms containing the *sid-1* promoter array have a three-fold increase in small RNAs targeting the *sid-1* locus. This increase is maintained even in the absence of the array indicating that these small RNAs are likely the transgenerational signal that epigenetically silences *sid-1* in the germline. Genetic analysis reveals that efficient initiation of *sid-1* silencing partially requires the piRNA-stabilizing Argonaute PRG-1 and that maintenance of *sid-1* silencing requires HRDE-1, an Argonaute that stabilizes secondary siRNAs in the germline nuclear RNAi pathway. Further, I identify the specific endogenous piRNA that is required for efficient initiation of silencing. While the germline nuclear RNAi pathway is required for promoter-mediated *sid-1* silencing in the germline, the somatic nuclear RNAi pathway is not required for *sid-1* silencing in the soma. Instead, I find that multi-generational somatic silencing of *sid-1* specifically requires several chromatin modifying enzymes, leading to the intriguing possibility that the mechanisms of transgenerational silencing in the soma and germline are distinct.

W459 The not so simple regulation of a simple cell death. *Barbara Conradt*. LMU Munich, Planegg-Martinsried, DE.

W460 PP1β controls ZYG-1 levels to ensure precise centrosome doubling. *J. Iyer*[1], N. Peel[2], A. Naik[2], M. Dougherty[1], M. Dekker[3], K. F. O'Connell[1]. 1) NIH, Bethesda, MD; 2) The College of New Jersey, Ewing, NJ; 3) Max Planck Institute of Molecular Biology and Genetics, Dresden, Germany.

Centrosome duplication (CD) is a highly regulated process that occurs only once during each cell cycle. Deregulation of this process yields an abnormal centrosome number. This can result in aneuploidy, a hallmark of cancer cells. The nematode *C. elegans* is an excellent model system to study the process of CD because the core components of the CD pathway in *C. elegans* are conserved in humans. One such protein is the master CD kinase ZYG-1 that is absolutely essential for CD. Depleting ZYG-1 prevents CD while increasing its levels or activity causes centrosome over-duplication. Our laboratory is interested in identifying novel regulators of CD. Through our studies, we have identified the phosphatase PP1β as a biologically important and novel inhibitor of CD. PP1β acts as a molecular brake for CD as decreasing its activity causes an over-duplication of centrosomes. Western blot analysis revealed that in mutant worms with low PP1β activity, ZYG-1 protein levels are elevated. Thus, PP1β normally functions to down-regulate ZYG-1. To determine if this regulation occurs at the transcriptional, translational or post-translational level, we monitored *zyg-1* transcription and translation in mutants with low PP1β activity. In spite of an observed increase in ZYG-1 protein levels, we found that *zyg-1* transcription and translation were unaltered in worms with reduced PP1β activity. Therefore, we conclude that PP1β decreases ZYG-1 levels post-translationally by promoting its proteasomal degradation. We hypothesize that PP1β physically interacts with ZYG-1 and dephosphorylates it to promote its degradation. ZYG-1 being a low abundance protein is very difficult to immunoprecipitate from worm extracts. Therefore, we utilized an innovative approach to determine if ZYG-1 and PP1β interact *in vivo*. We tagged endogenous ZYG-1 with a Biotin Acceptor Tag (BAT) and endogenous PP1β with the *E. coli* biotin ligase (BirA) using the CRISPR technique. Indeed, we found that BAT-ZYG-1 became biotinylated only in the presence of PP1β-BirA. This suggests that ZYG-1 and PP1β physically interact *in vivo.* Thus, we

have identified the master CD kinase ZYG-1 as a novel candidate substrate of PP1β. In summary, by controlling ZYG-1 levels, PP1β ensures that only one daughter centriole forms adjacent to each mother centriole during CD. Future studies will involve determining which amino acid residues of ZYG-1 are de-phosphorylated by PP1β and the mechanism by which this leads to ZYG-1 degradation.

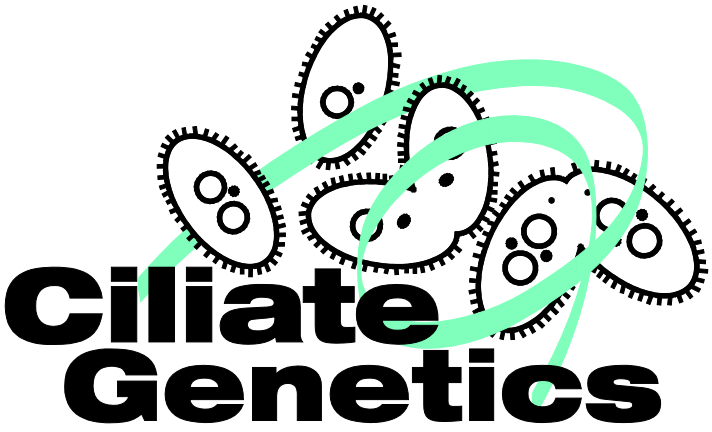
W461 Sumoylation and desumoylation in epidermal morphogenesis. *L. Broday*, A. Tsur, A. Raju, U. Bening Abu-Shach. Tel Aviv Univ, Tel Aviv, IL.

During morphogenesis junctions between epithelial cells are rapidly assembled and disassembled in order to allow cell movements and shaping of organs and tissues. We found that dynamic post-translational modifications and specifically the SUMO machinery play a role in the regulation of the flexibility of cell junctions during *Caenorhabditis elegans* epidermal morphogenesis. We identified the SUMO protease ULP-2 as a regulator of adherens junctions (AJ) assembly and show that dysregulated ULP-2 activity impairs epidermal morphogenesis in *C. elegans* embryos. The conserved cytoplasmic tail of HMR-1/E-cadherin is sumoylated and is a target of ULP-2 desumoylation activity. Coupled sumoylation and desumoylation of HMR-1 are required for its recruitment to the subapical membrane during AJ assembly and the formation of the linkages between AJs and the apical actin cytoskeleton. Sumoylation weakens HMR-1 binding to HMP-2/β-catenin. Our study provides a mechanistic link between the dynamic nature of the SUMO machinery and AJs plasticity and highlight sumoylation as a molecular switch that modulate the binding of E-cadherin to the actin cytoskeleton. We are currently studying how ULP-2 activity is regulated during epidermal morphogenesis.

W462 Conserved TRPA1-Nrf2 signaling mediates reactive alpha-dicarbonyl detoxification relevant for diabetic pathologies. *J. Chaudhuri*[1], N. Bose[1,2], J. Gong[3,4], D. Hall[1], A. Rifkind[1], D. Bhaumik[1], T. Peiris[1], M. Chamoli[1], C. Le[1], J. Liu[3], G. Lithgow[1], A. Ramanathan[1], X. Xu[4], P. Kapahi[1,2]. 1) Buck Institute for Research on Aging, Novato, USA; 2) University of California, Department of Urology, San Francisco, USA; 3) College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, China; 4) Life Sciences Institute and Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, USA.

Chronic hyperglycemia results in the accumulation of reactive α-dicarbonyls (α-DCs), such as methylglyoxal that leads to various diabetic complications. Conventional treatment regimens for long-term diabetics focus on lowering blood glucose levels with comparatively less focus on glucose derived by-products or the α-DCs**.** α-DCs are detoxified by the evolutionarily conserved glyoxalases; however their core biochemical regulation is still not clear. Using a *Caenorhabditis elegans* model with an impaired glyoxalase (*glod-4/GLO1*), we characterized a mechanism to broadly study α-DC-related stress. *glod-4* animals display phenotypes such as hyperesthesia, neuronal damage and early mortality recapitulating diabetic neuropathy related pathologies in two weeks. Our results demonstrate TRPA-1/TRPA1 as a sensor for α-DCs, conserved between worms and mammals. Additionally, TRPA-1 activates SKN-1/Nrf2 via calcium-modulated kinase signaling that regulates the glutathione dependent (GLO1) and independent (DJ1) glyoxalases to detoxify α-DCs. Phenotypic drug screen using *C. elegans* identified podocarpic acid, a novel *C. elegans* TRPA-1 activator, that rescues the α-DC-induced pathologies both in *C. elegans* and mammalian cells. Using a combination of *C. elegans* and mammalian cells, we propose that amelioration of α-DC stress would help identify novel pharmacological leads that in principle would help overcome diabetic pathologies and associated neurodegenerative conditions like Alzheimer's, and Parkinson's disease without manipulating glucose levels.

2016 CILIATE MOLECULAR BIOLOGY MEETING



Plenary and Platform Session Abstracts

2016 Ciliate Molecular Biology Conference

SCHEDULE AT-A-GLANCE

Wednesday, July 13		
2:00pm-9:30pm	Speaker Ready Room Open	Hall of Cities - Anaheim
7:00pm-9:00pm	Scientific Session: Genomics: Genome Structure and Organization	Palms Ballroom Canary 2
9:00pm-11:00pm	Opening Mixer with Exhibits	Cypress Ballroom
Thursday, July 14		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities -Anaheim
7:45am-10:00am	Genetics and Determinants of Health Joint Plenary Session	Palms Ballroom
8:00am-4:00pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:30am-12:30pm	Scientific Session: Programmed DNA Rearrangement I	Palms Ballroom Canary 2
12:30pm-1:30pm	Mentoring Roundtables #1	North Tower - Harbor Beach
12:30pm-1:30pm	Speaking Up for Genetics and Model Organism Research	Crystal Ballroom H
1:30pm-3:30pm	Poster Presentations 1:30pm-2:30pm: Even-numbered poster 2:30pm-3:30pm: Odd-numbered posters	Cypress Ballroom *(Posters must be removed by 1pm)*
1:30pm-3:30pm	GeneticsCareers Center and Job Fair	Cypress Ballroom 1C
4:00pm-6:00pm	Scientific Session: Evolution and Population Biology	Palms Ballroom Canary 2
4:00pm-6:00pm	Plenary Session and Workshop for Undergraduate Researchers	North Tower - Sawgrass
7:45pm-9:45pm	Scientific Session: Stability and Dynamics	Palms Ballroom Canary 2
10:00pm-11:30pm	*Science Cafe Event	Palms Ballroom Sabal
Friday, July 15		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-9:30am	Scientific Session: Programmed DNA Rearrangement II	Palms Ballroom Canary 2
8:00am-4:30pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:00am-12:00pm	Scientific Session: Chromatin Structure & Chromatin Modification	Palms Ballroom Canary 2
12:00pm-1:30pm	*Editor's Panel Discussion and Roundtable	North Tower - Harbor Beach

** Ticketed Event*

#TAGC16

Friday, July 15 (continued)		
1:30pm-3:30pm	Poster Presentations 1:30pm-2:10pm: "A" poster authors present 2:10pm-2:50pm: "B" poster authors present 2:50pm-3:30pm: "C" poster authors present	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center	Cypress Ballroom 1C
2:00pm-2:45pm	GeneticsCareers Workshop - Nailing the Job Talk	Cypress Ballroom 1B
4:00pm-6:00pm	Scientific Session: Signaling Systems: Signal Transduction, Protein Secretion, and Trafficking	Palms Ballroom Canary 2
6:00pm-7:30pm	*Women in Genetics Panel and Networking	North Tower - Harbor Beach
7:30pm-9:30pm	Development and Evolution Joint Plenary Session	Palms Ballroom
Saturday, July 16		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-10:00am	Workshops: See topics and descriptions under the Workshop Section	Multiple locations
8:00am-12:00pm	Exhibits Open	Cypress Ballroom
8:00am-9:00am	Trainee Bootcamp Workshops: Session 1	North Tower
9:00am-10:00am	Trainee Bootcamp Workshops: Session 2	North Tower
10:00am-12:00pm	Poster Presentations 10:00am-11:00am: Odd-numbered posters 11:00am-12:00pm: Even-numbered posters	Cypress Ballroom
10:00am-12:00pm	GeneticsCareers Center	Cypress Ballroom 1C
10:30am-11:15am	GeneticsCareers Workshop	Cypress Ballroom 1B
12:15pm-1:45pm	*Mentoring Roundtables #2	North Tower - Harbor Beach
1:45pm-3:45pm	Scientific Session: Cell Motility: Cilia, Basal Bodies, and Tubulin	Palms Ballroom Canary 2
4:00pm-6:00pm	Scientific Session: Cell Biology, Morphogenesis, & Development	Palms Ballroom Canary 2
7:30pm-9:30pm	Scientific Session: Community Resources: Current and Future Needs	Palms Ballroom Canary 2
Sunday, July 17		
8:00am-10:00am	Scientific Session: Ciliates in the Classroom and Undergraduate Ciliate Research Symposium	Palms Ballroom Canary 2
10:30am-12:30pm	Technology and its Application Joint Plenary Session	Palms Ballroom

** Ticketed Event*

C13 Maintenance and loss of duplicated genes by dosage subfunctionalization in *Paramecium*. *J. Gout*, M. Lynch. Indiana University, Bloomington, IN, USA.

The *Paramecium aurelia* complex is a group of 15 species so similar in morphologies that they were initially believed to be only one species. The sequencing of genomes from several species belonging to the *P. aurelia* complex has revealed that the members of the *P. aurelia* complex have diverged many million years ago, following a Whole-Genome Duplication (WGD) event that took place in the common ancestor of all *P. aurelia* lineages. WGDs have been rampant in the history of eukaryotes (yeast, plants, vertebrates, etc.) and it is well established that following a WGD, most pairs of duplicated genes eventually revert to a single copy state through pseudogenization. However, some pairs retain both duplicates for long evolutionary periods and it is still unclear why some duplicates are evolutionary successful while others are rapidly lost. Here, we analyzed genomics and transcriptomics data from three *P. aurelia* species and fond that dosage constraints are major factors opposing post-WGD gene loss in *P. aurelia*. We propose a model where a majority of WGD-derived duplicates preserve their ancestral function and are retained to produce enough of the proteins performing this same ancestral function. Under this model, the expression level of individual duplicated genes can evolve neutrally as long as they maintain a roughly constant summed expression, and this allows random genetic drift towards uneven contributions of the two copies to total expression. Our analysis suggests that once a high level of imbalance is reached, which can require substantial lengths of time, the copy with the lowest expression level contributes a small enough fraction of the total expression that selection no longer opposes its loss. Extension of our analysis to yeast species sharing a common ancestral WGD yields similar results, suggesting that duplicated-gene retention for dosage constraints followed by divergence in expression level and eventual deterministic gene loss might be a universal feature of post-WGD evolution. These results also illustrate the importance of using ciliates as a model organism for studying evolution following gene and genome duplications.

C14 De-Novo sequencing of the *Paramecium tetraurelia* macronucleolar (MAC) genome using Pacific Biosciences single molecule long reads for improvement of genome assembly and annotation. *R. Woycicki*, C. Hoehener, K. Schneeberger, E. Swart, S. Bhullar, M. Nowacki. University of Bern, Bern, Bern, CH.

De-novo sequencing of the *Paramecium tetraurelia* MAC genome, set previously its size to be of 72 Mbp. Since discovery of NGS methods the *Paramecium* MAC genome is routinely sequenced for mapping proposes during the course of studying the massive whole genome rearrangements system. Our recent estimation of the genome size made with Illumina 2x125 PE data (coverage > 100x) using the PreQC module from the String Graph Assembler software, showed that the actual MAC genome size can be about 20 percent larger. Unlike the first and second generation sequencing methods, Pacific Biosciences (PacBio) sequencing shows more equal coverage along the genome. No template amplification eliminates bias towards cloned/amplified sequences before the actual sequencing. Taking into consideration the above as well as the very long PacBio reads, we have chosen this system to de-novo sequence and assemble the *Paramecium tetraurelia* MAC genome with the goal of improvement both the genome assembly as well as the genes annotation.

Using the newest PacBio P6-C4 chemistry and 13 SMRT cells, the sequencing resulted in more than 250x coverage of the *Paramecium tetraurelia* strain 51 mating type 7 MAC genome. The reads error correction and assembly was conducted using first the PBcR pipeline from Celera Assembler ver. 8.3rc2 and later using Canu software (v1.0/v1.1).

Our preliminary de-novo assembly allowed us to map to it more shotgun Illumina and Sanger genomic, cDNA and MAC matching 25nt smallRNA reads, than to the reference 51 strain assembly. Based on cDNA mapping we estimate that our current assembly may have a few hundred more new genes.

We will present the current state of genome assembly and annotation which aims to reconstruct a more complete genome.

C15 Comparative genomics in the ciliate genus Paramecium. *Georgi Marinov*, Thomas G. Doak, Michael Lynch. Indiana University, Bloomington, IN.

The *Paramecium* genus provides a unique system for studying the effects of gene and genome duplication on organismal phenotype. It contains a number of distinct morphospecies together with numerous cryptic species, morphologically indistinguishable but biologically isolated from each other. The most famous such group is the *P. aurelia* species complex, consisting of ~15 distinct species, which is also remarkable for having undergone two whole-genome duplication (WGD) events not shared with other members of the *Paramecium* genus prior to its diversification. *Paramecium* is thus of great importance for our understanding of gene duplication and WGD events, their phenotypic effects, and the factors influencing the evolution of orthologous and paralogous genes and regulatory sequences. We address these issues by presenting a comprehensive comparative genomic analysis of members of the *P. aurelia* species complex together with several *Paramecium* species outside of the *aurelia* group.

C16 Programmed retention of germline-limited genes in *Oxytricha trifallax*. *Richard V. Miller*[1], Derek M. Clay[1], Laura F. Landweber[1,2]. 1) Princeton University, Princeton, NJ; 2) Columbia University Medical Center, New York, NY.

One of the defining characteristics of ciliates is nuclear dimorphism: two types of nuclei with distinct genome architectures exist within one cell. In *Oxytricha trifallax*, one nucleus contains a fragmented and scrambled germline copy of the somatic genome. During mating, a haploid germline nucleus transfers between cells. After fertilization, a zygotic germline nucleus matures into a new somatic nucleus by undergoing a process of DNA elimination and rearrangement. In addition to fragments of coding sequences that are retained in the somatic genome and noncoding sequences that are eliminated, 810 recently discovered germline-restricted genes also reside in the germline genome and are eliminated during development. These genes are only transcribed during the process of DNA elimination and rearrangement, and their elimination during development of the somatic genome may constitute a mechanism of developmental gene regulation. I have shown that

microinjection of RNA matching the sequences of germline-restricted genes reprograms the cell to retain these genes in a heritable manner. Furthermore, it appears that retained germline genes are transcribed outside of their normal developmental window. These data support a model in which germline-restricted genes are relegated to the germline nucleus to ensure that they are only expressed during the sexual cycle.

C17 Cell Cycle Transcriptome Analysis in the Binucleated Ciliate, *Tetrahymena thermophila*. *L. Zhang*[1*], M. Cervantes[1], K. Kronganti[1], X. Jie[2], W. Miao[2], G. M. Kapler[1]. 1) Department of Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX; 2) Institute of Hydrobiology, Chinese Academy of Sciences,Wuhan, China.

As a bi-nucleated eukaryote, *Tetrahymena thermophila* has two distinct nuclear cycles within each vegetative cell cycle. Rather than a mononucleate G1-S-G2-Mitosis cell cycle, the somatic nuclear cycle of Tetrahymena progresses through G1-S_{mac}-G2-Amitosis, while the germline nuclear cycle is S_{mic}-G2-M-S_{mic}. Furthermore, in contrast to mononucleated organisms, mitosis is not temporally coupled to cytokinesis. Gene expression data could reveal transcriptional programs critical to processes during the cell cycle. By use of centrifugal elutriation to synchronize the vegetative cell cycle, along with RNAseq and EdU labeling, we have begun to elucidate the transcriptional program across the vegetative cell cycle. At 30-minute intervals, we have deployed RNAseq to identify all genes that encode cell cycle regulated transcripts. We have also subjected elutriated cells to EdU labeling to assess mic and mac DNA synthesis, and in conjunction with other cytological markers have assessed synchrony and assign time points to stages of the cell cycle. What we have found is that of the ~27000 annotated genes (TGD.org), nearly 4500 (16%) exhibit a dynamic regulation in mRNA levels of a two-fold change above background. Of those 4500 genes, 2,166 (48%) map to an associated gene ontology (GO) term. At our most stringent criteria for cycling – above twice the median expression of all genes (around 50 counts after normalization) and at least a 4-fold change in expression between 2 time points – we find roughly 400 genes of which 139 (35%) map to associated GO terms. 69 (49%) of the GO terms assigned to our most stringent criteria fall in pathways involved in DNA replication, chromosome organization and microtubule anchoring. Examples of genes within these pathways that demonstrate a 4-fold change in expression include TPB1, a PiggyBack-like protein, DNA licensing factor MCM2, and separase protein ESP1. In regards to temporal regulation of mRNA production, we have found that mRNA levels of replicative genes and certain histone modifiers are up-regulated coordinately at the G1/S border and during S phase of the cell cycle. As for genes involved in cell cycle progression – better known as cyclin genes – we see that 13 of the 26 show dramatic regulation in mRNA levels and meet our criteria for cycling. Some cyclin profiles may suggest their involvement mic –or mac-specific events, warranting further investigation. The wide breath of pathways and genes covered by our data suggest its usefulness to the research community.

C18 RNA-seq analysis of stress response to silver nanoparticles in *Tetrahymena thermophila*. *A. Piersanti*[1], K. Juganson[2], W. Wei[3], J. Zhang[3], Z. Zhao[3], S. Pucciarelli[1], C. Miceli[1], W. Miao[3]. 1) School of Biosciences and Veterinary Medicine, University of Camerino, Italy; 2) Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia; 3) Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China.

Currently, silver nanoparticles are increasingly used as biocides in various consumer products and if released in the environment they can affect non-target organisms. Nanoparticles are defined as particles with at least one dimension between 1 and 100 nm. The specific surface area increases in reversal proportion to the particle size; thus, the smaller the particle, the greater the proportion of atoms that lay close to or at the surface resulting in higher reactivity of the particle. These properties, which make nanomaterials more efficient in industrial applications, might make them also more harmful to living organisms since they could be able to penetrate physiological barriers, travel throughout the body and interact with subcellular structures. Therefore, understanding the toxicity mechanisms is crucial for both the design of more efficient nano-antimicrobials and, at the same time, for the design of nanomaterials that are biologically and/or environmentally benign throughout their life-cycle.

Tetrahymena thermophila has been largely investigated and functional genomic databases are available. Therefore*, T. thermophila* provides an optimal model system for studying molecular bases of environmental responses, since molecular data obtained in different environmental conditions can be easily compared. We recently used *T. thermophila* to elucidate the environmental effects of silver nanoparticles by analysing *T. thermophila*'s gene expression profile by RNA-seq after exposure to collargol (protein-stabilized silver nanoparticles) and comparing with the effect of the soluble silver salt, $AgNO_3$. In order to see the effects of these substances in viable cells, only sub-lethal concentrations are used in gene expression studies. We tested two different toxicant concentrations at two time points, for 2 and 24 hours.

The experimental sequences are compared with the control to evaluate quantitatively the inhibition or increase of gene expression due to nanoparticles or silver ions. Then gene set enrichment is performed.

Some processes are targets of both toxicants. In addition to many similarities in affected genes, some effects were different for soluble silver ion with respect to collargol. We found that genes involved in mRNA splicing and translation processes appear differentially expressed only in collargol treated samples.

This research provides evidence that silver nanoparticles might be toxic due to combined effects of soluble silver ions released from the particles and the particles themselves.

C19 Preliminary analysis on genome and transcriptome data of two species of karyorelictids, *Loxodes* sp. and Trachelocercidae sp. (Ciliophora, Karyorelictea). *Y. Yan*[1,2], X. Maurer-Alcalá[2,3], A. Rogers[2], L. Katz[2,3]. 1) Ocean University of China, Qingdao, China; 2) Smith College, Northampton, MA, US; 3) University of Massachusetts Amherst, Amherst, MA, US.

Karyorelictea are an understudied class of ciliates characterized by the presence of postciliodesma fibers and non-dividing somatic macronuclei. Karyorelictids have both somatic macronucleus and transcriptionally silent germline micronucleus like other ciliates. However,

during vegetative growth, their 'paradiploid' macronuclei are unable to divide and their number of macronuclei can only be reestablished by division and differentiation from germline micronuclei (Raikov, 1985). All other ciliate classes have polyploid macronuclei dividing through amitosis (Raikov, 1985). Since the majority of Karyorelictids are currently uncultivable, few data are available compared to other groups of ciliates (i.e. only 72 nucleotide sequences including environmental samples and six protein sequences are in Genbank); and only a limited number of studies focusing on the karyorelictean nuclei have been published (Bobyleva et al., 1980; Raikov, 1963, 1972, 1989; Raikov and Karadzhan, 1985). Recently, we collected two karyorelictean species, ***Loxodes*** sp. and Trachelocercidae sp., to characterize genome features through single cell whole genome amplification and whole transcriptome amplification. We are currently analyzing over 39 million Illumina reads from these taxa. After the removal of bacterial contaminants and poor quality reads, we are assembling the protein-coding genes from these taxa and performing pilot analyses of genome structure.

C20 Genome Rearrangement and Organization in *Oxytricha*: A Complex Epigenome. *Laura Landweber*[1,2], Jaspreet Khurana[1,2], Leslie Beh[2], Kelsi Lindblad[2], Xiao Chen[2], Robert Sebra[3]. 1) Columbia University, New York, NY; 2) Princeton University, Princeton, NJ; 3) Icahn School of Medicine at Mount Sinai, New York, NY.

The ciliate *Oxytricha* possesses a dynamic pair of genomes. Massive DNA rearrangements produce a highly fragmented but functional somatic genome from a complex germline genome. This process eliminates nearly all noncoding DNA, including transposons, and rearranges over 225,000 short DNA segments to produce a mature, somatic genome containing over 16,000 gene-sized "nanochromosomes." In the precursor germline genome, the shattered segments of different genes often interweave with each other, frequently overlap and may combinatorially assemble. Noncoding RNAs guide the entire process of genome rearrangement, but DNA methylation of both cytosine (m5C and hm5C) and adenosine (m6A) also influence genome organization. We previously described a class of 27nt piRNAs that provide the critical information to mark and protect the retained DNA segments of the genome. We recently identified new classes of small RNAs involved in different aspects of genome regulation. Maternally-inherited, long RNA transcripts provide templates for genome remodeling and RNA-guided DNA repair, but they also regulate gene dosage and chromosome copy number, possibly together with a new class of small RNAs. Overall, *Oxytricha*'s elaborate epigenome, assembled and maintained through complex interacting networks of both long and small non-coding RNAs, encapsulates an RNA-driven world packaged in a modern cell. The mechanism for all of these dynamic actions bypasses traditional pathways of inheritance, hinting at the power of RNA molecules and DNA modifications to sculpt genomic information.

C21 Novel genetic manipulation approaches to investigate development-specific genes in *Oxytricha trifallax*. *D. M. Clay*[1], L. F. Landweber[1,2]. 1) Princeton University, Princeton, NJ; 2) Columbia University, New York, NY.

Oxytricha trifallax is a stichotrich which transforms a micronucleus into a macronucleus during sexual development by excising and rearranging its DNA. During this genomic rearrangement, more than 90% of the DNA sequence in the micronuclear chromosomes is deleted. The remaining sequences are converted into ~16,000 nanochromosomes with average length of 3 kb, forming the macronucleus. Development of the new macronucleus from a copy of the zygotic micronucleus requires a suite of non-coding RNAs. In *Oxytricha trifallax*, long RNA templates, together with 27-nucleotide Piwi associated RNAs, guide genome rearrangements. By exploiting these pathways, somatic gene knockouts in *Oxytricha* have been developed. In this study we investigated two highly expressed early sexual development-specific genes, Alba-like 1 and Alba-like 2, which encode Alba domain containing proteins. Alba domains are known to be nuclei acid binding proteins and are involved in translation regulation in *Trypansoma brucei* (Mani *et al*., 2011) and *Plasmodium falciparum* (Vembar *et al*., 2015). Both Alba-like 1 and Alba-like 2 genes were identified as co-precipitates of long dsRNA, possibly the RNA templates, in early sexual development. Knockouts of both genes were developed through exploiting the genomic rearrangement pathways of *Oxytricha* via different approaches. The introduction of artificial piRNAs programs the retention of normally eliminated sequences, which permits retention of DNA sequences that interrupt open reading frames. Another approach is the introduction of synthetic DNA templates which sometimes induces deletion of the targeted nanochromosome. For both genes, the knockout cell lines are viable during vegetative, asexual growth but unable to complete sexual development, with Alba-like 1 being trans-lethal when mated to wild type. To further probe the functions of these target genes, tagged proteins were generated using artificial nanochromosomes injected into the macronucleus of vegetative cells. This transformation approach results in artificial chromosomes that are maintained throughout the asexual life cycle at high copy number and show expression patterns that faithfully recapitulate their native counterparts and permit purification of the tagged proteins. Together, these experiments have increased the utility of *Oxytricha trifallax* as a model system for genetics.

C22 The prevalence of paralogous macronuclear DNA fragments aid in the formation of scrambled genes in *Oxytricha trifallax*. *J. Burns*[1,2], M. Saito[2], N. Jonoska[2], L. F. Landweber[1]. 1) Princeton Univ., Princeton, NJ; 2) USF, Tampa, FL.

A ciliated protozoa undergoes programmed genome rearrangements during nuclear development, forming a transcriptionally active somatic macronucleus from a copy of its germline micronucleus. In *O. trifallax*, this nuclear transition involves an RNA-mediated excision of 90% of the germline genome, after which, the remaining 225,000 DNA fragments are amplified and reorganized into gene-sized nanochromosomes via RNA templates. Early analysis of the sequenced *O. trifallax* genomes revealed instances of alternative fragmentation of longer chromosomes into shorter ones, and alternative processing of a single loci of conserved micronuclear material to form a variety of macronuclear genes. In this study we observe that the micronuclear genome of *O. trifallax* contains an abundance of paralogous macronuclear segments -- at varying levels of degeneration -- that contribute to the genetic variation of the micronuclear genome, promote the evolution of new macronuclear genes, and provide the means for a gene to become scrambled in the germline genome.

C23 Domesticated *piggyBac* transposases and DNA repair factors work hand in hand during programmed rearrangements in *Paramecium*. *Mireille Betermier*[1], Julien Bischerour[1], Simran Bhullar[2,3], Vinciane Régnier[1], Nathalie Mathy[1], Cyril Denby Wilkes[1], Olivier Arnaiz[1], Estienne Swart[2], Arthur Abello[1], Marc Guérineau[1], Linda Sperling[1], Mariusz Nowacki[2]. 1) I2BC, CNRS, CEA, Univ. Paris Sud, Gif-sur-Yvette, FR; 2) Inst. of Cell Biology, Univ. Bern, Bern, CH; 3) IBENS, Paris, FR.

Because of their nuclear dimorphism, ciliates constitute unique unicellular models for the study of the molecular mechanisms involved in programmed DNA elimination during somatic differentiation. During development of the macronucleus (MAC) in *Paramecium tetraurelia*, ~30% of the germline DNA is eliminated from the somatic genome. Eliminated sequences include ~45,000 short, single-copy Internal Eliminated Sequences (IESs), which are excised through a highly precise "cut-and-close" mechanism related to DNA transposition. PiggyMac (Pgm), a catalytic active domesticated piggyBac transposase, is essential for the introduction of initiating DNA double-strand breaks at IES ends. The DNA double-strand break repair proteins Ku70/Ku80 interact with Pgm and are required for DNA cleavage at IES ends, ensures tight coupling between DNA cleavage and repair. Because IESs do not carry a clearly conserved sequence motif that could be recognized specifically by the excision machinery, one major issue is whether other partners are required to target Pgm to IES ends.

We recently discovered that nine additional Pgm-like (PgmL) proteins are encoded by the MAC genome. *PGML* genes group in five families of whole-genome duplication (WGD) ohnologs. In contrast to Pgm, PgmLs do not harbor a conspicuous DDD catalytic triad, suggesting that they may not have retained full catalytic activity. However, high-throughput RNA sequencing revealed that all *PGML* genes are transcribed specifically during MAC development, and fluorescence microscopy data indicated that all PgmL proteins localize in the developing new MAC. Systematic RNAi experiments, and a genome-wide survey of IES retention in depleted cells, established that each PgmL family is essential for IES excision. Using a heterologous protein expression system, we demonstrated that PgmLs can interact with each other and with Pgm in cell extracts. However, the nuclear localization of each PgmL family is differentially affected upon RNAi-mediated inactivation of other *PGML* genes. Our results indicate that Pgm acts within a highly ordered multi-component protein complex to catalyze programmed DNA elimination.

C24 SDCP, a novel Paramecium protein involved in macronuclear development during autogamy. *A. Singh*[1,2], E. Swart[1], S. Gisler[1], M. Nowacki[1]. 1) IZB, University of Bern, Bern, CH; 2) GCB, University of Bern, Bern, CH.

In ciliates, massive genome reorganization occurs during autogamy where superfluous DNA such as Internal Eliminated Sequences (IESs), transposons, minisatellites and other repetitive sequences are eliminated from the developing macronucleus producing a streamlined functional somatic genome. Here we describe our current analyses of a novel *Paramecium tetraurelia* protein, *SDCP*, which is significantly differentially expressed during autogamy. *SDCP* contains an ATPase, a helicase domain, a SANT domain and a SLIDE domain. This architecture is characteristic of the conserved ISWI protein family. The domains of ISWI proteins are known to play multiple roles, regulating transcription, chromosome organization and DNA replication. This makes *SDCP* an interesting protein to study its putative role in the genome reorganization during sexual reproduction (autogamy).

Knockdown of SDCP during autogamy in *Paramecium tetraurelia* is lethal and affects both precise and imprecise DNA excision leading to the retention of maternally and non-maternally controlled IESs, and transposons. MAC genome sequencing data following *SDCP* knockdown shows substantial retention of IESs and a modest correlation between IESs retained following either *Nowa1* or *TFIIS4* silencing, two other genes involved in IES excision (Nowacki, 2005 & Maliszewska-Olejniczak, 2015). SDCP knockdown leads to similar IES end base frequency variation relative to IES retention in a similar manner to that seen for the other genes (Swart & Denby Wilkes, 2014). Interestingly, IES retention scores seem to peak at a regular base length interval corresponding to the size of the nucleosomes. Since the C-terminal of the protein contains a SLIDE domain, that is known to regulate ATP-dependent repositioning of the nucleosomes, we wished to determine, firstly whether SDCP is involved in nucleosome positioning, and secondly if the nucleosome position around an IES is important for its precise excision. To assess these possible roles for SDCP, we are therefore sequencing nucleosome-bound DNA from post-autogamous cultures.

C25 A mutagenesis screen based on mating-type switch reveals a small subset of IESs enriched in a 5-bp motif. *S. Bhullar*[1], O. Arnaiz[3], C. Denby Wilkes[3], G. Pellerin[1], S. Malinsky[1], L. Sperling[3], M. Nowacki[2], E. Meyer[1]. 1) Institute de Biologie l'Ecole Normale Superieure, 46 rue d'Ulm, 75005 Paris, France; 2) Institute of Cell Biology, University of Bern, Baltzerstrasse 4, 3012 Bern, CH; 3) nstitute of Integrative Biology of the Cell (I2BC), UMR 9198 CNRS CEA Université Paris-Sud, Gif-sur-Yvette, France.

Genome rearrangements during macronuclear development in *Paramecium tetraurelia* have been proposed to be guided by meiosis-specific small RNAs, called scnRNAs, which are produced from micronuclear transcripts by the Dicer-like proteins Dcl2 and Dcl3. ScnRNAs are likely bound by the Piwi proteins Ptiwi01 and Ptiwi09 and are required to target the excision of a subset of MIC-specific sequences in the developing MAC, including maternally controlled IESs: silencing of both Dicer-like or both Piwi genes during autogamy results in genome rearrangement defects and in cell death. To obtain viable mutants in scnRNA-pathway genes, we designed a mutagenesis screen based on mating type reversal, which relies on the hypersensitivity of the mtA promoter IES to the slightest perturbation of the scnRNA pathway. Mating type O is normally determined during MAC development by the excision of the promoter of the *mtA* gene as an IES. This excision event is impaired by the silencing of a single gene from the Dcl2/Dcl3 or Ptiwi01/Ptiwi09 pairs, which results in mating type E viable progeny, whereas other IESs are hardly affected. Furthermore, the promoter-retaining version of the mtA gene is 'dominant' over the promoter-excised version, since it allows expression of the gene, while retention of intragenic IESs results in non-functional genes. Thus, screening populations of mutagenized O cells for E-expressing progeny should allow us to recover non-lethal mutations that only partially impair the scnRNA pathway, such as null alleles of genes with redundant ohnologs or hypomorphic alleles of essential genes. This scheme allowed us to isolate 2 mutants: one is a null allele of a developmentally regulated zinc finger protein with a WGD1 ohnolog, called *mtG*, and the other is a mis-sense mutation in Ptiwi09. An mtG-GFP fusion protein was shown to localize in the new MAC, with an initially punctate pattern. As development proceeds, mtG-GFP progressively concentrates at a single spot in the DNA-poor region of the MAC, as does the heterochromatin mark, H3K9me3. RNAi-mediated silencing of

mtG and/or its ohnolog during autogamy of wild-type O cells impaired excision of the *mtA* promoter and caused progeny to switch to mating type E. In addition, resequencing of the MAC genome showed that a very small subset of ~100 IESs are retained in these conditions. These IESs are significantly enriched in a 5-bp motif, GCTAA, and have a specific size range. Preliminary analysis of *mtG* function in the sibling species *P. octaurelia* suggested that orthologous IESs also require *mtG* for excision, but only when the GCTAA motifs are conserved. I will discuss the possible mechanisms and biological significance of *mtG*-dependent IES excision.

C26 Transposable elements as vehicles of gene movement and duplication within and between eukaryotes. *Ellen Pritham*[1], Komal Vadnagara[2], Jainy Thomas[1]. 1) University of Utah, Salt Lake City, UT; 2) MD Anderson, Houston, TX.

Transposable elements are mutagens that can contribute directly and indirectly to genetic innovation. In bacteria, transposable elements carrying genes involved in cellular function such as antibiotic resistance, catabolism, and pathogenicity are commonly horizontally transferred. Contrary to their bacterial counterparts, eukaryotic transposable elements are not known to traffic cellular genes between species. Here we provide evidence that many different superfamilies of eukaryotic transposable elements are actively transducing and transporting cellular genes across species boundaries. Families of *Crypton*, *Helitron, Mutator, PiF-Harbinger and PiggyBac,* identified from the genome of the eukaryotic phyto-pathogenic microbe, *Phytophthora infestans* carry cellular genes, in addition to the genes encoding transposase (called supercharged in this study (SCTE). The supercharged genes are predicted to be involved in epigenetic gene regulation or pathogenicity and expressed during plant infection. We show that large gene families were spawned via SCTE transposition. These superfamilies are predominantly found in the dynamic genome locale populated by host recognition and infection genes as well as genes necessary for growth, *in planta*. Indeed, SCTE transposition facilitated the seeding of this region with genes typically found in more constrained locations. Finally, we present compelling evidence that transposable elements are transporting genes horizontally between *Phytophthora* species. Our results demonstrate that eukaryotic transposable elements, like their bacterial counterparts, are vehicles of gene movement both within and between genomes.

C27 Evolution of internal eliminated sequences in *Paramecium*. *Diamantis Sellis*[1], Frédéric Guérin[2], Olivier Arnaiz[5], Walker Pett[1], Nicole Boggetto[2], Sascha Krenek[3], Thomas Berendonk[3], Arnaud Couloux[4], Jean-Marc Aury[4], Karine Labadie[4], Sophie Malinsky[6], Simran Bhullar[6], Eric Meyer[6], Linda Sperling[5], Sandra Duharcourt[2], Laurent Duret[1]. 1) Université Lyon 1, CNRS, UMR 5558, LBBE, Villeurbanne, France; 2) Institut Jacques Monod, CNRS, UMR 7592, Sorbonne Paris Cité, Paris, France; 3) TU Dresden, Institute of Hydrobiology, Dresden, Germany; 4) Commissariat à l'Energie Atomique (CEA), Institut de Génomique (IG), Genoscope, Evry, France; 5) Institute of Integrative Biology of the Cell, UMR9198 CNRS CEA U Paris-Sud,91198 Gif-sur-Yvette, France; 6) Ecole Normale Supérieure, Institut de Biologie de l'ENS, IBENS, Paris, France.

The genome of *Paramecium* undergoes a remarkable reorganization during the development of the macronucleus. Tens of thousands of mostly short sequences are interspersed in the micronuclear genome, often interrupting protein reading frames. During the macronuclear development, these internal eliminated sequences (IESs) are precisely excised and thus the macronuclear genes are fully functional. The role, if any, of IESs, their evolutionary history and dynamics as well as the molecular mechanism(s) involved in their excision are still far from resolved. As a first step towards exploring these questions we here performed a large scale systematic reconstruction of the evolutionary history of IESs in *Paramecium*. We focused on 7 species from the aurelia species complex and used *P. caudatum* as an outgroup. We sequenced the micronuclear and, when not available, the macronuclear genomes of each species and annotated genes and IESs. We specifically focused on conserved genes that can be aligned across species in order to find homologous IESs. We inferred the phylogenies of each gene family and reconciled the resulting gene trees with the species tree. Using a Bayesian approach we inferred the ancestral states of presence and absence for each IES. The result is an unprecedented detailed description of the evolutionary history of tens of thousands of IESs. Preliminary results validate previous models of IES evolution. We find that there was a wave of insertion of IESs after the split of the aurelia species complex from *P. caudatum* and subsequently the rate of gain and loss of IESs was significantly reduced. Our detailed description of IES evolutionary history also enables us to compare the age of acquisition of different IESs with various genomic properties. For example we find that recently inserted IESs are on average longer. We believe that our results will fuel further studies to test models and gain new insights on the molecular mechanisms of genomic rearrangements in the developing macronucleus.

C28 Population genomics of *Paramecium* species. *P. Johri*[1], T. G. Doak[1], S. Krenek[2], G. K. Marinov[1], M. Lynch[1]. 1) Indiana University, Bloomington, IN; 2) Institute of Hydrobiology, Technische Universität Dresden, Dresden, Germany.

Population-genomic analyses are essential to understanding factors shaping genomic variation and revealing lineage-specific sequence constraints and adaptation. The dearth of such analyses for unicellular eukaryotes prompted us to assess variation in *Paramecium*, one of the most well-studied ciliate genera. The a*urelia* complex consists of ~15 morphologically indistinguishable species that diverged subsequent to two rounds of whole-genome duplications (WGDs), perhaps as long as 320 MYA, and are well known for their streamlined genomes and extremely short introns. We examine patterns of polymorphism by sequencing whole genomes of 10-13 worldwide isolates of each of three species belonging to the *Paramecium aurelia* complex: *P. tetraurelia*, *P. biaurelia*, *P. sexuarelia,* and two outgroup species that do not share the WGDs: *P. caudatum* and *P. multimicronucleatum*. An apparent absence of strong global geographic population structure suggests continuous or recent dispersal of *Paramecium* over long distances. Introns and intergenic regions are highly constrained relative to 4-fold degenerate sites, more so in species with smaller intergenic regions. Nuclear genomic diversity in the intergenic regions is reduced for ~100-150 bp, both upstream and downstream of genes, suggesting the presence of densely packed regulatory modules. The beginning and end (~70-100 bp) of protein-coding genes are more constrained than the rest of the gene. Comparison of sequence variation at non-synonymous and synonymous sites allows identification of possible candidates of duplicate genes that might be undergoing non-functionalization and provides insights

into differential evolutionary constraints on single-copy versus multi-copy genes. This study serves as a first attempt at a population-genomic analysis in *Paramecium*, and provides an excellent resource for future studies in evolutionary and functional genetics in ciliates.

C29 Transcriptome analysis in the Antarctic ciliate Euplotes focardii: molecular basis of cold adaptation and insights regarding the potential impact of climate change. *Cristina Miceli*, Sandra Pucciarelli, Patrizia Ballarini, Angela Piersanti, Kesava Pryan Ramasamy. School of Biosciences and Veterinary Medicine, University of Camerino, IT.

Ciliates provide optimal model systems to study environmental adaptation. Comparative transcriptome analysis of *Euplotes focardii*, a strictly psychrophilic ciliate isolated from Antarctic seawater, and the mesophilic congeneric species *E. crassus* revealed that in *E. focardii* the majority of the expressed genes code for proteins involved in oxidoreductase activity, as reported for Antarctic fishes and krill. These results confirm that a major problem of Antarctic marine organisms is to cope with increased O_2 solubility at low temperatures. They also suggest that an increased defense against oxidative stress likely provides an important evolutionary feature that allowed the adaptation of Antarctic organisms in their oxygen-rich environment. Gene ontology annotation also revealed that many of the transcripts encoded proteins involved in maintenance of protein homeostasis (e.g., chaperones). Quantitative PCR showed that expression of *Hsp70* genes was induced when *E. focardii* cells were subjected to oxidative stress, whereas thermal stress did not cause induction. These results argue that *E. focardii* in its current environment is well protected against reactive oxygen species and are consistent with prior reports of constitutive *Hsp70* expression as a defense against cold-induced protein denaturation. *E. focardii* appears to be poised to cope with the oxidative challenge that is likely to accompany oceanic warming over the next century, but the absence of a temperature-inducible chaperone response may place its proteome at risk.

The comparative analysis of the *Euplotes* species also revealed a rapid evolution and unusual plasticity of the programmed +1 ribosomal frameshifting, a process that allows the change of the reading frame during translation. This process appears pervasive in *Euplotes* as it affects decoding of over 3,000 genes in these genomes and it is not conserved in the affected genes of the two species. In addition, evidence for +2 frameshifting appeared from the analysis.

We are currently setting up reverse genetics in *E. focardii* in order to have a better understanding of the function of some genes that are expressed only in the cold adapted species.

C30 Comparison of adaptive mechanism between sexual and asexual reproduction in *Tetrahymena thermophila* based on the experimental evolutionary genomics. *W. Miao*, G. Wang, K. Chen, W. Yang, J. Xiong. Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, CN.

The evolution and maintenance of sex is an important problem in evolutionary biology. The advantage of sexual reproduction was thought that it increases the rate of adaptation to the novel environment comparing to the asexual reproduction, but the molecular basis of this adaptive advantage and the similarities/differences between sexual and asexual reproduction are still unclear. *Tetrahymena thermophila*, as a classical unicellular model organism, has made outstanding contributions to the discovery of foundational biology, such as telomere, telomerase and RNA self-splicing. Using its advantages including possessing sexual and asexual stages during its life cycle, culture in the lab, complete genomic and transcriptomic database and techniques of omics and bioinformatics, we dynamically detect the changes of DNA, transcriptional regulation and genome organization during the continuous culture in the lab. Five parallel cell lines were set up. Currently, 1100 asexual fissions and 11 times sexual reproduction had been completed. The fitness tests (growth rate) indicated sexual populations grew significantly faster than asexual populations. Moreover, base on the re-sequencing genome/transcriptome data, we found sexual populations can produce much more genetic variation and accumulate more beneficial mutations than those of asexual populations. In addition, as for sexual group, all the populations had losed mating ability after at most six rounds of sexual reproduction because of the purification of mating types, which resulted in them switching from sexual reproduction to asexual reproduction. Followed by this switching, the fixation of beneficial mutations produced by sex was completed in short 100-200 fissions through asexual reproduction.

C31 Diversities of endosymbiotic *Rickettsia* in the fish parasite *Ichthyophthirius multifiliis*. *Kassandra Ernestine Zaila*[1*], Thomas G. Doak[2,7], Hannah Ellerbrock[1], Che-Huang Tung[3], Mauricio L. Martins[4], Daniel Kolbin[5], Meng-Chao Yao[6], Donna M. Cassidy-Hanley[5], Theodore G. Clark[5], Wei-Jen Chang[1,6]. 1) Hamilton College, Clinton , NY; 2) Indiana University, Bloomington, IN; 3) National Chyai University, Chyai City 60004, Taiwan; 4) College of Veterinary Medicine, Cornell University, Ithaca, NY; 5) Universidade Federal de Santa Catarina, 88040-900, Florianópolis, Santa Catarina, Brazil; 6) Academia Sinica, Taipei 115, Taiwan; 7) National Center for Genome Assembly Support, Indiana University.

Although the presence of endosymbiotic bacteria—specifically *Rickettsia*—have been reported in the ciliated protozoan *Ichthyophthirius multifiliis*, an obligate parasite that infects freshwater fish, it remains unclear whether these bacteria are present in most, if not all, isolates of *I. multifiliis*. In this study we report identification of these endosymbiotic bacteria in 18 different isolates of *I. multifiliis*, from three distinct geographical regions worldwide (Brazil, Taiwan, and United States). In three isolates (Ark11, Ark12, and G15) the presence of endosymbiotic bacteria were determined by deep sequencing, and in the remaining 15 isolates bacteria were detected by PCR amplification of conserved regions of the bacterial 16S rDNA genes. All *Ichthyophthirius* isolates appear to contain at least one rickettsial rDNA sequence. This ubiquity suggests that *I. multifiliis*—which has a very reduced genome— may be dependent on this endosymbiotic relationship. Results derived from phylogenetic analyses based on rickettsial rDNA sequences show that the rickettsial bacteria can be clustered into four groups. Some *I. multifiliis* isolates—particularly those collected from pet stores—were infected by multiple groups of rickettsial bacteria, suggesting that the association between *Rickettsia* and *I. multifiliis* is more dynamic than previously thought. Our results also support the hypothesis that

endosymbiotic *Rickettsia* is transmitted both vertically and horizontally, although mechanisms accounted for horizontal transfer remain unknown.

C32 Genetic and epigenetic control of DNA replication in *Tetrahymena thermophila*. *Geoffrey Kapler*[1], Xiangzhou Meng[1], Chunxiao Ge[1], Yifan Liu[2], Shan Gao[3]. 1) Texas A&M University, College Station, TX; 2) University of Michigan, Ann Arbor, MI; 3) Ocean University of China, Qingdao, China.

The 21 kb *T. thermophila* ribosomal DNA (rDNA) minichromosome initiates DNA replication once per cell cycle during vegetative growth from origins that reside in the 5' non-transcribed spacer (5' NTS). Using Q-PCR, we show that the rDNA is replicated by two distinct modes during macronuclear development- locus-specific gene amplification, and genome-wide endoreplication. 5' NTS origins mediate the initial wave of replication- amplification from 2C to 800C in starved mating cultures. This occurs in a restricted temporal window, concurrent with genome-wide endoreplication of non-rDNA macronuclear chromosomes from 2C to 8C. Upon re-feeding, the rDNA is no longer selectively amplified. Instead, it is endoreplicated to ~9000C along with the remainder of the genome, which achieves a copy number of ~45C. ORC protein levels are reduced during the second wave of DNA synthesis and known origins in the rDNA 5' NTS are frequently bypassed. Aberrantly migrating rDNA replication intermediates (RIs) form at this time; their migration in 2D gels is consistent with the accumulation of stable RNA-DNA hybrids in the 5' NTS. These RIs are indistinguishable from intermediates generated in a vegetative TXR1 knockout mutant strain, defective in histone H3K27 monomethylation.

To explore the possible relationship between H3K27me1 and endoreplication, the average inter-origin distance (IOD) was determined by DNA fiber analysis. This genome-wide approach revealed significant differences in replication initiation in endoreplicating wild type cells and vegetative TXR1 mutants. Despite the down-regulation of Orc1p in endoreplicating mated/re-fed cells, IOD decreased. Paradoxically, more initiation events occur when ORC levels are reduced. In contrast, the IOD increased in the vegetative TXR1 knockout mutant. Thus, although the formation of aberrant RIs is conserved, the mechanism underlying their biogenesis may differ. Finally, RNA-Seq revealed that TXR1 mRNA levels peaks in G1, prior to the onset of DNA replication. In situ immunofluorescence confirmed cell cycle regulation at the protein level. We propose a model in which H3K27me1 facilitates the assembly of ORC-dependent pre-replicative complexes (pre-RCs) during G1 phase. We speculate that the failure to establish pre-RCs triggers an alternative DNA replication program that bypasses the requirement for ORC. A recently published study, in which vegetative Orc1p levels are transiently reduced 50-fold, is consistent with the existence of an alternative pathway for the initiation of DNA replication in Tetrahymena.

C33 Beyond condensation: novel roles for condensin in the polyploid somatic nucleus of *Tetrahymena thermophila*. *R. Howard-Till*, J. Loidl. University of Vienna, MFPL, Chromosome Biology, Vienna, AT.

In most organisms, condensin proteins play an important role in organizing chromosomes for orderly segregation. This is also true in the ciliate *Tetrahymena thermophila*, where separate germline and somatic nuclei exhibit markedly different chromosomal organization and modes of division. The germline nucleus has 10 metacentric chromosomes that divide by mitosis and meiosis. The polyploid somatic nucleus divides by amitosis, a poorly understood process that results in random segregation of the chromosomes during a closed division. The *Tetrahymena* genome encodes one homolog each of the Smc2, Smc4, and CapG subunits of the condensin complex, two homologs of CapD, and four homologs of the kleisin (CapH/Barren). Smc2, Smc4, and CapG are shared between all the condensin complexes in the cell, but the CapD and kleisin subunits show nuclear specific localizations. As expected, condensin is required for condensation and segregation of germline chromosomes. However, previous work has also shown an unexpected requirement for condensin in the somatic nucleus. The somatic nucleus contains approximately 45 copies each of 181 chromosomes ranging in size from ~100 kb to ~2 mb. These chromosomes do not visibly condense prior to division, but condensin is clearly required for normal DNA segregation. Depletion of Smc2, CapD1 or Brn3 subunits results in aberrant divisions in which the nuclear membrane elongates and pinches off, but the chromatin is not distributed. Thus one daughter cell receives the majority of the DNA, while the other has very little. To better understand how condensin contributes to division, we have begun to investigate the organization of chromosomes in the somatic nucleus. FISH labeling of whole somatic chromosomes in wild-type cells shows distinct foci that are distributed fairly evenly throughout the nuclear space. In the absence of condensin, this distribution is disrupted, and the foci are clustered. Two color FISH of two different chromosomes shows that clusters do not overlap, indicating that chromatin is not just more dense in one part of the nucleus. These results indicate that condensin is involved in the organization of chromosomes in the somatic nucleus. Additional results indicate that a specialized condensin may also be involved in the generation of the somatic nucleus after sexual reproduction. Depletion of CapD2 during mating disrupts the process of somatic nuclear development, resulting in the retention of DNA elements that are normally eliminated. Nuclear development arrests without amplification to the normal DNA levels. Work is ongoing to determine the exact role of condensin in these processes, and promises to reveal novel roles for condensin in the generation and maintenance of an unusual polyploid nucleus.

C34 Repair of a fragile site in the mating type genes using an episomal template in *Tetrahymena*. *Marcella D. Cervantes*[1], Michael J. Lawson[2], Linying Zhang[1], Eileen Hamilton[3], Eduardo Orias[3], Geoffrey Kapler[1]. 1) Texas A&M Health Science Center, College Station, TX; 2) Uppsala University, Uppsala, Sweden; 3) University of California Santa Barbara, Santa Barbara, California.

The *Tetrahymena thermophila* somatic mating type genes offer an opportunity to understand an unusual mechanism for repairing fragile sites using episomal DNA as a template. *Tetrahymena* has seven possible mating types, and each is determined by the MTA and MTB gene pair. In the somatic nucleus, the gene pair is oriented head to head, sharing a presumed promoter region. Both genes are transcribed at a very low level during growth, but are induced to a high level of transcription by starvation. In the germline nucleus, the mating type locus contains an

array of truncated gene pairs. While the terminal exons of each gene are conserved between mating types, each copy of the conserved terminal exons contains polymorphic nucleotides unique to that particular copy. The germline array of genes is rearranged during somatic nuclear development by homologous recombination. PCR experiments suggests that recombination produces circular episomal byproducts that are retained in progeny for more than 120 fissions. Homologous recombination reduces the mating type locus to one complete gene pair in the new somatic nucleus. The pattern of polymorphic nucleotides shows up to 89% of the newly assembled MTB genes are produced by a single recombination event. Unexpectedly, older vegetative cells, do not exhibit a single recombination junction because the terminal exons have a mosaic of polymorphic nucleotides - presumably resulting from multiple gene conversion events. I propose that the template for these gene conversion events are episomal circular byproducts generated during mating type determination. I am addressing two questions regarding the episomes. The first question is whether the promoter region of the mating type gene pair can serve as an autonomously replicating sequence (ARS). To test the promoter region as an ARS, a plasmid with an *E.coli* backbone, a *Tetrahymena* selection cassette and regions of interest was transformed into vegetative *Tetrahymena*. We have obtained stable transformants that harbor a plasmid with the mating type VII promoter region. The second question is whether gene conversion within the conserved terminal exons occurs at a particular site due to replication fork pausing. Although the MTA and MTB genes acquire a mosaic of polymorphic nucleotides, they do not acquire mutations. This suggests homologous recombination. A pause site of DNA replication could explain the propensity for gene conversion in the terminal exons of MTA and MTB. The presence of pause sites in the terminal exons is being determined by 2D gel electrophoresis.

C35 Identification and Characterization of *Tetrahymena thermophila* Snf2/Swi2 ATPase Homologs involved in DNA Repair. *A. F. Morin*, J. J. Smith. Missouri State University, Springfield, MO.

The Snf2/Swi2 ATPase family of proteins is composed of different subfamilies with distinct functional roles, which can be classified based on the structure of their ATPase domains and other domains within the proteins. The Rad5/16 subfamily of Snf2/Swi2 ATPases is composed of Rad5 and Rad16, both of which play vital roles in different DNA repair pathways. Despite their activity in different repair pathways the structures of Rad5 and Rad16 are quite similar. Both contain the characteristic SNF2/SWI2 ATPase domains and, situated between the two ATPase domains, a Zinc-finger RING E3 ubiquitin ligase domain. Functionally, both Rad5 and Rad16 have ATPase activity, which is associated with chromatin remodeling to allow different proteins access to DNA, specifically at damaged sites. They also have E3 ubiquitin ligase activity, which is used to transfer ubiquitin molecules onto target substrates as a way of signaling for degradation or stability. The main difference between the two proteins is a characteristic HIRAN DNA binding domain, which is present at the N-terminus of Rad5, but absent in Rad16. Both proteins were initially discovered and characterized in *Saccharomyces cerevisiae*. Rad16 was demonstrated to be involved in nucleotide excision repair (NER), specifically global genome NER (ggNER). Rad5 was found to act in a variety of different instances, including: acting as a catalyst for translesion synthesis, acting in post replication repair as well as DNA double strand break (DSB) repair. Due to their roles, both proteins are extremely important as factors which help to enhance genome stability and promote ongoing genome integrity. *Saccharomyces* contains one constitutively silenced genomic locus, and since ggNER takes place in silenced areas of the genome this makes the study of ggNER proteins, such as Rad16, difficult. In contrast, *Tetrahymena thermophila* has a transcriptionally silent micronucleus, which provides a large platform for the study of Rad16 and ggNER. The goal of this work is to identify the homologs of both Rad16 and Rad5, while analyzing both their interactions and functions. Four putative homologs of Rad5/Rad16 were identified in *Tetrahymena* through bioinformatic analyses to elucidate sequence differences and similarities. Additionally, qRT-PCR analyses of homologs were conducted to determine expression in response to different DNA damaging agents; UV radiation to generate bulky adducts and induce NER, and methyl methanesulfonate (MMS) to induce DSB repair. To determine function in DNA repair and genomic integrity, shRNA constructs were created and transformed into cells to analyze the phenotypic consequences of decreased expression following UV and MMS damage.

C36 Interplay between the Homologs Rad51 and Dmc1 in Cell Division, Sexual Reproduction, and Homologous Recombination Repair. *Amaal Abulibdeh*[1], Allie Maltzman[1], Daniel Romero[2], Joshua Smith[1]. 1) Missouri State University , springfield, MO; 2) University of Minnesota, Minneapolis, MN.

Rad51 and Dmc1 promote the homologous and strand exchange steps of homologous recombination. The amino acid composition of both proteins is similar to that of RecA, containing a region of consensus sequence for nucleotide binding ss-and ds-DNA binding, and strand exchange. Dmc1 is a Recombinase involved in meiosis-specific repair of double strand breaks (DSBs) via homologous recombination, whereas Rad51 has been found to be involved in meiotic and non-meiotic DSBs repair. Microarray and RT-PCR data from previous studies in *Tetrahymena* have demonstrated that expression of DMC1 increases during meiosis and that the protein localizes to the micronucleus. Some studies in budding yeast show that when RAD51 is overexpressed, interhomolog recombination still occurs even when DMC1 is knocked out. . It is not known whether Dmc1 and Rad51 interact directly, but evidence suggests that they somehow work together. Rad51 and Dmc1 have not been fully characterized in the ciliate *Tetrahymena thermophila*. Previous studies demonstrated that without Rad51, DSBs were not repaired, but meiotic DSB repair is dramatically reduced in the absence of Dmc1 and the function of Dmc1 can be compensated by replacing with Rad51 promotor. The aim of this study is to further define the role of Rad51 verses Dmc1 in *Tetrahymena* and to determine if they interact with each other in a complex during the cell cycle and DNA repair. Overexpression of the RAD51 causes macronuclear division and chromosome copy number defects. Bioinformatics was used to compare Dmc1 and Rad51 homologues from various species. RT-PCR studies showed an increase in DMC1 mRNA expression after treatment with MMS, UV, and H2O2, suggesting a possible role for Dmc1in DNA repair outside of meiosis. Survivability studies show that knocking out DMC1 makes *Tetrahymena* more sensitive to MMS treatment, but not to UV or H2O2. Data suggests they may play both similar and distinct roles in meiotic recombination. The role of *Tetrahymena* Dmc1 in DNA repair will be further elucidated through immunoprecipitation, fluorescence microscopy, and overexpression studies.

C37 Both maternal and paternal scnRNAs can target excision of transposon-derived sequences during *Paramecium* development. *E. Meyer*, Guillaume Pellerin. IBENS, Paris, FR.

Like all ciliates, *Paramecium tetraurelia* entirely rearranges its genome during development of the somatic macronucleus from the germline micronucleus, in each sexual generation. Rearrangements include the precise excision of IESs (Internal Eliminated Sequences), single-copy intervening sequences likely derived from transposon insertions, from coding and non-coding regions of the genome. Correct genome rearrangements depend on a meiosis-specific class of small RNAs called scnRNAs. Initially produced from the entire maternal germline genome during meiosis, scnRNAs are thought to scan the maternal macronuclear genome which acts as a sponge to remove homologous molecules from the active pool, resulting in the selection of germline-specific scnRNAs that later allow the zygotic macronucleus to reproduce the same deletions. This genome scanning model raises a theoretical problem during conjugation between polymorphic strains : a heterozygous F1 cell should be unable to excise a scnRNA-dependent IES from the incoming (paternal) allele if that IES is absent from the maternal allele, or if its sequence is too divergent. Our results, however, indicate that divergent paternal alleles are correctly rearranged, using scnRNAs produced by the paternal cell. Germline-specific scnRNAs may be exchanged between conjugants along with gametic nuclei, or they may program IES excision at a very early stage, by targeting the deposition of unknown genomic imprints in gametic nuclei. I will present an update on experiments designed to test these hypotheses.

C38 Analysis of development-specific Piwi proteins in Paramecium. *D. Furrer*, E. Swart, M. Kraft, M. Nowacki. University of Bern, Bern, CH.

In ciliates, Piwi-bound small RNAs are involved in massive DNA elimination that takes place during sexual development. In Paramecium, we know so far of two classes of small RNAs involved in the excision of germline-limited DNA such as IESs and transposons. Scan RNAs (scnRNAs) are involved in the first round of the DNA elimination process. The later arising iesRNAs originate from eliminated IESs and are required to complete the DNA elimination.

We provide experimental evidence that Ptiwi01 and Ptiwi09 are essential for the scnRNA pathway and the elimination of maternally-controlled IESs. In addition we demonstrate that Ptiwi10 and Ptiwi11 are required for iesRNA-specific DNA elimination. Using deep sequencing we show that in both pathways the Ptiwi proteins select guide RNA strands from dsRNA Dicer cleavage products. Sequencing of DNA from different Ptiwi knock-downs also demonstrates that only one of the two small RNA pathways affects the excision of transposons.

Additionally we show that the late expressed Ptiwi10 and Ptiwi11, among many other proteins, are expressed from the new macronucleus and that their transcription depends on IES excision. We propose that the excision of IESs from proteins expressed in the new MAC may function as a checkpoint that maintains developmental timing.

In addition we are characterizing the remaining development-specific Ptiwi proteins to get a more detailed view of the complex role of small RNA-Ptiwi interactions in Paramecium development.

C39 Regulation of DNA elimination boundaries requires novel DNA-binding proteins that define heterochromatin domains. *Douglas Chalker*, Christine Carle, Vita Jaspan. Washington Univ in St. Louis, St. Louis, MO.

During development of the ciliated protozoan *Tetrahymena thermophila*, germline-derived DNA undergoes extensive reorganization including the removal of ~50Mbp in segments called internal eliminated sequences (IES's) from the differentiating somatic genome. The eliminated sequences are targeted for excision by small-RNA-directed heterochromatin formation. To ensure that the reorganized, gene-dense genome maintains all coding and regulatory sequences, the boundaries of the eliminated DNAs must be accurately defined. We have identified developmentally expressed proteins that define the boundaries of specific subsets of IESs. First, we discovered that the guanine-quadruplex binding protein, Lia3, specifically regulates the rearrangement of loci that are flanked by G-rich (5'-AAAAAGGGGG-3') boundary controlling sequences. Second, we characterized the Lia3-like protein 1 (Ltl1) and found that the IES's regulated by Ltl1, which include the D IES, are distinct from those regulated by the Lia3. Initial binding assays show that Ltl1 has a high affinity for double-stranded DNA, but does not bind single-stranded DNA. The IESs regulated by these two proteins comprise only a fraction of the thousands of eliminated loci. These results suggest that multiple proteins are required to accurately control the specification of heterochromatin domains that are targeted for removal from the somatic genome.

C40 Transiently maintained somatic chromosomes of *Tetrahymena* contain development-specific genes. *Y. Liu*[1], L. Feng[1], E. Hamilton[2], J. Xiong[3], G. Wang[3], W. Dui[1], L. Khadr[1], W. Miao[3], E. Orias[2]. 1) Department of Pathology, University of Michigan, Ann Arbor, MI 48109, USA; 2) Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, CA 93106, USA; 3) Key Laboratory of Aquatic Biodiversity and Conservation, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China.

In the ciliate *Tetrahymena*, programmed genome rearrangement events accompany the differentiation of the somatic macronucleus (MAC) from the germline micronucleus (MIC). Internal eliminated sequences (IES), most of which are likely derived from transposable elements, are excised by an RNAi-dependent pathway. Furthermore, the 5 MIC chromosomes are fragmented into ~200 MAC chromosomes at highly conserved chromosome breakage sequences (CBS), followed by *de novo* telomere addition. Intriguingly, some MAC chromosomes are lost soon after MAC differentiation, thereafter referred to as non-maintained macronuclear chromosomes (NMC).

Systematic comparison between the MIC and MAC genome sequences reveals several large NMC. In contrast to IES, NMC are still present in developing MAC during late conjugation. More importantly, these NMC generate high levels of mRNA, but very low levels of small RNA. Many NMC-contained genes encode conserved proteins, potentially involved in development-specific events. Here we focus on NMC-3 (~12 kb), which contains TPB3, a putative PiggyBac transposase with potential roles in IES excision. NMC-3 is quickly lost in mature MAC during asexual propagation, attributable to lack of efficient replication origins. Deleting either the left or right flanking CBS effectively prevents its loss. TPB3 is

expressed late in conjugation, during developing MAC formation of the progeny. Deleting NMC-3 from the germline MIC of parental cells—and consequently developing MAC of the progeny—abolishes TPB3 expression. Conjugation progress delay and progeny growth defects are also observed. Sequencing developing and mature MAC genomes from these progeny reveals that a small subset of IES is affected. We conclude that these NMC contain development-related genes, providing a novel mechanism for achieving a highly specific gene expression pattern.

C41 Cell cycle control of histone methyltransferase TXR1 levels is required for proper DNA replication in *Tetrahymena*. *Shan Gao*[1], Yifan Liu[2], Geoffrey Kapler[3], Jie Huang[1], Xiaolu Zhao[1,2], Xiao Chen[1]. 1) Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao 266003, China; 2) Department of Pathology, University of Michigan, Ann Arbor 48109, USA; 3) Department of Molecular and Cellular Medicine, College of Medicine, Texas A&M Health Science Center, College Station, TX 77843, USA.

DNA replication is tightly controlled by histone-modifying enzymes. In the ciliate *Tetrahymena,* knockout of histone methyltransferase TXR1 (TXR1 KO), specific for H3 lysine 27 mono-methylation (H3K27me1), leads to severe replication stress and DNA damage response. Here we investigate the mechanism by which DNA replication is regulated by TXR1. We demonstrate a cell cycle-dependent oscillation of TXR1 at mRNA and—more strikingly—protein levels. TXR1 peaks in G1 phase but is quickly depleted upon entering S phase. Correspondingly, H3K27me1 is built up slowly in newly synthesized histones, showing a much higher level in G1 phase than G2 phase. TXR1 degradation in S phase is most likely mediated by PCNA, as point mutations at the PCNA-interacting protein (PIP) motif cause TXR1 to accumulate at S phase. Strains with TXR1 PIP mutations as well as TXR1 overexpression show replication defects, which are distinct from the TXR1 KO phenotype. We therefore conclude that cell cycle control of TXR1 levels ensures proper DNA replication in *Tetrahymena*.

C42 Functional analysis of the Ibd1 protein in *Terahymena thermophila*. *A. Saettone*[1], A. Burtch[1], J. Garg[2], J. Lambert[3], A. Gingras[3], R. Pearlman[2], J. Fillingham[1]. 1) Department of Chemistry and Biology, Ryerson University, Toronto, Ontario, Canada; 2) Department of Biology, York University, Toronto, Ontario, Canada; 3) Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada.

The objective of this research is to identify and initiate functional characterization of the SWI/SNF complex in the unique chromatin environment of *Tetrahymena thermophila*. Through affinity purification followed by mass spectrometry (AP-MS), we identified the first SWI/SNF complex in protists through the reciprocal interaction of Snf5 and a small bromodomain containing protein (Ibd1). Through AP-MS of Ibd1 I found Ibd1 is versatile and interacts with several additional putative chromatin remodeling complexes such as a HMT, and SAGA and SWR. Bromodomains have affinity for acetylated lysine residues. Our peptide array experiment suggests that Ibd1 has affinity to multiple acetylated PTM related to highly transcribed regions. We found that indirect immunofluorescence (IF) of Ibd1 hints at a role in transcription. We are developing a protocol to use ChIP-Seq to identify the Ibd1 binding sites in the genome during growth.

C43 GCN5, ESA1, and CHD1: More Than Just Transcription Regulators? *J. J. Smith*, L. A. Andreas. Missouri State University, Springfield, MO.

The packaging of chromatin plays a vital role in the ability of the genes to be accessible or inaccessible to transcription which leads to expression or prevention of protein formation. Acetylation decreases the DNA-histone interaction allowing the chromatin to become euchromatin or transcriptionally active DNA. GCN5, ESA1, and CHD1 are three proteins that are involved in this process. GCN5 and ESA1 are histone acetyltransferases (HATs) and CHD1 is a bromodomain containing protein that is recruited to sites of acetylation. Tetrahymena thermophila are the ideal organism to study chromatin packaging because they contain a macronucleus and micronucleus that separate acetylation and transcription. It has been shown that chromatin modification can also allow access for DNA repair mechanisms. This research specifically focuses on GCN5, CHD1, and ESA1's function in Tetrahymena in chromatin regulation and DNA repair. The characterization of these proteins in Tetrahymena will allow for a better understanding of their exact role in DNA repair and genome stability. An interesting phenotype of GCN5 overexpression has been observed when cells are under starved conditions and was also studied in this research.

C44 MAC-specific Chromatin Remodelers bind a Zinc Finger Protein and Diverse RNAs throughout the *Tetrahymena* Life Cycle. *E. F. DeRango-Adem*[1], J. Garg[1], C. Mudalige[2], A. Saettone[2], J. P. Lambert[3], A. C. Gingras[3], J. Fillingham[2], R. Pearlman[1]. 1) York Univ., Toronto, Canada; 2) Ryerson Univ., Toronto, Canada; 3) Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada.

In a screen for ATP-dependent chromatin remodeling proteins in *Tetrahymena thermophila*, we identified and partially characterized two conserved Chromodomain Helicase DNA-binding (Chd) proteins of the SNF2 superfamily, Chd3 and Chd7. *Tetrahymena* Chd proteins have essential and diverse functions in growth and development. These proteins contain chromo-, bromo-, and PHD- domains and classically bind DNA. Chromodomain proteins and other ATP-dependent chromatin remodelers can also bind mRNAs, as well as ncRNAs during transcriptional silencing. These nucleic acid binding complexes are called DNA- and RNA-binding Proteins (DRBPs). DRBPs likely play a role in promoting recognition and removal of Internal Eliminated Sequences (IES) and irreversible genome silencing. A bioinformatic analysis of developmental-specific *Tetrahymena* proteins with DNA *and* RNA binding domains identified Chd3 and Chd7 among candidate DRBPs. To study three-way DNA:RNA:protein interaction, a three-way immunoprecipitation protocol was used. **P**rotein **I**mmuno**p**recipitation (PIP), **Ch**romatin **I**mmuno**p**recipitation (ChIP), and **R**NA **I**mmuno**p**recipitation (RIP) were used to identify DRBP interactions in an experimental setup called IP3.

FZZ-tagged constructs of Chd3 and Chd7 were transformed into *Tetrahymena* for expression analysis, localization, and purification. Indirect immunofluoresence in vegetative growth localized these proteins to the macronucleus (MAC). During development, Chd3 and Chd7 expression was lost in the old MAC, and localized exclusively in the developing MAC from 6-8 hours of conjugation. There was no localization to micronuclei (MIC) at any time. This is indicative of zygotic expression in anlagen, consistent with microarray and RNA-seq expression data. Affinity purification/mass spectrometry (APMS) of Chd3 and Chd7 in vegetative and conjugating cells indicate interaction with core histone proteins, heat shock protein 70, and an RNA-recognition motif protein. We have focused on and will discuss the interaction of a previously

uncharacterized MIZ Ring zinc finger (Znf) protein with Chd3. RNA immunoprecipitation and Urea-PAGE show Chd3 and Chd7 bind diverse RNAs, including multiple long RNAs, a 100nt ssRNA and an 80nt ssRNA species. Experiments in progress to be presented include RNAseq to identify RNA species immunoprecipitated, ChIP analysis to identify loci the DNA-binding chromatin remodelers are recruited to, and protein microarray analysis to identify histone modifications bound by Chd proteins.

C45 Homology dependent heterochromatin formation by *trans* acting RNAi in *Paramecium tetraurelia*. *M. Simon*[1], M. Cheaib[1], U. Götz[1], S. Marker[1], R. deWijn[1], A. Rodrigues[1], D. Durai[2], V. Oruganti[2], K. Nordström[2], S. Karunanithi[1,2], M. Schulz[2]. 1) Molecular Cell Dynamics Saarland University, Centre for Human and Molecular Biology, Campus A2 4, 66123 Saarbrücken, Germany; 2) Cluster of Excellence, Multimodal Computing and Interaction and Max Planck Institute for Informatics Saarland University, Department for Computational Biology and Applied Algorithmics, Campus E1 4, 66123, Saarbrücken, Germany; 3) Department for Genetics, Saarland University, Centre for Human and Molecular Biology, Campus A2 4, 66123 Saarbrücken, Germany.

Across kingdoms, RNA interference (RNAi) has been shown to control gene expression at the transcriptional- or the post-transcriptional level. Here, we describe mechanisms in which different classes of siRNAs trigger heterochromatin formation *in trans*. First, injection of truncated transgenes, unable to produce translatable mRNA, produce predominantly antisense siRNAs which depend on a variety of RNAi components. These include RDR3, HEN1 & PTIWI14 as well as a second set of components, which are also involved in post-transcriptional silencing: RDR2, PTIWI13, DCR1 and CID2. Our data indicates differential processing of nascent un-spliced and long, spliced transcripts. As transgene derived siRNAs trigger repressive histone marks at the remote loci, this suggests a functional interaction between post-transcriptional and co-transcriptional RNAi: next to a loss of H3K9ac and H3K4me3, increased levels of histone occupancy and H3K27me3 can be observed. This is accompanied by secondary siRNA accumulation, strictly limited to the open reading frame of the remote locus. Next to this exogenously triggered RNAi, also the endogenously controlled mutual exclusive expression of the surface antigen family depends on RDR3, HEN1, DCR1 and DCL4. Here, silent genes show surprisingly high levels of H3K4me3 and they exhibit very low levels of 23nt antisense RNAs. Surprisingly, actively transcribed antigen genes produce high levels of 23nt sense siRNA produced from the entire length of the mRNA. As silencing of RDR3 disrupts mutual exclusive expression as all surface antigens are co-expressed, this is accompanied with loss of sense siRNAs and accumulation of antisense siRNAs of the entire coding genome. As the data suggests that RDR3 and RDR2 compete for different classes of precursor RNAs, we classified siRNA clusters of the entire genome by their dependency on different RNAi components and compared these with chromatin data. This indicates that similar mechanisms to transgene induced silencing and serotype regulation contribute to transcriptome regulation by post-transcriptional and transcriptional silencing.

C46 The Enhancer of zeste like protein Ezl1 is required for scnRNA selection and transcriptional repression of transposon-derived sequences in *Paramecium tetraurelia*. *Andrea Frapporti*[1], Olivier Arnaiz[2], Maoussi Lhuillier-Akakpo[1], Augustin de Vanssay[1], Caridad Miro Pina[1], Raphaël Margueron[3], Sandra Duharcourt[1]. 1) Institut Jacques Monod, CNRS, UMR 7592, Sorbonne Paris Cité, Paris, France; 2) Institute of Integrative Biology of the Cell, UMR9198 CNRS CEA U Paris-Sud,91198 Gif-sur-Yvette, France; 3) Institut Curie, Paris Sciences et Lettres Research University, INSERM, U934, CNRS, UMR3215, Paris, France.

In the unicellular eukaryote *Paramecium tetraurelia,* differentiation of the somatic macronucleus (MAC) from the zygotic nucleus is characterized by massive and reproducible rearrangements of germline DNA, that include the elimination of transposable elements and 45,000 short, single-copy internal eliminated sequences (IESs). A specific class of small RNAs, the scnRNAs that are produced by the germline micronucleus (MIC) through a meiosis-specific RNAi pathway, is essential for DNA elimination of homologous sequences in the developing MAC. The mechanisms that allow specific recognition of germline-limited sequences within chromatin remain an open question. We identified recently a *Paramecium* homolog of mammalian Enhancer-of-zeste, Ezl1 that is required for the establishment of both H3K27me3 and H3K9me3 epigenetic modifications during development and for programmed genome rearrangements (Lhuillier-Akakpo, 2014). Here, we show that the Ezl1 protein is a *bona-fide* histone methyl-transferase, whose catalytic activity is necessary *in vivo* for histone H3 trimethylation on lysines 27 and 9 and for correct elimination of germline-limited DNA. We used RNA sequencing to measure steady state RNA levels from *EZL1* knockdown (KD) cells at different time points during development. Analysis of small RNA datasets revealed that scnRNA biogenesis is not affected upon Ezl1 depletion as compared to control. Yet elimination of MAC-specific scnRNAs from the initial pool of scnRNAs, a process that normally leads to MIC-specific scnRNA enrichment during development, is abrogated in *EZL1* KD. Ezl1 depletion also caused a sharp rise in transposon-derived transcripts. Altogether our data suggest that the histone methyltransferase Ezl1 is required for MIC-specific scnRNA selection and for transcriptional repression of transposon-derived sequences.

C47 Proteomic Characterization of *Tetrahymena thermophila* Chromatin Assembly Proteins. *J. Garg*[1], S. Nabeel-Shah[2], J.-P. Lambert[3], A.-C. Gingras[3,4], R. P. Pearlman[1], J. Fillingham[2]. 1) York University, Toronto, Ontario, CA; 2) Department of Chemistry and Biology, Ryerson University, Toronto, Ontario, Canada; 3) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto M5G 1X5, Canada; 4) Department of Molecular Genetics, University of Toronto, Toronto M5S 1A8, Canada.

In humans, transport of histones H3/H4 from the cytoplasm to the nucleus occurs in a stepwise fashion and is mediated by protein factors including HSP90, NASP, Asf1 and Importin4. Cells can adopt two different pathways to assemble chromatin, namely replication dependent and replication independent pathways which are mediated respectively by “Chromatin Assembly Factor 1 (CAF1)” and “Histone Regulator A (HIRA)” protein complexes. Despite their identification, the mechanistic details of H3/H4 transport and subsequent deposition onto DNA remains unclear. To address this, we have performed proteomic characterization of Asf1, NASP, CAF1 and Hat1 in *Tetrahymena* using affinity purification combined with mass spectrometry approach. *Tetrahymena* is an excellent model system to study chromatin related processes

providing that it features nuclear dualism in the form of a transcriptionally active macronucleus and a silent micronucleus. Our results indicate that NASP physically interacts with Asf1 and HSP90 and thus likely functions in H3/H4 transport suggesting an evolutionarily conserved nature of this pathway. We also show that the three protein CAF1 complex (Cac1, Cac2, Cac3) interacts with H3/H4 as well as with Casein Kinase 2 (CK2), an evolutionary conserved SER/THR protein kinase. Our MS data also indicates that Hat1 co-purifies with H4 and a putative Hat2 protein. Interestingly, we found that Hat2 subunit of HAT complex is also shared by CAF1 complex as its Cac3 subunit. We have named this protein SSCH1 (Shared Subunit of CAF-1 and Hat1). This suggest that SSCH1 exists in two separate pools of protein complexes and is capable of carrying functions that are divided between two separate proteins in higher eukaryotes such as humans.

C48 Signaling and Cell Cycle Studies in *Tetrahymena thermophila*. *R. E. Pearlman*[1], W. Ahmad[1], J. Garg[1], A. Kume[1], M. Anafi[1], W. Glowacka[1], J.-P. Lambert[2], A.-C. Gingras[2], J. Fillingham[3]. 1) York University, Toronto, Ontario, CA; 2) Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, CA; 3) Ryerson University, Toronto, Ontario, CA.

Phosphotyrosine signaling in response to growth factors and its involvement in cell cycle regulation is widely believed to be a key signal in vertebrate and invertebrate cells to determine their fate. In multicellular organisms, protein tyrosine phosphorylation is well known to have a pivotal role in the coordination of signal transduction in response to extracellular stimuli involved in the control of a wide range of cellular processes. Until recently, tyrosine phosphorylation signaling was thought to be limited to multicellular organisms and most of the studies in this field are therefore with multicellular organisms. Some recent publications however suggest a role for tyrosine phosphorylation in signaling in unicellular eukaryotic organisms as well. Recent work in our laboratory with *Tetrahymena thermophila* has provided both experimental and bioinformatic computational evidence for tyrosine phosphorylation. The dynamics of tyrosine phosphorylation in *T. thermophila* in a mating type specific manner and the role such phosphorylation might play in this unicellular eukaryote have been examined. Key questions addressed in this work are to identify the pathway(s) of tyrosine phosphorylation in *Tetrahymena* including proteins associated with pathway components already identified and to identify sites of tyrosine phosphorylation on proteins that we have suggested to be involved in tyrosine phosphorylation in *Tetrahymena*.

Genes of interest were obtained using PCR and the available *Tetrahymena* complete genome sequence (www.ciliate.org) and 3' tagged with FZZ. Constructs in this study include 4 protein tyrosine kinases and two protein tyrosine phosphatases. Plasmid DNAs were introduced into *Tetrahymena* by biolistic transformation and gene replacements were made homozygous using phenotypic assortment. Western blot and indirect immunofluorescence (IF) were used to confirm expression and localization of the tagged proteins. Affinity purification/mass spectrometry (AP/MS) to identify protein/protein interactions has been carried out and will be described. Bioinformatic including gene network analysis is used in combination with biological approaches such as gene knock out and gene knock down to describe the network of interacting proteins to gain additional insight into biological function.

C49 A potential role for TtSNX4 in macronuclear degradation in *Tetrahymena thermophila* Conjugation. *S. Guerrier*[1], A. Ariatti[1], B. McField[1], M. Cervanted[2], G. Kapler[2]. 1) Millsaps College, Jackson, MS; 2) Texas A and M Health Science Center, College Station, TX.

Organellophagy is the selective degradation of organelles by autophagosomes. Alterations in organellophagy are associated with several disorders including neurodegeneration, heart disease, obesity, and cancer. Selectivity in organellophagy is achieved by the specific binding autophagosome protein ATG8 to proteins on the target compartment. A distinct set of proteins is then used by selective autophagy pathways to expand the autophagosome around the specific target. Nucleophagy, the selective degradation of the nucleus by autophagosomes, is up regulated in laminopathies and in response to oncogenic stress. But whether nucleophagy utilizes proteins that are required for selective autophagy patways remains unclear. Our primary goal is to understand the molecular mechanisms underlying how the nucleus is specifically degraded by autophagosomes. In order to uncover these mechanisms we plan to exploit nuclear degradation during mating in *Tetrahymena thermophila*. *Tetrahymena* selective degrade their entire (macronucleus) nucleus in an autophagy dependent manner, as part of normal mating. Importantly this process can be induced in the lab and occurs in predictable and identifiable stages making nucleophagy in *Tetrahymena* straightforward to monitor. Preliminary work in our lab has shown that nucleophagy in *Tetrahymena*, may employ selective autophagy proteins. Specifically, we identified a *Tetrahymena* homolog of SNX4 (TtSNX4), a protein that is required for the selective degradation of mitochondria by autophagosomes (mitophagy) in yeast as candidate for regulating nucleophagy in *Tetrahymena*. We find that TtSNX4 localizes specifically to the degrading nucleus in a phosphoiositide 3 kinase (PI3K) dependent manner and deletion of TtSNX4 results in impaired nuclear condensation, an early stage in the degradation process that is dependent on nucleases provided by mitochondria that have been engulfed by autophagosomes. Importantly, proteins like SNX4, that possess PX-BAR domains have been shown to bind PI(3)P, a peoduct of PI3K. Therefore we hypothesize that TtSNX4 may regulate the delivery of mitochondrial nucleases to the nucleus in a PI(3)P dependent manner.

C50 Characterization of the ubiquitin-like modifier *Urm1* in the Ciliate *Tetrahymena thermophila*. *J. Copeland*, J. Smith. Missouri State University, Springfield, MO.

Ubiquitin-Related Modifier (Urm1) is a small protein with the characteristic ubiquitin Beta-grasp fold and Carboxyl termini diglycine motif. It has been implicated in two cellular functions: conjugation to substrate proteins during oxidative stress, such as pAhp1 and in the thiolation of wobble uridines in certain cytosolic tRNAs. Both processes require activation by E1-like enzyme Uba4, which has a C-terminal rhodonase domain and demonstrates both sulfurtransferase and acetyltransferase activity. Once Urm1 is activated by Uba4, the other members of the Urm1 tRNA-thiolation pathway: Ncs6, Ncs2, Tum1, Nsf1 will help mediate the transfer of the sulfur onto the uridine. Urm1 Thiolation of tRNAs is ubiquitous to life and highly conserved. It has been linked to various cellular stress responses: heat shock, misfolded protein response and

oxidative stress as well as several signal transduction pathways: notably mTOR and JNK. However, it's been shown several times that Urm1 knockouts have minimal phenotypic consequences. Urm1 is particularly interesting because it believed to be the most ancient Ubiquitin-like Protein (Ubl), and appears to be an evolutionary link between Eukaryotic Ubiquitination and Molydopterin Cofactor (MoCo) synthesis, which is present in Prokarea and most of Eukarya. This is supported by evidence that the Uba4 homolog in Arabidopsis thaliana is responsible for activating both Urm1 and MoCo Synthesis Pathways. Urm1 was first characterized in Saccharomyces cerevisiae, but there is a need to demonstrate tRNA thiolation activity of homologs in other organisms. This work will characterize Urm1 in Tetrahymena thermophila, a model organism useful for its quick growth as well as its relatively easy genetic engineering through homologous recombination. There will be bioinformatics data focusing on protein structure and function, as well as sequence homology to support the role of Urm1 in ubiquitin evolution. Expression profiles of Urm1 will be performed using qRT-PCR of T. thermophila grown in different growth conditions, focusing particularly on oxidative stress and heat shock. Fluorescence microscopy co-localization studies will be performed for Urm1 and the other pathway members, using genes that have been endogenously tagged with fluorophores.

C51 Early stages of diversification in the Rab GTPase gene family revealed by genomic and functional studies in *Paramecium* species. *L. Bright*, J.-F. Gout, M. Lynch. Indiana University, Bloomington , IN.

New gene functions arise within existing gene families as a result of gene duplication and subsequent diversification. However, the evolutionary forces that act on gene duplicates (paralogs) over time, as well as the intermediate steps on the path to functional change, are not well understood. In an effort to understand functional diversification in paralogs on both the genomic and subcellular level, we tracked duplicate retention patterns and subcellular markers of functional diversification in the Rab GTPase gene family in *Paramecium aurelia* species. Rab GTPases are on the whole more highly expressed and more highly retained than other genes in *Paramecium* genomes after whole genome duplication (WGD). Additionally, consistent with early steps in functional diversification, expression levels of these recent Rab paralogs appear to be diverging more rapidly from one another than other genes in the genome. We uncovered evidence of diversification at the subcellular level by localizing GFP-tagged paralogs from the Rab11 subfamily. Because the functionally diversifying paralogs are closely related to and derived from a clade of functionally conserved paralogs, we were able to pinpoint two specific amino acid residues that may be the drivers of the change in localization and, thus, function. Interestingly, the functionally conserved proteins label compartments involved in both endocytic recycling, the conserved Rab11 function, and phagocytic recycling, revealing evolutionary links between the two pathways.

C52 The detection of intracellular cAMP fluctuations – a sensitive *in vivo* assay to investigate signal transduction pathways in *Tetrahymena thermophila*. *D. P. Romero*. University of Minnesota, Minneapolis, MN.

Cyclic adenosine 3',5'-monophosphate (cAMP) is an important 2nd messenger involved in signal transduction pathways in diverse organisms from bacteria to mammals. Extracellular stimuli, including variable ionic conditions and drugs, can modulate cytoplasmic adenylyl cyclase activity via their effect on transmembrane proteins such as ion channels and G-protein coupled receptors. Through its influence on downstream signaling pathways, cAMP modulates various biological processes including metabolism, differentiation, cell adhesion and neuronal signaling. Standard methods to determine intracellular cAMP levels include radioimmunoassay (RIA) and competitive enzyme-linked immunosorbant assay (ELISA). Although quite sensitive, these immunological methods are time consuming and entail cell lysis and extensive processing steps. In an effort to streamline the detection of intracellular cAMP in *Tetrahymena thermophila,* and to make it possible to measure fluctuations of cAMP *in vivo* and in real time, the GloSensor™ cAMP Assay (www.promega.com), designed for transient expression in mammalian cells, has been adapted for use in *Tetrahymena.* The assay is based on a chimeric protein that includes a cAMP binding domain fused to mutant forms of *Photinus pyralis* luciferase. Upon binding intracellular cAMP, conformational changes alter the chimeric reporter tertiary structure, resulting in a reconstituted luciferase activity and a large increase in light output in the presence of the GloSensor™ substrate. *Tetrahymena t.* strains expressing the GloSensor™ reporter from the constitutive *BTU1* transcriptional promoter have been constructed. The stable transformant strains are able to take up the GloSensor™ substrate readily. Using 96-well plates (approx. 40,000 cell/well) and a plate reader with luminometer capabilities, cAMP levels can be measured to gauge the effect of various chemical compounds and growth conditions in a live-cell, non-lytic assay format. It is possible to monitor changes in cAMP levels in cells responding to stimuli in real time. This system will make it possible to screen complex compound libraries for ligands that affect G-protein coupled receptor (GPCR) function in *Tetrahymena.* An initial screen of the Pathogen Box (http://www.pathogenbox.org), composed of 400 diverse, drug-like molecules active against neglected diseases of interest, is currently ongoing.

C53 Polycystin-2 (Pkd2) and its unexpected role in Mg^{2+} permeability in *Paramecium*. *M. S. Valentine*, J. Yano, J. L. Van Houten. University of Vermont, Burlington, VT.

The non-selective cation channel polycystin-2 (Pkd2) is a member of the transient receptor potential (TRP) family of proteins and when mutated, can lead to autosomal dominant polycystic kidney disease. Here, we use the ciliated cell *Paramecium tetraurelia*, to examine Pkd2 in both the cilia and the cell membrane, together or separately, using deciliated or intact cells. *Paramecium* has two paralogs for Pkd2: *PKD2a* and *PKD2b*, which are 85% identical at the nucleotide level. Depletion of the mRNA for these paralogs using RNA interference (RNAi) results in reduced backward swimming, specifically in Mg^{2+} solutions, and a resistance to heavy metal toxicity. Our results suggested Pkd2 may play a role in the Ca^{2+}-dependent Mg^{2+} pathway because the results are similar to the phenotype of *eccentric* (XntA1), a mutant that is defective in Mg^{2+} conductance. We localized the Pkd2 and XntA proteins by expressing the genes using a plasmid containing a 3' terminal 3×FLAG or 3×myc sequence. The Pkd2-FLAG and XntA-FLAG (or XntA-myc) proteins localize in the cell membrane and in the cilia, as confirmed by immunoprecipitation (IP), immunofluorescence, and Western blot. Using FLAG and myc affinity agarose, we IP'd the epitope-tagged versions

of the proteins and demonstrated that XntA-myc co-IPs Pkd2-FLAG, but not *vice versa*. These data suggest a potential C-terminal interaction of the Pkd2-FLAG protein with XntA-myc, where the FLAG epitope is probably masked by the Pkd2 protein. To tease apart the contributions of the Pkd2 channel at the cell membrane and in the cilia, we utilized electrophysiology and membrane potential measurements to assess the permeability of *Paramecium's* membrane to Mg^{2+}. Our data suggest that Pkd2 is both necessary and sufficient for Mg^{2+} permeability of the cell membrane and that Pkd2 in the cilia may require the XntA protein; that without XntA, Pkd2 cannot function in the cilia. We propose that the Pkd2 channel is responsible for membrane permeability to Mg^{2+} while the role of the XntA protein, which is still under investigation, appears to be important for channel positioning or communication among channels in the membrane.

This project was supported in part by NIH Grant Numbers GM59988 and 5 P30 RR032135 and NIGMS grant 8 P30 GM103498. DNA sequencing performed in the VT Cancer Center supported in part by IDeA from NIGMS of NIH under grant P20GM103449.

C54 Whole genome sequencing of a Tetrahymena mutant reveals that VPS8, a subunit of the CORVET complex, is essential for biogenesis of mucocysts. *D. Sparvoli*[1], C. Kontur[2], X. Lan[3,4], J. K. Pritchard[3,4], A. P. Turkewitz[1]. 1) The University of Chicago, Chicago, IL; 2) Yale University, New Haven, CT; 3) Stanford University, Stanford, CA; 4) Howard Hughes Medical Institute, Stanford, CA.

In the ciliate *Tetrahymena thermophila*, regulated secretion of peptides occurs from dense core granules named mucocysts. Prior work from our laboratory revealed that mucocyst biogenesis depends on several proteins associated with lysosomes and lysosome-related organelles, namely cathepsins and VPS10/sortilins. To identify additional factors required in this pathway, we have used whole genome sequencing to analyze a recessive Mendelian mutant, called UC616, that is defective in mucocyst biogenesis. In an F2 mutant pool derived by outcrossing and then backcrossing UC616, we identified a homozygous single nucleotide variant predicted to alter a splice junction within a *Tetrahymena* homolog of the *VPS8* gene, which we call TtVPS8a. The mutation in TtVPS8a indeed inhibits intron excision and introduces a premature stop codon in the N-terminal domain of the protein. Importantly, the mucocyst biogenesis defect in UC616 can be fully rescued by endogenous-level expression of a full length, GFP-tagged copy of TtVPS8a. Yeast VPS8 encodes a subunit of CORVET, a complex shown to mediate vesicle tethering in the endolysosomal system in yeast and mammals. In UC616, mucocysts are absent and a protein that forms the wildtype mucocyst core instead accumulates in heterogeneous cytoplasmic compartments. Interestingly, *T. thermophila* and related ciliates have multiple paralogs of VPS8 and other CORVET-complex subunits, while lacking subunits that are specific to the related HOPS complex in fungi and animals. *Tetrahymena* maintains several distinct organelles, in addition to mucocysts, which appear related to lysosomes, and our results suggest that specialized paralogous CORVET tethering complexes may play an important role in their biogenesis. Our results also highlight the accessibility of this organism to forward genetic approaches, to interrogate this and other pathways.

C55 Using Chlamydomonas to understand cilia assembly. *S. K. Dutcher*, Huawen Lin, Suyang Guo, Robyn Roth. Washington Univ Sch Med, St Louis, MO.

The eukaryotic cilium is a complex machine with over 600 polypeptides. Motile cilia move cells or fluids. Motile and primary cilia play roles in signaling. Cilia both send and receive signals from the environment. Defects in these organelles cause a wide range of human diseases that include cancer, obesity, retinal degeneration, kidney and bone diseases as well as neurocognitive defects. We use *Chlamydomonas reinhardtii*, to understand the assembly and function of cilia. Ciliary proteins are highly conserved throughout the eukaryotic lineage and model organisms have been key in identifying and understanding human ciliopathies. We have been addressing three key questions using electron tomographic, biochemical, and genetic approaches. First, how are the large protein assemblies of motile cilia assembled in the cytoplasm? Second, how are cytoplasmic proteins kept out of cilium? Third, how are membrane vesicles used for ciliary signaling? Primary ciliary dyskinesia (PCD) is a disease in which the motility of cilia on the multi-ciliated epithelia of the respiratory tract is compromised. We are examining the roles of several proteins (HEATR2, DYX1C1, and IDA3) in the cytoplasmic assembly of the dynein arms. Proteins that localize to the transition zone, which spans from the triplet microtubules of the basal body, a modified centriole, to the doublet microtubules of the ciliary axoneme cause a variety of ciliopathies characterized by kidney defects, polydactyly, and retinal degeneration. We have found that some of these transition zone proteins (NPHP4, CEP290, RPGRIP1L) as well as proteins needed for intraflagellar transport (IFT172, IFT144, IFT52) are needed for the assembly of highly organized arrays of transmembrane proteins in the ciliary necklace, which is in the ciliary membrane surrounding the transition zone. It has been postulated that the transition zone and/or the ciliary necklace provide the barrier to the entry of cytoplasmic proteins into cilia. Using mass spectroscopy of isolated flagella, we find that that the ciliary necklace does not provide barrier function. The absence of transition zone proteins affects the barrier. Loss of RPGRIP1L has the strongest effect on the barrier; the 26S proteasome and the TRiC/CCT chaperonin complex enter the mutant cilia. Understanding the consequences of the entry of these proteins is underway. Studies in *Chlamydomonas* and *C. elegans* showed that membrane vesicles referred to as ectosomes are released from the ciliary membrane (Wang et al., 2015; Wood et al., 2013; Cai et al., 2015). These ectosomes carry signaling activity. We have found that the transition zone protein, RPGRIP1L, plays a role in the production of signal-containing ectosomes. The identification of new mutants is being pursued to understand the role of the transition zone in ectosome production and signaling.

C56 Role of molecular motors and microtubule-binding proteins in cell polarity and regeneration of Stentor. *T. Makushok*, W. Marshall. UCSF, San Francisco, CA.

Cells rely heavily on their polarity establishment and maintenance mechanisms, because proper polarization is required for performing a wide range of cell functions. The unicellular ciliate Stentor is an excellent model system for studying polarity due to its giant size, extraordinary level of structural regularity of its cytoskeleton, and amenability to microsurgical manipulation. Importantly, Stentor has the capacity to regenerate any of its parts, which gives access to studying the re-establishment of polarity perturbed in a wide variety of ways. In this study, we used RNAi

to analyze the role of Stentor cytoskeleton and molecular motors in cell motility, cell polarity, dynamic cell shape control, and regeneration. We show that cytoplasmic dyneins are essential for cell polarity maintenance, with abnormal cell morphologies observed when the corresponding genes are knocked down. We also observed that when kinesin gene is knocked down, Stentor cells are unable to extend to their full length. This suggests that kinesin may be involved in the sliding of microtubule sheets within microtubule rows running parallel to the main axis of the cell. Sliding within this complex cytoskeletal structure is thought to provide the mechanistic basis for Stentor dynamic shape changes. In addition to cytoplasmic dyneins and kinesin, we uncovered B subunit of the protein phosphatase PP2A as a major player in Stentor morphology control. Thus, our results provide insights into the molecular basis of Stentor morphology maintenance and dynamic cell shape control. Better molecular understanding of Stentor amazing ability to regenerate is essential, because it may lead to advancements in regenerative medicine.

C57 Kinome analysis in the giant ciliate *Stentor coeruleus*. *S. B. Reiff*, P. Sood, J. G. Ruby, M. Slabodnick, J. DeRisi, W. Marshall. Univ of CA, San Francisco, San Francisco, CA.

The giant unicellular ciliate *Stentor coeruleus* has the ability to fully regenerate after being cut in half, in a way that perfectly preserves cell polarity and structure. This regenerative ability has made it a classical model system for studying regeneration at the cellular level. So far, however, the molecular details behind this incredible phenomenon have remained largely unstudied. Recently, our laboratory has developed a system for RNAi knockdown of Stentor genes, and additionally sequenced the *Stentor coeruleus* genome. Interestingly, not only do Stentor's introns appear to possess the smallest average intron length of any organism described to date at 15 bp, but Stentor also seems to use the standard genetic code, unlike other ciliates. We wish to understand how the regeneration process is coordinated at the molecular level. Some of the details of the regeneration process parallel the events of cell division, and thus we additionally wish to understand whether the cell co-opts certain cell division signaling pathways for regeneration.To identify candidates for RNAi knockdown we analyzed the kinome of Stentor by looking for protein kinase domains among the predicted protein coding genes. Stentor was found to encode more than 2000 kinases, making up 6% of the total protein coding genes. Many of these consist of expansions in mitotic kinase families such as PLKs, NDRs, and NEKs. There are also expansions of families absent in animals and yeast; over 12% of the kinome consists of the calcium-dependent CDPK family, originally identified in plants. We also analyzed additional protein domains found on kinase genes in Stentor, revealing a few novel domain architectures. The most notable example is an adenylate kinase fused to a calcium-dependent protein kinase, with a large region in between containing a AAA+ ATPase and other protein domains. RNAi screening of kinase genes is ongoing, and will ultimately reveal which of these kinases help to coordinate the many different precisely timed cellular events required for successful regeneration. In the future, a better understanding of the mechanisms behind single cell regeneration will have important implications for basic biology as a whole, and will reveal how these single cells can establish and maintain their polarity and cortical organization with such a high degree of precision.

C58 Forward Genetics in *Tetrahymena thermophila* by a Modified Pooled Linkage: Identification of Causative Mutations Related to Cell Division and Ciliogenesis. *Yu-Yang Jiang*[1], Gregory Minevich[2], Jacek Gaertig[1]. 1) University of Georgia, Athens, GA; 2) Columbia University Medical Center, New York, NY.

We developed an improved protocol for identification of causative mutations in *Tetrahymena* using pooled linkage and comparative whole genome sequencing. The workflow involves an outcross of a mutant of interest, uniparental cytogamy of a single F1 clone, pooling of a number of wild-type and mutant F2 clones, whole genome sequencing of mutant and wild-type pools, variant subtractions from the mutant set, and filtering for nitrosoguanidine-induced transition mutations. Based on a few mutants we have analyzed thus far, this workflow reveals either a single or a small number of closely linked variants containing 100% of mutant reads, as candidates for the causative mutation. The procedure can be used for both recessive and dominant alleles. We successfully used this approach for identification of the causative mutation for *cdaI-1*, a temperature-sensitive "hammerhead" mutant, in which the oral primordium and the cleavage furrow shift toward the anterior end, resulting in an incomplete and unequal cell division (isolated by L.M. Jenkins and J. Frankel, University of Iowa; Frankel J., *Eukaryotic Cell* 2008,10:1617-1639). The *cdaI-1* cause is a substitution in a STE20-related kinase encoded by TTHERM_00971920. A wild-type fragment of TTHERM_00971920 rescued the mutant phenotype of the *cdaI-1* cells grown at the restrictive temperature. We applied the same strategy to a more challenging case, identification of a genetic suppressor of a cilia length regulation mutant. A loss of function of a *Tetrahymena* ortholog of the "long flagella 4" (LF4) protein of *Chlamydomonas reinhardtii* leads to longer cilia. LF4 encodes a conserved protein kinase. We aim at identifying the mechanism by which LF4 regulates the length of cilia and its kinase substrate(s). An MTT1-driven cadmium-induced overexpression of LF4 shortens cilia, which leads to cell paralysis, an easily selectable phenotype. A suppressor locus of the LF4 overexpression could encode a phosphorylation substrate or a regulator of LF4. We isolated a number of suppressors of the LF4 overexpression, all of which but one are intragenic (due to a loss of the overproduced LF4 kinase activity). For the single extragenic suppressor, we identified a candidate causative mutation in a conserved ciliary protein gene. We are currently testing whether an introduction of the mutant candidate gene fragment suppresses the consequences of LF4 overexpression.

C59 *Paramecium* as a model to study human ciliopathies: study of a transition zone protein, MKS2. *A. Tassin*, A. Aubusson-Fleury, M. Lemullois, S. Abdallah, J. Cohen, F. Koll. CNRS, Gif sur Yvette , FR.

Ciliogenesis is a multi-step process, including centriole/basal body assembly, maturation and docking to the cell surface where it templates the growth of the cilium. The structural junction between the basal body and the axoneme (transition zone) acts as a filter between the cellular and the ciliary compartment and houses many proteins involved in human ciliopathies. Two transition zone modules (MKS and NPHP modules) are required for cilium formation/function that act together with the Cep290 as a ciliary gate. The transmembrane protein MKS2/TMEM216 protein is one of the MKS module. In order to get a better understanding of the function of MKS2/TMEM216 in motile cilia formation, we

turned to *Paramecium*, which is covered with thousands of motile cilia.

Three *Paramecium* genes encoding three expressed MKS2 proteins were identified in the *Paramecium* genome as a result of whole genome duplication. These three proteins are highly similar. Using GFP-tagged MKS2 expression we demonstrated, using confocal microscopy and electron microscopy, that MKS2 is localized specifically at the transition zone of ciliated basal bodies on Triton-X100 membrane remnants at the level of the axosomal plate, most probably corresponding to the ciliary necklace. To get an understanding of the MKS2 function, we performed two kinds of experiments: knock-down of the three MKS2 genes by bacterial feeding and expression of one *Paramecium* gene carrying one of the two human mutations (R 73L and L114R) causing respectively Joubert and Meckel syndromes. RNAi knocked-down cells showed the presence of cilia and, unlike control cells, all basal bodies were ciliated. This result suggests a deregulation of the ciliogenesis after knocking-down MKS2. Ultrastructural analyses of MKS2-deficient cells revealed an excess of membrane protrusions and an accumulation of vesicles, suggesting defective membrane trafficking in these cells. We demonstrate that introduction of the human mutations in one Paramecium gene leads to mislocalization of the expressed protein.

Altogether, the *Paramecium* model allowed us to localize precisely MKS2 in the transition zone. We demonstrate its involvement in the regulation of ciliogenesis. The mislocalization of the human mutated protein opens the way to understand the mechanisms leading to ciliogenesis defects in the human pathology.

C60 Sfr proteins that transiently localize to the basal bodies during assembly. *Mark Winey*, Alex Stemm-Wolf, Wes Heydeck. Univ Colorado, Boulder, CO.

Basal bodies organize cilia, which are microtubule-based cellular projections responsible for fluid flow and involved in signaling pathways. In Tetrahymena, cilia are arranged along cortical rows that propel the cells and into specialized structures within the oral apparatus that is used for feeding. Basal bodies are comprised of nine-triplet microtubules and hundreds of additional proteins. This structure, and many of the protein components in Tetrahymena are conserved in centrioles of vertebrate cells. We are interested in understanding the assembly of new basal bodies in hopes of revealing mechanisms conserved between basal bodies and centrioles. We have previously reported the basal body functions of the widely conserved component, centrin. Centrin binds a short repetitive sequence first found in the yeast centrosomal protein Sfi1. Human Sfi1 is found at centrioles. Centrioles also contain a related centrin-binding protein, Poc5. We have identified Tetrahymena orthologs of both Sfi1 and Poc5. These proteins are members of a large family of Tetrahymena proteins containing the centrin-binding motif, the Sfr family, on which we have previously reported. Many of the Sfr proteins localize asymmetrically around the basal body and play roles in basal body positioning, a role also demonstrated for centrin. As expected, TtSfi1 and TtPoc5 are found at basal bodies. Interestingly, both proteins only appear transiently at basal bodies during their assembly. Among the many Tetrahymena basal body components that have been examined, this behavior of transient localization is unique to TtSfi1 and TtPoc5. We believe that such localization is suggestive of important roles for these proteins in basal body assembly. TtPoc5 localizes to the basal body distal end, similar to hPoc5 in centrioles, while TtSfi1 localizes asymmetrically and is offset from the basal body microtubule scaffold. Preliminary data from a TtSfi1 macronuclear null strain suggests significant basal body defects. Micronuclear null strains for both TtSfi1 and TtPoc5 have been constructed and the basal body phenotypes of these strains will be examined.

C61 Stabilizing basal bodies to resist asymmetric ciliary forces. *C. G. Pearson*[1], J. Meehl[2], I. M. Cheeseman[3], J. Gaertig[4], M. Winey[2], B. A. Bayless[1]. 1) University of Colorado, Aurora, CO; 2) University of Colorado, Boulder, CO; 3) Whitehead Institute for Biomedical Research, Cambridge, MA; 4) University of Georgia, Athens, GA.

Motile cilia of multi-ciliary arrays undulate in a concerted and asymmetric fashion to produce unidirectional fluid flow. Ciliary forces are resisted and translocated to the cell via radially symmetric basal bodies that remain stable in the face of constant ciliary mechanical forces. The Poc1 protein stabilizes such asymmetric forces by organizing and maintaining the radially symmetric triplet microtubules by linking neighboring triplet microtubules. Specific triplet microtubules at the posterior side of the basal body are preferentially lost in poc1Δ basal bodies. This suggests that asymmetric ciliary beating may contribute increased forces on specific triplet microtubules. We find the Poc1 interacting protein, Fop1 to also stabilize basal bodies. Interestingly, Fop1 localizes asymmetrically to the basal body microtubules that preferentially disassemble in poc1Δ and that are predicted to experience high ciliary forces. Finally, Poc1 and Fop1 alter the level of microtubule glutamylation at triplet microtubules, a modification that is predicted to stabilize microtubules. Like Fop1, basal body microtubule glutamylation is asymmetrically localized and, indeed, required for basal body stabilization. However, Poc1, Fop1 and triplet microtubule post-translational modifications appear to act in separate, but redundant, pathways to stabilize basal bodies against asymmetric ciliary forces.

C62 Organ sculpting in the Drosophila ovary. *D. Bilder*. University of California, Berkeley, Berkeley, CA.

My lab uses Drosophila to investigate the biology of epithelia, the fundamental cell type of metazoan animals. Our interests range from the cell biology of apicobasal polarity to exploiting the fly in order to model mammalian cancer and its associated lethality. In this talk I will focus on our work concerning tissue morphogenesis, exploring how genetic programs create physical forces to specify the distinct organ forms that underlie their functions. Our system is the egg chamber of the Drosophila ovary, a simple acinus-like organ that undergoes elongation coincident with a remarkable tissue-wide rotation. I will discuss our work which combines genetic approaches with imaging and biophysical tools to address issues of microtubule-based symmetry-breaking, planar-polarized cellular organization, and ECM-directed morphogenesis.

C63 Answer to the Puzzle: Why *Paramecium* Pawn Mutants Cannot Swim Backward. *J. L. Van Houten*, J. Yano, S. Lodh, M. S. Valentine. Univ Vermont, Burlington, VT.

More than forty years ago, important new mutants of *Paramecium tetraurelia* were named Pawns (*pw)* for the chess piece because they had lost the ability to swim backward (Kung, Chang et al. 1975). Subsequently they were shown to lack the calcium conductance of the voltage gated calcium channels (Ca_V), which have been shown by electrophysiology to be limited to the ciliary membrane. Otherwise, pawns have normal conductances (Satow and Kung 1974). The genes for *pwA* and *pwB* were identified by complementation 25 years later, but they did not appear to code for channels or subunits. The channels were also elusive until 2012 when we identified three Ca_V1 in the ciliary membrane proteome. We cloned, epitope tagged and expressed these very large (>250 kd) proteins, and showed that they are in the ciliary membrane. These advances allowed us to demonstrate by RNAi that these channels were responsible for most if not all of the backward swimming in depolarizing conditions. This in turn set the stage for answering the question of why *pwA* and *pwB* mutants cannot swim backward. When we express tagged Ca_V1 a, b, or c in wild type cells, these channels can be found in the ciliary membrane. When these tagged proteins are expressed in *pwA* or *pwB* cells, these channels are not detected in the ciliary membrane. However, these channels can be found again in the ciliary membranes, when these mutants are "rescued" with the wild type copy of their mutant gene. Therefore, *pwA* or *pwB* mutants do not swim backward because they do not have Ca_V channels in their cilia where they must reside in order to generate the action potential that drives backward swimming.

Kung, C., S. Chang, Y. Satow, J. Van Houten and H. Hansma (1975). "Genetic dissection of behavior in *Paramecium*." Science **188**: 898-904.
Satow, Y. and C. Kung (1974). "Genetic dissection of active electrogenesis in *Paramecium aurelia*." Nature **247**: 69-71.
Acknowledge P20GM103449.

C64 Mitochondrial contributions to behavioral and developmental phenotypes in *Paramecium tetraurelia*. *W. E. Bell*, E. A. Thompson, O. M. Emery. Virginia Military Institute, Lexington, VA.

Mitochondria have long been recognized as the primary energy conversion organelle in Eukaryotic cells. More recent studies have illuminated other critical processes in cellular development that involve mitochondria, specifically roles in programmed cell death and cell division. Ciliates have a long history of contributing to our understanding of cellular processes including chemosensory behavior, genetic reorganization, calcium regulation, and ciliary function. Little work has been done on assessing the role of mitochondria in the cellular dynamics of Ciliates. *Paramecium* are excitable cells whose swimming behavior is regulated by membrane potential. Backward swimming responses can be initiated by depolarizing the cell with an increase in external K^+ added to the bathing solution. We have identified a ryanodine-sensitive calcium flux in *Paramecium* that can be observed under such depolarizing conditions. When *Paramecium* were treated with ryanodine receptor antagonists, backward swimming responses initiated by K^+ depolarization were significantly reduced, but not eliminated. Our attempts to localize these channels led not to the endoplasmic reticulum (the expected site of ryanodine receptor/channels) but to mitochondria. Increasing evidence suggests that ryanodine sensitive channels can be found in mitochondria. In addition, recent sequence analysis of the *Paramecium* genome reveals several channels with conserved ryanodine domains, although none with significant overall homology. Since Ca^{2+} is a mediator of the backward swimming response, we used the mitochondrial calcium-sensitive dye Rhod-2 to assess mitochondrial calcium contributions to depolarization. During a K^+ – induced depolarization, Rhod-2 fluorescence in the mitochondria decreased by approximately 30%. When cells were treated with the ryanodine receptor antagonist dantrolene prior to depolarization, the loss of calcium from mitochondria was significantly attenuated. We conclude that mitochondrial calcium stores can extend backward swimming responses in *Paramecium*. We have also utilized MitoTracker dyes to observe mitochondrial phenotypes displayed during autogamy and autophagy in *Paramecium*. Disruption of mitochondrial outer membrane proteins result in increased autophagy and disruption of cell division in *Paramecium*.

C65 Role of Aurora Kinases in Single-Cell Regeneration of *Stentor*. *A. Lin*, W. Marshall. UCSF, San Francisco, CA.

Single cells are capable of developing complex patterns and shapes, but the mechanism by which cells develop shape is largely unknown. *Stentor coeruleus* is a classical model system to study the development and regeneration of cell shape due to the large size ($1mm^3$), the presence of distinct cortical features that define body axes, and most importantly due to the fact that we can surgically manipulate the cells and visualize their regeneration. When the oral apparatus is regenerated in *Stentor*, the macronucleus undergoes shape changes identical to those that occur during cell division at the point in division when a new oral apparatus is formed. This observation has suggested that the timing of distinct steps of oral apparatus formation might be regulated by the same molecules that regulate the timing of division, possibly suggesting that mitosis mechanisms are integral to the processes of regeneration. To study whether there is a connection between regeneration and mitosis, we looked at the role of Aurora kinases in regeneration. Aurora kinase A (AurkA) is a kinase known to regulate spindle assembly. Aurora kinase B (AurkB) is known to guide kinetochore attachment to the spindle. We studied the role of AurkA and AurkB in single-cell regeneration using Aurora kinase inhibitors. We have observed that AurkA inhibitor, MLN8237 and VX-680, accelerates regeneration. Surprisingly, AurkA and AurkB inhibitor, PF03814735, suppresses regeneration entirely. Thus, we show that Aurora kinases are involved in single cell-regeneration and a link between regeneration and mitosis.

C66 Transcriptional dynamics of single-cell regeneration in the ciliate *Stentor coeruleus*. *P. Sood*[1], M. Slabodnick[2], J. G. Ruby[3], S. Reiff[1], S. Roy[4], W. Marshall[1]. 1) UCSF, San Francisco, CA; 2) UNC Chapel Hill, Chapel Hill, NC; 3) Calico, South San Francisco CA; 4) San Francisco State University, San Francisco CA.

The ciliate *Stentor coeruleus* is a remarkable cell–individuals can reach up to 1 mm in length and exhibit great subcellular complexity, including its beautiful wineglass shape. *Stentor* additionally has incredible regenerative abilities: almost any portion of the cell, when removed

through excision, will give rise to a normally proportioned cell with intact subcellular organization. Pioneering studies of *Stentor* elucidated morphological principles of its regeneration (Morgan, 1901; Tartar, 1961), but we know little about the molecular basis of *Stentor*'s regenerative and healing abilities. In general, little is known about regeneration and healing at the level of an individual cell. Because of its large size and its subcellular coordinate system where the anterior and posterior are clearly marked by the oral apparatus and holdfast, respectively, *Stentor* is a very useful system for understanding single cell regeneration and wound repair.

We have recently sequenced and annotated the genome of *Stentor coeruleus*, which will serve as a foundation for future molecular studies. I am currently using RNA-seq to study the transcriptional dynamics underlying the regeneration of the oral apparatus (OA), one of the most prominent features of the cell and a complex organelle composed of thousands of cilia. Upon subjecting cells to sucrose shock, the OA is induced to shed, and 8 hours later a new one forms. We show that genes are organized into five groups of gene expression with distinct temporal signatures, corresponding to the known morphological changes taking place during OA regeneration. During the earliest waves of gene expression, conserved genes involved in centriole production and ciliogenesis are expressed, necessary to replace the OA. Also during these earliest stages, genes involved in RNA metabolism are expressed, including Pumilio, suggesting the requirement for large scale transcriptome alteration through regeneration. The detailed transcriptional time course, in combination with RNAi manipulations, will further reveal the genetic networks that regulate OA regeneration, and will help elucidate the fundamental principles of cell regeneration and healing at the scale of a single cell.

C67 Quantifying HAP2-mediated cellular fusion in a sexual ciliate. *Jennifer F. Pinello*, Donna Cassidy-Hanley, Theodore G. Clark. Cornell Univ., Ithaca, NY.

Although the mechanisms underlying gamete fusion during sexual reproduction remain obscure, recent studies have implicated the conserved transmembrane protein Hapless 2 (HAP2) as an ancestral gamete fusogen. In this regard, genetic disruption of the *HAP2* locus in a variety of eukaryotic organisms, ranging from the ciliated protist *Tetrahymena thermophila*, to flowering plants and malaria parasites, has been shown to lead to a decisive block to fertilization due to the hapless cells' inability to fuse their gametic membranes. To better study the role of HAP2 in membrane fusion we developed a flow cytometry-based assay for the quantification of cellular fusion during *T. thermophila* sexual conjugation. In this assay, complementary *T. thermophila* mating types are first labeled with fluorescent cell tracers (either carboxyfluorescein diacetate succinimidyl ester or cell trace far red) and then allowed to undergo mating. When membrane fusion occurs, the cells in a mating pair actively exchange a portion of their fluorescently-labeled cytoplasmic proteins. After mating, exconjugant cells disengage enabling easy quantification of the overall percentage of cells that exchanged cytoplasm along with the relative amount of cytoplasm transferred using flow cytometry. This assay allowed us to validate our previous finding that HAP2 is necessary for membrane fusion during *T.thermophila* fertilization and provided a rapid way to test for essential functional domains in the HAP2 protein using deletion analysis. We found that disruptions of core domains within the N'-terminus severely restricted membrane fusion events between mating cells, whereas deletions at the C'-terminus had little impact on fusogenicity. Importantly, through the use of homology modelling, we identified a region within the N'-terminal ectodomain that appears to have a high degree of structural similarity to the Dengue virus envelope protein (a class II viral fusogen). This region contains a peptide loop used by the viral protein to perturb target membrane bilayers enough to initiate fusion, allowing viral entry into host cells during infection. We found that deletion of either this 'fusion loop' or the entire Dengue envelope protein homology domain in *T. thermophila* HAP2 completely eliminated cytoplasmic exchange between mating *Tetrahymena* cells. In addition to raising interesting questions regarding the evolutionary origin of HAP2, these results may provide new insights into the mechanistic function of this key developmental protein.

C68 The Role of Extracellular Microvesicles During Conjugation in *Tetrahymena thermophila*. *E. S. Cole*[1], O. Dmytrenko[1], A. Ripeckyj[1], L. Higgins[2], T. Markowski[2], T. Giddings[3], C. Ozzello[3], R. Anderson[3], M. Winey[3]. 1) St. Olaf College, Northfield, MN; 2) Center for Mass Spectrometry & Proteomics, U.MN, St. Paul, MN; 3) MCDB, Univ. Colorado, Boulder, CO.

Presenting author: Eric S. Cole[1]

Tetrahymena thermophila undergo mating that involves adhesion of two cell partners, formation of a mating junction between conjugating cells, and the reciprocal exchange of nuclei across the mating junction. Cell adhesion happens early during pair formation and is followed by cell fusion initiated at hundreds of sites in the future exchange junction. Fusion loci begin as small membrane protrusions that span the intercellular gap, make contact and fuse with membrane of the mating partner. The resulting junction pores expand to create a membrane "curtain" that accommodates bi-directional nuclear exchange. During pore expansion one observes microvesicles extruded into the extracellular space within the mating junction. This study aims to understand the role of these extracellular microvesicles (EMVs). Two hypotheses were explored: 1) EMVs could participate in membrane excavation during pore expansion; 2) EMVs could be involved in signaling between mating cells relevant to advancement of the developmental program. EMVs were isolated by disrupting mating pairs of *T. thermophila* 2-3 hours into conjugation and performing differential high-speed centrifugation. The presence of vesicles was confirmed by negative staining and transmission electron microscopy. Both RNA and protein were detected in purified EMV material. Protein identification from isolated vesicles was performed using capillary liquid-chromatography and tandem mass spectrometry. The results of proteomic analysis (specifically the presence of proteins associated with ubiquitination and formation of multivesicular bodies), were consistent with the hypothesis suggesting that EMVs participate in membrane remodeling during pore-expansion. Proteasome components, typically associated with protein recycling, were also found. Finally, we found Argonaute proteins normally associated with RNAi, suggesting that EMVs could be involved in down-regulating the small "scan" RNAs expressed from the germ-line nucleus early during meiosis. These findings suggest that EMVs represent a complex recycling mechanism targeting cytoplasmic proteins, miRNAs and membrane components during early stages of conjugation. Preliminary evidence also suggests that EMVs secreted early during early conjugation may play a role in cell signaling with

developmental consequences. We are currently pursuing RNA analysis of the EMV contents, and following up on their potential role as mediators of developmental signaling. .

C69 TetraMine and Web Apollo at Tetrahymena Genome Database. *Naomi Stover*, William Chu. Bradley University, Peoria, IL.

Tetrahymena Genome Database (TGD; Ciliate.org) has provided information on the genes, proteins, and genomes of T*etrahymena thermophila* and related ciliate species since 2004. The latest additions to the tool set at this community website are TetraMine and Web Apollo. TetraMine is built using InterMine, an open source software package developed for the integration and analysis of complex biological data. We have incorporated the data from TGD Wiki, our community annotation and data display service, into TetraMine and schedule regular updates to maintain user annotations at both sites. Web Apollo at TGD utilizes the open source Web Apollo package, which allows update of genome sequence annotations by members of the Tetrahymena research community. Web Apollo has been loaded with the final sequences produced from JCVI and is now accepting community annotations of gene start/stop sequences and intron/exon boundaries. Combining these two new tools with the existing resources at TGD, including TGD Wiki and the Student/Unpublished Research Database (SUPRDB), Tetrahymena researchers have access to the advanced search and editing techniques necessary to capture the latest annotations based on published and unpublished research. .

C70 TetraExpress™: A Breakthrough Protein Expression Technology. *Janna Bednenko*[1], Yelena Bisharyan[1], Ashot Papoyan[1], Joanna Cardarelli[1], Alka Agrawal[1], Miguel Pineros[2], Paul Colussi[1], Ted Clark[1]. 1) Tetragenetics Inc. 91 Mystic Street, Arlington, MA, 02474; 2) Robert W. Holley Center for Agriculture and Health USDA-ARS Ithaca, NY 14853.

Membrane proteins are involved in various cellular processes, and many of them serve as therapeutic targets for human diseases. Conventional (mammalian, insect, microbial) cell lines often fail to produce recombinant membrane proteins in large quantities. Tetragenetics, Inc. has developed TetraExpress™, a protein expression platform that takes advantage of the unique biology of *Tetrahymena thermophila*, to overcome the historical limitations of current recombinant systems. TetraExpress™ technology was utilized to tackle an extremely challenging task – rapid production of correctly folded and functional mammalian ion channels in *Tetrahymena* membranes or in purified form. For example, tetrameric (4 x 64 kDa) potassium voltage-gated channel Kv1.3, a therapeutic target implicated in autoimmune disorders, and a 221 kDa sodium voltage-gated channel Nav1.8, a well-validated target for pain treatment, were shown to localize on plasma membrane and in endoplasmic reticulum when expressed in *Tetrahymena*, with the levels of expression 50-100 fold higher than those observed in commercially available mammalian cell lines. Both ion channels were isolated at >90% purity, with the yield of 0.5-2 mg from a liter of culture, and reconstituted into phospholipids. Correct folding and functionality of human Kv1.3 were confirmed by binding assays with fluorescent ShK toxin and voltage clamp recordings in *Xenopus* oocytes injected with Kv1.3 proteoliposomes. Ion channel activity of purified Nav1.8 was demonstrated by electrophysiology in giant unilamellar vesicles using a Nanion Port-a-Patch system. Thus, TetraExpress™ represents an attractive platform that can advance a variety of downstream applications including therapeutic antibody discovery, drug screening, and structure-based drug design. In addition, Tetragenetics, Inc. has partnered with the Tetrahymena Stock Center at Cornell University to facilitate the application of TetraExpress™ to basic and translational research in the form of providing academic community with the opportunity to order starting material for purification of membrane, intracellular or secreted proteins.

C71 Construction of a Gateway fluorescent tagging plasmid system for integration into the *btu1-1* locus. *Jeremy Y. Tee*, Joshua J. Smith. Missouri State University, Springfield, MO.

Tetrahymena thermophila is a ciliate single-celled eukaryote that is extensively studied as a model organism, particularly in the area of chromatin remodeling and DNA repair, where key discoveries have elucidated nucleosome structure and telomerase function. Fluorescence tagging with GFP and RFP is a common way to study protein interactions in *T. thermophila* and typically makes use of recombination of the fusion gene product into the RPL29 locus (exogenous tagging system). GFP/RFP localization along with immunoprecipitation and gene expression studies can elucidate unknown interactions and uncover pathways previously uncharacterized. This project was to develop constructs that are directed to a beta tubulin 1 locus and exogenously express fluorescent-tagged genes (GFP and RFP), while maintaining compatibility with Gateway cloning technology. This will give the ability to visualize protein dynamics while inducing protein expression in live cells, easily moving genes into the plasmid and examining co-localization of tagged genes expressed in different loci. Our plasmids contain a metallothionein (MTT-1) inducible promoter and fluorescence tags of GFP or RFP. Through homologous recombination, genes will be inserted into the beta tubulin 1-1 (*btu1-1*) locus, and following transformation cells are selected by paclitaxel resistance. Of particular interest to this work are proteins involved in DNA repair, in which studying sirtuin and NER-related homologues and making comparisons with previous data provide a benchmark to judge the effectiveness of our plasmid construction.

C72 Research and Cloning of *Tetrahymena thermophila UBE2S* in an Introductory Science Lab. *E. M. Schmoll*, L. N. Allen, J. J. Smith. Missouri State University, Springfield, MO, MO.

The model organism *Tetrahymena thermophila* is used in many modern research experiments due to its simplicity and the numerous homologues that it shares with other more complex organisms. In this freshman honors course, Introduction to the Biomedical Sciences, students with little laboratory experience or background were expected to complete several original research projects throughout the semester, though the primary focus was on learning to analyze, understand, and ultimately clone genes such as *UBE2S* found in *Tetrahymena thermophila*. *UBE2S*, or Ubiquitin Conjugating Enzyme E2S, is a gene present in numerous organisms and active within several cellular processes. The gene aids in a wide variety of functions, most notably mitosis regulation and protein degradation. At the start of the lab,

students were introduced to techniques to isolate and concentrate genomic DNA from *T. thermophila*. The targeted gene was then researched through bioinformatics using several databases and molecular computational tools. This procedure was followed by amplification using polymerase chain reactions, visualization through agarose gel electrophoresis, and cloning using pENTR plasmid TOPO cloning and transformation into *E. coli*. The isolated plasmids were then screened for correct insertion through restriction enzyme digestion. A final gel electrophoresis at the conclusion of the semester confirmed the successful cloning of *T. thermophila UBE2S*. All positive clones were then cryopreserved for use in future research projects in upper division biomedical sciences labs, specifically Recombinant DNA Techniques.

C73 Characterization of the SIRT2 and SIRT3 homologs in *Tetrahymena thermophila*. *Kyle Cook*, Joshua Smith. Missouri State University, Springfield, MO.

The ciliate *Tetrahymena thermophila* contains 18 histone deacetylase (HDAC) homologs, which are responsible for removing acetyl groups from acetylated lysines on histones and other proteins. There is a class of HDACs called Sirtuins (Class III HDACs), which have been implicated in various cellular processes like cancer, diabetes, aging, apoptosis, and transcription regulation. The model organism *Tetrahymena thermophila* has 11 homologs of Sirtuins (four more than humans and other vertebrates even). The scope of this research is to investigate the genes homologous to human SIRT2 and SIRT3, Tetrahymena Histone Deacetylases (THDs) 13, 15, and 16. This study will investigate their expression levels within the cell under various conditions including genotoxic stressors, starvation, and conjugation using qRT-PCR. Localization studies will be done through cloning these genes into plasmids to encode for GFP and 2HA tags. These tagged constructs were then transformed into *T. thermophila* to be used in future studies. Characterizing the function, localization, and the proteins interacting with THD13, THD15, and THD16 could help us better understand the various roles of SIRT2 and SIRT3 histone deacetylases.

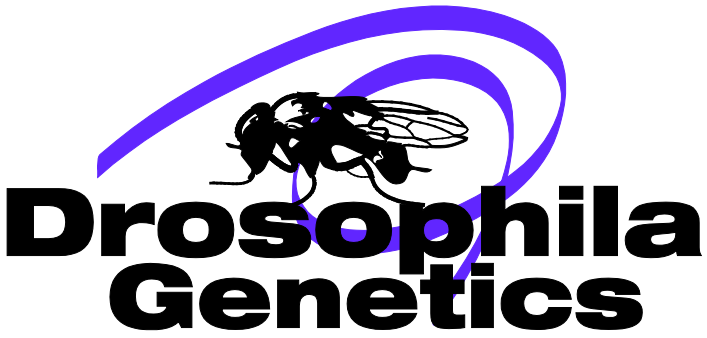
C74 Gene expression changes during infection of *Paramecium caudatum* by *Holospora undulata* bacteria. *C. Kagemann*[1], L. Bright[1], T. Doak[1], O. Kaltz[2], M. Lynch[1]. 1) Indiana University, Bloomington, IN; 2) Institut des Sciences de l'Evolution (ISEM), Montpellier, France.

Holospora is a genus of endosymbiotic bacteria that only grow and divide within one of the two nuclei of their *Paramecium caudatum* hosts. For our study, we focus on *Holospora undulata*, a species that infects the micronucleus of specific *P. caudatum* strains, while other *P. caudatum* strains are resistant. In previous studies, it was shown that the presence of *Holospora* in *Paramecium* benefits the host, especially under stress conditions: certain genes that protect *Paramecium* against both osmotic stress and excess heat are expressed in the presence of *Holospora*. At the same time, infection comes at a significant growth-rate cost to the *Paramecium* host, and we expect that there are host defense mechanisms that are activated. *Holospora* have two forms: a reproductive form and an infectious form. During infection of *P. caudatum, H. undulata* are engulfed and released from the phagosome into the cytoplasm, where they begin to move to the micronucleus. Once the *Holospora* have successfully entered the micronucleus, binary fission occurs to produce reproductive forms of the bacteria. Upon starvation, the reproductive forms transform into infectious forms until they are able to break out of the micronucleus, to find a new host. The movement of *Holospora* within the host suggests that factors such as signaling and membrane trafficking mediate *Holospora* infection. Currently we are focused on determining what genes are associated with membrane trafficking and what stages of infection the resistant strains of Paramecium are blocked at. Total mRNA (RNA seq) from infected and uninfected *Paramecium* will be sequenced to find the total set of genes that are up-regulated during various stages of *H. undulata* infection. To find the genes that are up-regulated at specific time points during infection, we are staging infections and isolating mRNA though the infection cycle.

C75 Towards the Identification of Genomic Targets of MED 31 in *Tetrahymena thermophila*. *C. ThuppuMudalige*[1], J. Garg[2], J. Lambert[3,4], A. Gingras[3], R. Pearlman[2], J. Fillingham[1]. 1) Ryerson University, Toronto, Ontario, Canada; 2) York University, Toronto, Ontario, Canada; 3) Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada; 4) University of Toronto, Ontario, Canada.

Tetrahymena thermophila is a unicellular protist that has a nuclear dimorphism with a polyploid somatic macronucleus (MAC) and a diploid germline micronucleus (MIC) within its single cell. During the vegetative growth of *Tetrahymena*, the two nuclei replicate and divide independently of each other; MIC divides by mitosis and the MAC divides by amitosis. The MIC undergoes meiosis during the sexual life cycle of *Tetrahymena* known as conjugation. The MIC is transcriptionally inactive during the vegetative state and active during meiosis while the MAC is active during vegetative growth and conjugation. The MIC genome is extensively remodeled to form the transcriptionally active MAC genome. The Med31 protein belongs to the transcriptional coactivator complex, Mediator. Med31 protein is small and highly conserved in T. thermophila and most eukaryotes at the amino acid level. While Mediator complexes are well characterized in plants, fungi and animals, it is yet to be characterized in protists. In humans and yeast, Mediator has been shown to interact with the C-terminal domain (CTD) of RPB1 subunit of RNA Polymerase II. Since T. thermophila lacks a canonical CTD in its RPB1 subunit, it raises the question whether the organism has a canonical Mediator. Affinity purification using epitope tag added to the Med 31 and subsequent mass spectrometry analysis represented a potential Mediator complex in T. thermophila. Indirect immunofluorescence has indicated that during vegetative growth, Med 31 localizes to the MAC and to the MIC during meiosis, consistent with function in transcription. To test this hypothesis, we are working towards mapping the genomic targets of Med31 in growth and development using Chromatin Immunoprecipitation combined with next generation sequencing (ChIP-Seq). ChIP is a powerful molecular method where crosslinked chromatin extracts are immune depleted of DNA-protein complexes with specific antibodies against the DNA-binding proteins. The DNA is subsequently analyzed using a variety of methods including direct sequencing. The ChIP method was initially developed for *Tetrahymena* model organism by Dedon et al. (1991) using purified MACs as starting material. We have modified the protocol to use whole cells as starting material and intend to use highthroughput sequencing to identify the genomic targets of Med31.

57TH ANNUAL DROSOPHILA RESEARCH CONFERENCE



Plenary and Platform Session Abstracts

57th Annual Drosophila Research Conference

SCHEDULE AT-A-GLANCE

Wednesday, July 13		
2:00pm-9:30pm	Speaker Ready Room Open	Hall of Cities - Anaheim
7:00pm-9:00pm	Scientific Session: Opening General Session	Crystal Ballroom M
9:00pm-11:00pm	Opening Mixer with Exhibits	Cypress Ballroom
Thursday, July 14		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities -Anaheim
7:45am-10:00am	Genetics and Determinants of Health Joint Plenary Session	Palms Ballroom
8:00am-4:00pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:30am-12:30pm	Scientific Session: Plenary Session I	Palms Ballroom Sago/Sabal/Royal
12:30pm-1:30pm	Mentoring Roundtables #1	North Tower - Harbor Beach
12:30pm-1:30pm	Speaking Up for Genetics and Model Organism Research	Crystal Ballroom H
1:30pm-3:30pm	Poster Presentations 1:30pm-2:30pm: Even-numbered posters 2:30pm-3:30pm: Odd-numbered posters	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center and Job Fair	Cypress Ballroom 1C
4:00pm-6:00pm	Concurrent Scientific Sessions: Cell Division and Growth Control Neural Development Organogenesis & Gametogenesis	 Palms Ballroom Sago Palms Ballroom Sabal Palms Ballroom Royal
4:00pm-6:00pm	Plenary Session and Workshop for Undergraduate Researchers	North Tower - Sawgrass
7:45pm-9:45pm	Concurrent Scientific Sessions: Cell Cycle and Cell Death Evolution & Quantitative Genetics I Pattern Formation	 Palms Ballroom Sago Palms Ballroom Sabal Palms Ballroom Royal
10:00pm-11:30pm	*Science Cafe Event	Palms Ballroom Sabal
Friday, July 15		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-9:30am	Concurrent Scientific Sessions: Cell Biology & Cytoskeleton Evolution & Quantitative Genetics II Chromatin & Epigenetics	 Palms Ballroom Sago Palms Ballroom Sabal Palms Ballroom Royal
8:00am-4:30pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4

** Ticketed Event*

#TAGC16

Friday, July 15 (continued)		
10:00am-12:00pm	Concurrent Scientific Session: Physiology, Organismal Growth & Aging Techniques & Resources RNA Biology	 Palms Ballroom Sago Palms Ballroom Sabal Palms Ballroom Royal
12:00pm-1:30pm	*Editor's Panel Discussion and Roundtable	North Tower - Harbor Beach
1:30pm-3:30pm	Poster Presentations 1:30pm-2:10pm: "A" poster authors present 2:10pm-2:50pm: "B" poster authors present 2:50pm-3:30pm: "C" poster authors present	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center	Cypress Ballroom 1C
2:00pm-2:45pm	GeneticsCareers Workshop - Nailing the Job Talk	Cypress Ballroom 1B
4:00pm-6:00pm	Concurrent Scientific Sessions: Cell Biology & Signal Transduction Models of Human Disease I Regulation of Gene Expression I	 Palms Ballroom Sago Palms Ballroom Sabal Palms Ballroom Royal
6:00pm-7:30pm	*Women in Genetics Panel and Networking	North Tower - Harbor Beach
7:30pm-9:30pm	Development and Evolution Joint Plenary Session	Palms Ballroom
Saturday, July 16		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-10:00am	Workshops: See topics and descriptions in the Workshop Section	Multiple locations
8:00am-12:00pm	Exhibits Open	Cypress Ballroom
8:00am-9:00am	Trainee Bootcamp Workshops: Session 1	North Tower
9:00am-10:00am	Trainee Bootcamp Workshops: Session 2	North Tower
10:00am-12:00pm	Poster Presentations 10:00am-11:00am Odd-numbered posters 11:00am-12:00pm Even-numbered posters	Cypress Ballroom *(Posters must be removed by 1pm)*
10:00am-12:00pm	GeneticsCareers Center	Cypress Ballroom 1C
10:30am-11:15am	GeneticsCareers Workshop	Cypress Ballroom 1B
12:15pm-1:45pm	*Mentoring Roundtables #2	North Tower - Harbor Beach
1:45pm-3:45pm	Concurrent Scientific Sessions: Organelles & Trafficking Models of Human Disease II Gene Expression & Chromatin	 Palms Ballroom Sago Palms Ballroom Sabal Palms Ballroom Royal
4:00pm-6:00pm	Workshops: See topics and descriptions in the Workshop Section	Multiple Locations
7:30pm-9:30pm	Concurrent Scientific Session: Immunity and Pathogenesis Neurophysiology and Behavior Stem Cells	 Palms Ballroom Sago Palms Ballroom Sabal Palms Ballroom Royal
Sunday, July 17		
7:55am -8:00am	Poster Awards Presentation	Palms Ballroom
8:00am-10:00am	Scientific Sessions: Plenary Session	Palms Ballroom
10:30am-12:30pm	Technology and its Application Joint Plenary Session	Palms Ballroom

** Ticketed Event*

D76 Image Award Presentation. *Michelle Arbeitman*. Florida State University, Tallahasse, FL.
no abstract submitted

D77 Presentation of Larry Sandler Award and Lecture. *Daniella Drummond-Barbosa*. Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.
no abstract submitted

D80 Organizing the contraction that changes tissue shape. *A. C. Martin*, F. M. Mason, J. S. Coravos. Massachusetts Institute of Technology, Cambridge, MA.

Cells generate forces in tissues to sculpt the myriad of tissue shapes that occur in an organism. An engine that is associated with cell and tissue shape changes in diverse contexts is a dynamic actomyosin meshwork or cortex underlying the plasma membrane. In epithelial cells, contraction of the apical actomyosin cortex can drive apical constriction. How apical actomyosin meshworks contract is not well understood. In particular, the organization and polarity of actin filaments in the cortex are not well defined in contractile epithelial cells. Here, we show that in constricting non-muscle cells of the Drosophila embryo, actin filament pointed ends and myosin are enriched near the center of the apical cortex and actin filament barbed ends are enriched at the junctions. Thus, the apical cortex of non-muscle cells can adopt a polarized structure that topologically resembles a muscle sarcomere. Importantly, we provide evidence that depolarized apical Rho-kinase (ROCK) activity is insufficient to contract actin networks or to promote apical constriction, suggesting that targeted myosin activation by ROCK at the center of the cell apex is required for cell shape change. Polarized ROCK accumulation requires dynamic regulation of its upstream activator RhoA, and we identified an uncharacterized RhoA GAP that is critical for establishing ROCK polarity and apical constriction. ROCK activity is continuously required to sustain its own polarity and also the junctional enrichment of E-cadherin within the apical cortex, demonstrating ROCK's key role in organizing a contractile apical cortex. In conclusion, we identified that establishing ROCK polarity and a sarcomere-like organization to the apical cortex requires a dynamic upstream signaling network that surprisingly involves, not only activation, but inhibition of the RhoA GTPase.

D81 Hox Transcription Factors and their Cell type-specific Role in Development. *I. Lohmann*. Ruprecht-Karls University, Heidelberg, Heidelberg, DE.

The highly conserved and essential class of transcription factors encoded by the *Hox* genes specifies cell fates along the anterior-posterior axis of all bilaterian animals. Besides early patterning functions, Hox proteins are also involved in the morphogenesis of various organs and in the homeostasis of cell lineages in adults. This functional diversity is achieved through a high level of transcriptional specificity, since Hox proteins trigger developmental programs with extreme spatial and temporal resolution, even at the level of single cells. One of the processes controlled by Hox proteins is feeding, a crucial behaviour of all animals, which depends on the rhythmic activity of feeding muscles stimulated by specific brain neurons. We identified the Hox transcription factor Deformed (Dfd) to be expressed and functional in specific neurons and muscles, which are essential for feeding in *Drosophila*. Using genetic, molecular, genomic and behavioural approaches we demonstrate that Dfd is required at subsequent phases in the formation of the feeding unit by directly controlling in a stage-specific manner target genes of motor neuron development, from the initial specification to the establishment of functional synapses. Furthermore, the synchronous regulation of cell adhesion molecules in motoneurons and muscles critical for feeding uncovers Hox proteins as an important factor that might guide the recognition of the interacting synaptic partners. In order to provide vigorous proof for this hypothesis, we use now cell type-specific genomic approaches (INTACT, ChIPseq, RNAseq) to identify the full complement of synaptic molecules in two tissues, the mesoderm and the CNS. By interfering large-scale with synaptic specificity molecules and Hox gene expression, we aim to formulate a Hox-based synaptic specificity code critical for motoneuronal targeting, synaptic wiring and consequently regional motor outputs. Taken together, our multi-scale approach allows us to decipher how the developmentally critical class of Hox transcription factors acquires context-dependent activity and function at the mechanistic level.

D82 Modelling Intellectual Disability Disorders in *Drosophila* - from Genes to Functional Modules and Clinical Applications. *Annette Schenck*, & laboratory. Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Nijmegen, the Netherlands.

Intellectual Disability (ID) disorders are, due to their high frequency and lifetime long expenses, the biggest unmet challenge in clinical genetics and among the largest cost factors of health care in Western countries. ID disorders are largely monogenic, and disease gene identification over the past decade has been highly successful. More than 800 causative genes ('ID genes') have been reported, providing unique stepping stones into the molecular basis of cognition. However, the role of most ID genes, particularly in the nervous system, is poorly understood. A highly efficient model organism is needed to make use of the available information and advance our knowledge in this field.

I will present a synopsis of our work in humans and *Drosophila,* which aims to identify novel pathways and mechanisms that are commonly disrupted in ID and clinically & genetically overlapping Autism Spectrum Disorders. Large-scale phenotyping in *Drosophila* revealed novel roles of ID genes, such as regulation of basal neurotransmission, synapse development and learning. Our data present experimental evidence that ID disorders converge on highly connected, evolutionarily conserved functional modules. Consistent with conserved modularity, *Drosophila* phenotype groups mirror human disease similarity, establishing *Drosophila* as a model for human phenomics. I would also like to discuss how

Drosophila can contribute to disease gene validation in the era of Next Generation Sequencing, and report on our ongoing effort to establish parallel outcome measures in human patients and *Drosophila* models to enhance the translational impact of our model.

Our goal is to provide conceptual advance in understanding of higher brain function in health and disease and to significantly contribute to the development of 'Next Generation' diagnostic and therapeutic strategies for ID and related disorders.

D83 Hippo Signaling in Growth Control and Beyond. *D. Pan*. Johns Hopkins Univ, Baltimore, MD.

The Hippo signaling pathway regulates organ size and tissue homeostasis in diverse species from *Drosophila* to mammals. This pathway comprises several tumor suppressors acting in a kinase cascade that ultimately phosphorylates the oncoprotein Yorkie (Yki) or its mammalian counterpart YAP/TAZ. Much of the recent research on Hippo signaling has focused on identifying upstream inputs into the Hippo kinase cascade, elucidating the mechanisms of Yki/YAP/TAZ in transcriptional regulation, and developing small molecule probes and inhibitors targeting the Hippo pathway. Recent work has also expanded the physiological function of Hippo signaling to many biological processes beyond growth control. I will present recent progress in these areas at the GSA meeting.

D84 Centrosomes and the Spindle Assembly Checkpoint cooperatively ensure proper growth and organization of the developing fly brain by promoting genome stability and viability of neural stem cells. *John Poulton*, John Cuningham, Mark Peifer. University of North Carolina, Chapel Hill.

Proper assembly of the mitotic spindle ensures accurate transmission of genetic material into the two daughter cells. In animals, centrosomes are the primary microtubule organizing centers of mitotic spindles. Intriguingly, most cases of human primary microcephaly (MCPH) are caused by mutations in centrosomal proteins, though the mechanism(s) by which centrosome dysfunction leads to MCPH remains unclear. Although most cells can build mitotic spindles in the absence of centrosomes, we previously demonstrated that acentrosomal epithelial cells of the fly wing disc are prone to mitotic errors and subsequent cell death. In contrast, reports indicate that acentrosomal cells of the developing fly brain do not suffer significant mitotic error or cell death. We therefore explored the underlying basis for the ability of fly brain cells to tolerate centrosome loss. Our data indicate that one reason brain cells avoid detrimental consequences of centrosome loss is the activity of the Spindle Assembly Checkpoint (SAC), which delays anaphase until microtubule-kinetochore attachments form. We found that loss of both centrosomes and the SAC (*mad2,sas-4* double-mutant) disrupts brain development, resulting in dramatically reduced brain size, disorganized architecture, and loss of neural stem cells. Importantly, either single-mutant brains are essentially normal in size and appearance. We also found that cells of these double-mutant brains experience significant increases in mitotic error (~80% of cells are aneuploid/polyploid). Double-mutant brains displayed dramatic increases in rates of DNA damage and cell death, including apoptosis of neural stem cells. Cell death appears to be an important contributor to reduced brain size in these animals, as blocking apoptosis partially rescues double-mutant brain size. Together, our data suggest epithelial cells of the wing imaginal disc are sensitive to mitotic perturbation and quickly eliminated by apoptosis, perhaps because they can be easily replaced. However, irreplaceable central brain neuroblasts may have evolved robust checkpoints to ensure greater tolerance for cellular insults such as aneuploidy.

Drosophila models of microcephaly have been limited because mutations in most fly homologues do not dramatically disrupt brain development. Our data indicate the SAC is one reason fly brains do not mirror the microcephaly observed in humans. This genetic model can now be used to test additional hypotheses regarding the roles of centrosomes and mitotic fidelity during brain normal development and the pathology of microcephaly.

D85 The GATOR2 Complex Uses TORC1 Dependent and Independent Pathways to Regulate Cellular Metabolism. *W. Cai*, Y. Wei, M. Jarnik, J. Reich, M. Lilly. NICHD, National Institute of Health, Bethesda, MD.

TORC1 is a master regulator of metabolism in eukaryotes that responds to multiple upstream signaling pathways. The GATOR complex is a newly defined upstream regulator of TORC1 that contains two sub-complexes, GATOR1, which inhibits TORC1 activity in response to amino acid starvation and GATOR2, which opposes the activity of GATOR1. While the GATOR1 complex has been implicated in a wide array of human pathologies including cancer and hereditary forms of epilepsy, the *in vivo* relevance of the GATOR2 complex remains poorly understood in metazoans. We have defined the *in vivo* role of the GATOR2 component Wdr24 in *Drosophila*. Using a combination of genetic, biochemical, and cell biological techniques we demonstrate that Wdr24 has both TORC1 dependent and independent functions in the regulation of cellular metabolism. Through the characterization of a null allele, we show that Wdr24 is a critical effector of the GATOR2 complex that promotes the robust activation of TORC1 and cellular growth in a broad array of *Drosophila* tissues. Additionally, epistasis analysis between *wdr24* and genes that encode components of the GATOR1 complex revealed that Wdr24 has a second critical function, the TORC1 independent regulation of lysosome dynamics and autophagic flux. Notably, we find that two additional members of the GATOR2 complex, Mio and Seh1, also have a TORC1 independent role in the regulation of lysosome function. These finding represents a surprising and previously unrecognized function of GATOR2 complex components in the regulation of lysosomes. Taken together our data support the model that Wdr24 is a key effector of the GATOR2 complex, required for both TORC1 activation and the TORC1 independent regulation of lysosome acidification and autophagic flux.

D86 Vamana couples Fat signaling to the Hippo pathway. *J. R. Misra*[1,2], K. D. Irvine[1,2]. 1) Howard Hughes Medical Institute; 2) Waksman Institute, Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ.

Coordinated Growth and morphogenesis is critical to development of tissues of specific size and shape. The protocadherins Dachsous (Ds) and Fat initiate a signaling pathway that regulates both growth, through regulation of Hippo signaling, and morphogenesis, through regulation of planar cell polarity (PCP). Ds-Fat signaling regulates Hippo signaling and PCP by controlling the membrane localization of the atypical myosin

Dachs. Many studies have provided important insights both into how Dachs influences Hippo signaling, and how it influences PCP. In contrast, the mechanism by which Fat signaling actually controls Dachs has remained less well understood. Here we report isolation and characterization the *vamana (vam)* gene as playing a crucial role in regulating membrane localization of Dachs, and in linking Fat to Dachs. Loss of *vam* function decreases growth, whereas overexpression of *vam* promotes growth. These effects are mediated through regulation of the Hippo pathway. Epistasis experiments indicate that *vam* functions genetically downstream of *fat*, as *vam* mutations can suppress lethality, overgrowth and PCP phenotypes elicited by loss of *fat*. Vam localizes to the apical region of epithelial cells in a polarized manner, co-localizing with Dachs, and is required for proper membrane localization of Dachs. Vam physically interacts with both the carboxy-terminal domain of Dachs, and with a region of the Fat intracellular domain that is essential for controlling Hippo signaling and levels of Dachs. Structure-function analysis of Vam argue that Fat negatively regulates Dachs by displacing Vam from the membrane. Our findings establish Vam as a crucial component of the Dachsous-Fat pathway and identify a mechanism for transmission of Fat signaling.

D87 Genetic regulation of cell-fate plasticity in *Drosophila* imaginal discs. *M. I. Worley*, I. K. Hariharan. Univ California, Berkeley, Berkeley, CA.

During the development of an organism, the continued maintenance of determined cell fates is contingent upon the stability of networks of gene expression. Classical experiments using disc transplantation by Ernst Hadorn discovered the phenomenon of transdetermination in regenerating *Drosophila* imaginal discs. Damaged discs do not always regenerate the appropriate structures, and occasionally generate tissue appropriate for other discs or other parts of the same disc. Transdetermination results when cells with one determined state switch to a different determined state. Some signaling pathways, including Wg and Dpp have been shown to be important for this process. However, the underlying events that cause damage-induced transdetermination remain largely unknown, including the genes that regulate this process and the source of cells that change fate.

To address this question, we have conducted a deficiency screen for modifiers of regeneration that lead to the appearance of inappropriate structures following the ablation of the wing pouch by the temporally and spatially controlled expression of the TNF-alpha homolog *eiger.* Using this screen, we identified multiple loci that significantly increase the frequency of transdetermination. From one such locus, we identified the responsible gene as *C-terminal Binding Protein* (*CtBP*), which encodes for a transcriptional co-repressor that interacts with many different transcription factors. Inactivating mutations in the *CtBP* gene result in a high frequency of notum-to-wing transdetermination following damage, indicating that *CtBP* functions to maintain cell-fate identity during imaginal disc regeneration. The notum-to-wing fate change occurs following the generation of a secondary or ectopic blastema in the notum that is dependent on JNK activity. The new wing pouch arises from multiple cells and grows near the boundary that separates the anterior and posterior compartments, which is reminiscent of normal wing pouch development and suggests that cells at specific locations of the notum are more prone to undergo transdetermination into wing-pouch fated cells. In addition, during transdetermination multiple cells of the notum can also re-specify their compartmental identities, which under normal conditions are stably maintained. We have also observed that notum-to-wing transdetermination is significantly enhanced by increased expression of the cytokine Unpaired, and suppressed by reducing STAT activity, thereby demonstrating a role for the JAK/STAT pathway in triggering cell-fate plasticity. Taken together, our studies indicate that CtBP stabilizes cell fates and limits cell plasticity during regeneration and, especially when CtBP levels are reduced, damage-induced activation of JNK and JAK/STAT signaling can promote cell-fate plasticity and respecification.

D88 Oxidative Stress in Oocytes During Mid-Prophase Induces Premature Loss of Cohesion and Chromosome Segregation Errors. *A. T. Perkins*, T. M. Das, E. M. Morse, S. E. Bickel. Dartmouth College, Hanover, NH.

In humans, errors in meiotic chromosome segregation that produce aneuploid gametes increase dramatically as women age, a phenomenon termed the maternal age effect. During meiosis, cohesion between sister chromatids keeps recombinant homologs physically attached and premature loss of cohesion can lead to missegregation of homologs during meiosis I. A growing body of evidence suggests that meiotic cohesion deteriorates as oocytes age and contributes to the maternal age effect. One hallmark of aging cells is an increase in oxidative damage caused by reactive oxygen species (ROS). Therefore, increased oxidative damage in older oocytes may be one of the factors that lead to premature loss of cohesion and segregation errors. To test this hypothesis we used a Gal4/UAS RNAi strategy to induce oxidative stress in Drosophila oocytes and measured the fidelity of chromosome segregation during meiosis. Knockdown of the ROS scavengers SOD1 (cytoplasmic) or SOD2 (mitochondrial) caused a significant increase in segregation errors. This increase occurred primarily because recombinant chromosomes missegregated during meiosis I at a significantly greater frequency, consistent with oxidative damage causing premature loss of meiotic cohesion. FISH analysis confirmed that arm cohesion is disrupted in SOD knockdown genotypes. Together these results provide the first *in vivo* demonstration that oxidative stress during meiotic prophase induces chromosome segregation errors and support the model that accelerated loss of cohesion in aging human oocytes is caused, at least in part, by oxidative damage.

D89 Yorkie, a transcriptional co-activator that regulates growth, also functions at the cell cortex to promote cytoskeletal tension. *Jiajie Xu*[1], Pamela Vanderzalm[2], Ting Su[1], Misha Ludwig[1], Richard Fehon[1]. 1) University of Chicago, Chicago, IL; 2) John Carroll University, University Heights, OH.

Yorkie (Yki) is well known as a transcriptional co-activator that functions downstream of the Hippo pathway to positively regulate transcription of genes that promote tissue growth. Recent studies have shown that increased cytoskeletal tension activates both Yki and YAP (a mammalian orthologue of *Drosophila yki*), resulting in increased nuclear localization and tissue growth. To better understand the effects of tension, as well as upstream pathway activity, on Yki function in living tissues, we generated a tagged *yki* transgene that is expressed at

endogenous levels. Using this Yki reporter, we find that tension, generated either mechanically or genetically, results in increased nuclear Yki accumulation in the wing epithelium. Unexpectedly, we also find that tension induces Yki to accumulate in the cell cortex at the apical junctional region (AJR) in live cells. To ask if Yki might have a previously unrecognized, non-transcriptional function at the cell cortex, we added a myristoylation signal to the Yki N-terminus to tether Yki to the membrane. Remarkably, when expressed transgenically in the wing myristoylated Yki promotes cytoskeletal tension and folding in the epithelium by activating the *Drosophila* myosin regulatory light chain Spaghetti squash (Sqh). In addition, we found that activating Yki by genetically inactivating the Hippo pathway also causes Yki to accumulate at the AJR and increases Sqh activity. Conversely, depletion of *yki* using RNAi resulted in reduced Sqh activity. Based on these results, we suggest that active Yki functions in a feed forward 'amplifier' loop that promotes cytoskeletal tension, and thereby greater Yki activity, in response to tension such as that generated in the peripheral regions during imaginal disc growth. We are testing this hypothesis by generating *yki* alleles that lack the ability to activate Sqh, and are also dissecting the molecular mechanism underlying this novel function of Yki.

D90 Notch signaling promotes cell proliferation and controls cell identity in developing imaginal ring cells. *S. Yang*, W. Deng. Florida State University, Tallahassee, FL.

Imaginal rings are larval tissues composed of precursor cells that are essential for the formation of adult foreguts, hindguts and salivary glands. Imaginal ring cells are specified from subsets of the ectoderm at embryonic stage. These cells kept quiescent until late second instar and then undergo rapid cell proliferation during the third instar to attain adequate number of cells that will replace apoptotic larval tissues for adult organ formation. Therefore, cell specification, cell identity maintenance and cell proliferation are tightly associated with the development of imaginal rings, but the regulatory mechanisms are poorly understood. Here, we show that Notch signaling is activated in all three imaginal rings from middle embryonic stage to early pupal stage. Our mutant clonal analysis, knockdown and gain-of-function studies indicate that canonical Notch signaling positively is required and sufficient for cell proliferation in all three imaginal rings during the third instar. We also found that Serrate (Ser) but not Delta (Dl) from neighboring cells is the ligand for Notch activation. Ser predominantly *trans*-activates Notch signaling, but also plays a role in *cis*-inhibition to modulate the level of Notch activity. In addition, we demonstrate that Notch signaling is required for cell specification of all imaginal ring cells from primordium at middle embryonic stage and maintains imaginal ring cell identity during first and second instars. Taken together, our studies indicate that Notch acts as a growth-promoting and development-regulating signal in *Drosophila* larval precursor cells, and that imaginal rings are excellent *in vivo* models to decipher how Notch regulates cell proliferation and organogenesis.

D91 Regulating the regulator of cell cycle, Xpd. *R. NAG*, S. NIGGLI, S. GUIMARAES, B. SUTER. UNIVERSITY OF BERN, BERN, CANTON OF BERN, CH.

Xpd, a DNA helicase, classically known to play a role in transcription and nucleotide excision repair, has also been shown to be involved in cell cycle regulation in young Drosophila embryos (Chen et al., 2003, Li et al., 2010). Xpd has been shown to perform its functions as part of the TFIIH, the Cdk Activating Kinase (CAK) complex and the Crumbs/Galla-2 complex (Yeom et al., 2014). In our study of early Drosophila embryos, we report the interaction of Xpd with the Mms19 protein, which together form a complex consisting of Xpd/Mms19/Galla-2. Further investigations of the *mms19* gene revealed that the knockdown of the maternal *mms19* product in early embryos causes mitotic defects. We observed mainly chromosome segregation defects and spindle abnormalities. Interestingly, homozygous *mms19* mutants fail to develop imaginal discs during the larval stages, a typical phenotype of a loss-of-function mutation in a mitotic gene. Epistasis studies conducted in these larvae led to the rescue of some of the *mms19* phenotypes by the over expression of CAK. Our research indicates that the interactions of Xpd with Mms19 and/or Cdk7 at different stages of the cell cycle are crucial for the cell to undergo normal cell divisions.

D92 Formin3 regulates dendritic architecture via microtubule stabilization and is required for somatosensory nociceptive behavior. *R. Das*[1], A. A. Patel[1], H. M. Bobo[1], J. M. Harris[1], I. Foldi[2], S. Nanda[3], G. A. Ascoli[3], J. Mihaly[2], D. N. Cox[1]. 1) Georgia State University, Atlanta, GA; 2) Institute of Genetics, Szeged, Hungary; 3) George Mason University, Fairfax, VA.

The cytoskeleton is a defining component of eukaryotic cells including neurons and constitutes the foundation of their inner architecture. Despite many significant advances, it is not yet clear how genetically encoded growth rules are dynamically expressed through the local molecular interactions of cytoskeletal components driving dendritic arborization. Cell-type specific dendritic morphologies emerge via complex growth mechanisms modulated by intrinsic signaling involving transcription factors that mediate neuronal identity, as well as functional and morphological properties of the neuron subtype. Thus, in pursuit of identifying a convergent nodal point of combinatorial transcription factor regulation and modulation of the actin and microtubule cytoskeletons in *Drosophila* dendritic arborization (da) sensory neurons, we found that Formin3 (Form3) is a target of both Cut and Knot, two key transcription factors known for their modulatory role of cytoskeletal components. To investigate the role(s) of *form3* in dendritic development, we conducted *in vivo* neurogenetic analyses of loss-of-function mutants, together with Form3 overexpression analyses and immunohistochemistry (IHC) studies of Form3 localization. Intriguingly, among the six *Drosophila* Formins, only disruptions in *form3* function elicit strong defects on dendritic development, whereas disruption of the other five fly Formins had mild or no effect on class IV (CIV) dendrite morphogenesis. We demonstrate that Form3 is differentially expressed in da neuron subclasses and functions cell-autonomously in CIV neurons to stabilize distal higher order branching along the proximal-distal axis of dendritic arbors. Furthermore, live confocal imaging of cytoskeletal multi-fluor reporters and fixed tissue IHC analyses reveal that *form3* mutation leads to collapse of the microtubule (MT) cytoskeleton. Biochemical analyses indicate Form3 directly interacts with MTs via the FH1/FH2 domains. Form3 is predicted to interact with two ATAT1 alpha-tubulin N-acetyltransferases suggesting it may promote MT stabilization via acetylation. Analyses of acetylated dendritic MTs supports this hypothesis as defects in *form3* lead to reductions, whereas overexpression promotes increases in MT acetylation. Neurologically, *INF2* (the human ortholog of *form3)* mutations have been demonstrated to be causative

for dominant intermediate Charcot-Marie-Tooth (CMT) disease E. CMT sensory neuropathies lead to distal sensory loss resulting in a reduced ability to sense heat, cold, and pain. Intriguingly, disruption of Form3 function in CIV nociceptive neurons results in a severe impairment in nocifensive behavior in response to noxious heat suggesting that *INF2* and *form3* may share a primordial function in regulating nociception.

D93 Heparan sulfate proteoglycans promote dendritic growth of *Drosophila* sensory neurons through receptor protein tyrosine phosphatase Ptp69D. *Amy Poe*, Lingfeng Tang, Bei Wang, Chun Han. Weill Institute for Cell and Molecular Biology, Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Dendrite morphogenesis is essential for the formation of neural circuitry. As dendritic architectures profoundly affect neuronal functions, establishing cell type-specific dendrite morphologies is critical for diverse functions of neurons. A key question in dendrite morphogenesis is how extracellular cues direct dendritic patterning. In particular, many so-called "space filling" neurons have the capacity to extensively and uniformly cover the receptive field. Whether these neurons are self-sufficient in innervating their target fields or they rely on extracellular permissive signals is unknown. To answer this question, we use *Drosophila* class IV dendritic arborization (da) neurons as a model system. The dendrites of class IV da neurons completely and non-redundantly cover the larval epidermis. Using RNA interference (RNAi) in epidermal cells, we discovered that the innervation of receptive fields by class IV da neurons require epidermis-derived heparan sulfate proteoglycans (HSPGs), a type of extracellular or membrane-associated glycoproteins. This suggests the existence of an extracellular permissive signal for the dendritic growth of class IV da neurons. Our loss-of-function (LOF) analyses of HSPG genes demonstrated that Syndecan (Sdc) and Dally act redundantly to promote the local growth of high order dendritic branches. By conducting time-lapse imaging, we found that HSPGs are not required for short-term dynamics of terminal dendrites but are necessary for stabilizing dendritic microtubules. Rather than promoting dendritic growth by binding to secreted ligands, our data suggest that HSPGs themselves may serve as the permissive signal. Supporting this hypothesis, we found that receptor protein tyrosine phosphatase Ptp69D, a HSPG receptor expressed in class IV da neurons, is required for the growth of and the microtubule stabilization in class IV da dendrites. Together, our results demonstrate that HSPGs are permissive signals for dendritic growth of space-filling class IV da neurons.

D94 *nejire*-mediated transcriptional regulation of dendritic growth and arborization complexity. *S. G. Clark*, A. A. Patel, J. Lott, S. Bhattacharya, D. N. Cox. Georgia State University, Atlanta, GA.

nejire (*nej*), the *Drosophila* homolog of CREB Binding Protein, has been studied as a regulator of embryonic pattern formation and of circadian rhythms in adult *Drosophila*, however its potential role in dendritic development remains largely unexplored. In a genetic screen for transcriptional regulators of dendritic development in complex arbor neurons, we identified *nej* as a novel regulator of dendritic development. Here, we investigate the role of *nej* as an essential regulator of dendritic growth and branching complexity using dendritic arborization (da) sensory neurons of the *Drosophila* larva. Loss-of-function studies implicate *nej* in regulating dendritic growth and proximal-distal branch order distribution as disruptions lead to a reduction in dendritic field coverage and a shift from distal terminal branching complexity to more proximal dendritic tufting in class IV (CIV) da neurons. Conversely, *nej* overexpression in CIV neurons leads to severe disruption in higher order dendritic growth and branching whereby nearly all branches above second order are absent. To identify putative functional domains required for *nej*-mediated arbor development, domain-specific deletion constructs were expressed in CIV neurons revealing that deletion of the N-terminal region (ΔNZK) leads to defects that largely phenocopy those observed with *nej* knockdown, whereas deletion of the C-terminal region (ΔQ) generates phenotypic defects consistent with overexpression of full-length *nej*, albeit even more severe. To gain further insight into *nej*-mediated signal transduction in dendritic development, we have investigated putative *nej* interacting molecules. These analyses reveal a regulatory relationship between *nej* and *dar1*, a transcription factor known to affect dendritic growth by regulation of the microtubule severing protein Spastin. *dar1* overexpression produces a phenotype that is strikingly similar to that of *nej* knockdown and changes in *nej* expression cause shifts in the subcellular localization of Dar1 in CIV neurons. We also demonstrate a potential relationship between *nej* and *shaggy* (GSK3), which is known to affect microtubule dynamics in axons, as the expression of a kinase-dead *shaggy* results in CIV dendritic morphology that is phenotypically nearly identical to that caused by expression of the *nej* ΔNZK construct. Furthermore, our analyses suggest potential links to the NF-κB pathway, as *relish* overexpression phenocopies *nej* knockdown and *relish* knockdown causes a reduction in branching complexity that is phenotypically consistent with *nej* overexpression. Collectively, these analyses extend our understanding of molecular mechanisms of transcription factor activity at a class-specific level and how this regulation contributes to specification of distinct neuronal morphologies that underlie the establishment of complex neural networks.

D95 A Functionally Conserved Gene Regulatory Network Module Governing Olfactory Neuron Diversity. *S. Barish*[1], Q. Li[2], S. Okuwa[1], A. Maciejewski[1], A. Brandt[3], D. Reinhold[4], C. Jones[3], P. Volkan[1]. 1) Duke University, Durham, NC; 2) Stanford University, Palo Alto, CA; 3) University of North Carolina, Chapel Hill, NC; 4) Clark University, Worcester, MA.

Drosophila uses 50 different olfactory receptor neuron (ORN) classes that are clustered in combinations within distinct sensilla subtypes to decipher a complex chemical environment. Each sensilla subtype houses 1-4 ORN identities that arise through asymmetric divisions from a single multipotent sensory organ precursor (SOP). How each class of SOPs acquires a unique differentiation potential that accounts for ORN diversity is unknown. Here, we show that Rn, along with BarH1/H2 (Bar), Bric-à-brac (Bab), Apterous (Ap) and Dachshund (Dac), is part of a conserved proximodistal (PD) gene regulatory network module that patterns the antennal disc into seven concentric rings and diversifies SOP identities. Each ring expresses a unique combination of the aforementioned transcription factors, and encodes the differentiation potentials for a limited number of sensilla subtypes. Genetic perturbations of the network lead to predictable changes in ORN diversity. These data suggest that the diversification of precursor fields by the prepatterning network is the first step to neuronal diversification, followed by SOP selection

by proneural genes, and Notch-mediated neurogenesis. As each step operates in a context-dependent manner, deployment of the same transcription factor network module may regulate neuronal diversity in parallel systems with completely different fate outputs.

D96 Developmental programs and olfactory receptor signaling in structural and functional development of *fruitless* positive olfactory neurons. *P. C. VOLKAN*, C. E. HUESTON, D. OLSEN, Q. LI, S. OKUWA, J. WU, B. PENG. DUKE UNIVERSITY, DURHAM, NC.

During development, sensory neurons must choose identities that allow them to detect specific signals and connect with appropriate target neurons. Ultimately, these sensory neurons will successfully integrate into appropriate neural circuits to generate defined motor outputs, or behavior. This integration requires a developmental coordination between the identity of the neuron and the identity of the circuit. The mechanisms that underlie this coordination are currently unknown. Here we describe two modes of regulation that coordinate the sensory identities of Drosophila melanogaster olfactory receptor neurons (ORNs) involved in sex-specific behaviors with the sex-specific behavioral circuit identity marker fruitless (fru). The first mode involves a developmental program that coordinately restricts to appropriate ORNs the expression of fru and two olfactory receptors (Or47b and Ir84a) involved in sex-specific behaviors. This regulation requires the chromatin modulatory protein Alhambra (Alh). The second mode relies on the signaling from the olfactory receptors through CamK and histone acetyl transferase p300/CBP to maintain ORN-specific fru expression. Our results highlight two feed-forward regulatory mechanisms with both developmentally hardwired and olfactory receptor activity-dependent components that establish and maintain fru expression in ORNs. Such a dual mechanism of fru regulation in ORNs might be a trait of neurons driving plastic aspects of sex-specific behaviors.

D97 Inhibition of mitochondrial calcium entry in mushroom body neurons during pupariation causes memory impairment and neuronal structural defects in adult flies. *Ilaria Drago*, Ronald Davis. The Scripps Research Institute-Scripps Florida, Jupiter, FL.

Mitochondria are one of the key players in cellular calcium signaling. Upon an increase in cytoplasmic calcium concentration, the mitochondrial calcium uniporter protein complex is activated allowing calcium to enter the mitochondrial matrix. Mitochondrial calcium entry is critical to a plethora of cellular processes including cytoplasmic calcium buffering, regulation of ATP production, apoptosis and autophagy (Kamer and Mootha, 2015).

Components of the mitochondrial calcium handling machinery were found among the hits of a pan-neuronal RNAi screen performed to discover new genes that are required for normal olfactory memory formation in *Drosophila* (Walkinshaw et al., 2015). Subsequent olfactory conditioning experiments showed that inhibiting mitochondrial calcium entry specifically in the mushroom bodies (MB) causes a memory impairment without altering the ability of the flies to learn. Surprisingly, impairing mitochondrial calcium entry in MB only during pupariation - but not during earlier developmental stages or adulthood- recapitulates the phenotype observed when mitochondrial calcium entry is impaired both during development and adulthood. Global structural analyses of the MB in flies where the mitochondrial calcium uniporter is silenced failed to reveal any gross structural defect. Nevertheless, a more detailed analysis revealed that the number of synaptic vesicles in the MB of these flies is significantly decreased when compared to control flies. Moreover, single MB neuron analyses using photoactivatable GFP showed that the axonal length of a specific sub-population of MB neurons is increased when mitochondrial calcium entry is inhibited.

Our results uncover a novel role for mitochondria calcium handling *in vivo* and reveal a relationship between developmental mitochondrial calcium homeostasis, olfactory memory formation, and MB neuronal structure in adult flies.

D98 Syncrip regulates *prospero* stability during neuroblasts division and differentiation. *Lu Yang*[1], Francesca Robertson[1], Aino Jarvelin[1], Tamsin Samuels[1], Yoav Arava[2], David Ish-Horowicz[3], Ilan Davis[1]. 1) University of Oxford, Oxford, United Kingdom; 2) Department of Biology Technion, Israel Institute of Technology, Haifa, Israel; 3) MRC for Molecular Cell Biology, University College London, London, United Kingdom.

Brain development depends on precise regulation of the balance between neural stem cell proliferation and differentiation. One of the most extensively studied regulators of neural stem cells is Prospero (Pros). In *Drosophila* larval brains, it had been demonstrated that Pros level must be precisely regulated both spatially and temporally to allow correct brain development. However, how this is achieved remains unknown. In the current study, we directly test whether differential Pros level is regulated at transcriptional or post transcriptional level by performing *in situ* using a combination of intron and exon probes against *pros.* We showed although Pros is expressed at low level in neuroblasts (NB) and gradually increase in level in NB progeny over time, *pros* mRNA is transcribed at equal level in all cells types. This suggests post-transcriptional regulation is likely to be involved in achieving the differential Pros expression. We have identified a novel interactor of *pros* mRNA - Syncrip. In *syp* mutants, we observed significantly enlarged optic lobes along with increased number of nBs and increased nB division rate. Both Pros protein and mRNA level are decreased in *syp* mutants. Surprisingly, our data showed *pros* transcription is not affected in *syp* mutants but the stability of the RNA is significantly decreased. Mapping the poly(A) site of *pros* mRNA revealed *pros* mRNA harbours two distinct 3'UTR variants, one 3 kb and one 15 kb 3'UTR with the 15 kb UTR being the predominant form. By using *in situ* probes directly against the 15 kb 3'UTR, we showed the 15 kb variant is only transcribed in mature neurons and this is accompanied by an up-regulation of Pros protein. Interestingly, both forms for *pros* mRNA are made in *syp* mutants but not maintained. From the current data, we propose a Syncrip dependent model for regulating differential Pros protein expression through choice and maintenance of 3'UTR variants.

D99 Spontaneous grooming and other activity phenotypes resulting from Neurofibromin loss of function in Drosophila. *L. B. King*[1], M. Koch[1,2], K. Murphy[1], Y. Velazquez[1], W. W. Ja[1], S. M. Tomchik[1]. 1) The Scripps Research Institute, Jupiter, FL; 2) Center for the Biology of Disease, VIB, Leuven, Belgium.

Neurofibromatosis I is a common genetic disorder that results in tumor formation and predisposes individuals to a range of cognitive/behavioral symptoms, including deficits in attention, visuospatial skills, learning, language development, sleep, and autism spectrum

disorder-like traits. The *nf1*-encoded neurofibromin protein (Nf1) exhibits high conservation, from the common fruit fly, *Drosophila melanogaster*, to humans. *Drosophila* provide a powerful platform to investigate the signaling cascades upstream and downstream of Nf1, and the fly model exhibits similar behavioral phenotypes to mammalian models. In order to understand how loss of Nf1 affects motor behavior in flies, we combined traditional activity monitoring with video analysis of grooming behavior. In *nf1* mutants, spontaneous grooming was increased up to 7x. This increase in activity was distinct from previously-described dopamine-dependent hyperactivity, as dopamine transporter mutants exhibited slightly decreased grooming. Finally, we found that relative grooming frequencies can be compared in standard activity monitors that measure infrared beam breaks, enabling the use of activity monitors as an automated method to screen for grooming phenotypes. Overall, these data suggest that loss of *nf1* produces excessive activity that is manifested as increased grooming, providing a platform to dissect the molecular genetics of neurofibromin signaling across neuronal circuits.

D100 Mediator subunit *skuld* is required sex specifically for ovary development. *H. Sultana*, H. Yang, B. Oliver. NIDDK, National Institutes of Health, Bethesda, MD.

Organogenesis requires spatio-temporal regulation of gene expression. We use development of the female gonads as a model to understand gene expression regulation. In *Drosophila melanogaster*, female gonads consist of a pair of ovaries, which contain both germ cells and somatic cells. Somatic cells are indispensible for proper growth, differentiation, and sex determination of germ cells. Here we took two independent approaches to identify the genes that potentially affect germ cells nonautonomously. In the first approach, we performed a reverse genetic screen using the UAS-Gal4 system to knockdown genes in Traffic Jam positive somatic cells and screen for female sterility. We tested 1,119 genes in this screen and found 169 to be female sterile. In the second approach, we performed transcriptional profiling of the anterior ovariole consisting of germaria and round previtellogenic egg chambers, which are regions with close contact between germ cells and somatic cells. We performed RNA-Seq on 12 biological replicates of anterior ovariole and compared it to whole ovary. We found expression of 3,999 genes enriched in anterior ovariole compared to whole ovary *(Padj<= 0.05)*. Out of these 73 were female sterile in the genetic screen. We selected 67 genes for further characterization that were either female sterile in the genetic screen or were enriched in the anterior ovariole RNA-Seq experiment. Development of both testes and ovaries was affected in the knockdown of 18 genes, whereas knockdown of three genes *skuld*, *broad* and *misshapen* gave ovary specific phenotypes. *skuld (skd)* is a component of the kinase domain of the mediator complex along with *kohtalo (kto), Cyclin C* and *Cdk8*. *skd* knockdown in somatic cells of the gonad resulted in developmental arrest of the ovaries but not the testes. In L3 larval ovaries, germ cells were not present in a zone like control ovaries but were spread across the ovary suggesting a nonautonomous role of *skd* in germ cell organization. *skd* and *kto* have been shown to function in a similar context in several tissues. When we knocked down *kto* we found an effect on the development of both testes and ovaries. Both at L3 and adult stages, mutant ovaries for *skd* and *kto* looked similar. However, *kto* mutant testes were thin and sterile at 29°C unlike *skd* mutant testes, which were fertile. We cross-validated this phenotype by investigating gene expression of *skd* and *kto* in ovaries vs. testes from different publically available RNA-Seq datasets. We found significant enrichment of *skd* expression (*Padj<0.0001*) in the ovaries compared to the testes whereas *kto* is expressed at comparable levels in both male and female gonads. Our study suggests differences in function of mediator components *skd* and *kto* in gonad development and also proposes a sex specific role of *skd* in ovary development.

D101 Neuropeptide-dependent control of female germline stem cell proliferation after mating in *Drosophila melanogaster*. *T. Ameku*[1], Y. Yoshinari[1], S. Kondo[2], R. Niwa[1,3]. 1) University of Tsukuba, Tsukuba, Ibaraki, JP; 2) National Institute of Genetics, Mishima, Shizuoka, JP; 3) Japan Science and Technology Agency, Kawaguchi, Saitama, JP.

Gametogenesis and mating are two essential components of animal reproduction. Gametogenesis must be modulated by the need for gametes, yet little is known of how mating, a process that consumes gametes, may modulate the process of gametogenesis at the cellular level.

Here we report that mating stimulates female germline stem cell (GSC) proliferation via neuronal and endocrine systems in *Drosophila*. Mating-induced GSC proliferation is not simply owing to the indirect effect of emission of stored eggs, but rather is caused by a sex peptide (SP) and its receptor SPR, the canonical pathway to transduce the signal from male seminal fluid to female flies. We also identify ecdysteroid, the principal insect steroid hormone, as a key mediator of GSC proliferation after mating. Importantly, ovarian ecdysteroid biosynthesis is induced by neuronal SP signaling, suggesting that GSC proliferation is under the control of the characterized neuroendocrine system in response to an external stimulus, mating.

Previous studies and our own analysis, however, suggest that *SPR* gene is not expressed in GSC cells or GSC niche cells. Therefore, neuroendocrine mechanisms transmitting SP signals to GSCs still remain to be elucidated. To address this question, we carried out a transgenic RNAi screen to search genes that are required for transmits SP signals to GSCs. We examined GSC phenotypes in females in which a wide variety of membrane receptor genes in neurons as well as GSC niche cells have been knocked down. We identify 50 candidate genes, including 13 neuropeptide receptors, which are potentially involved in controlling mating-induced GSC proliferation. We also show that genes encoding ligands for some of the neuropeptide receptors are expressed in the midgut and are required for mating-induced GSC proliferation. Moreover, overexpression of the ligand genes are sufficient to induce GSC proliferation. These results suggest that neuropeptide signaling mediates mating-induced GSC proliferation via the hormonal interactions among different tissues. Our work reveals a novel role of neuropeptide signaling in regulating GSC proliferation that coordinates gametogenesis in response to the stimulus of mating.

In this presentation, the relationship between SP, ecdysteroid and neuropeptide in regulating GSC will be discussed.

D102 Electron Transport Chain Remodeling by GSK3 during Oogenesis Connects Nutrient State to Reproduction. *Matthew Sieber*[1], Michael Thomsen[1], Allan Spradling[1,2]. 1) Carnegie Institution for Science, Baltimore, MD; 2) Howard Hughes Medical Institute.

Reproduction in all systems is heavily influenced by nutrition and the metabolic state of the organism. Furthermore, metabolic syndrome is thought to be a major factor in a number of reproductive disorders in humans. For example, PolyCystic Ovary Syndrome (PCOS), the most common cause of infertility in women, is strongly associated with type-2 diabetes and obesity. However, despite these intimate relationships, the precise metabolic mechanisms that support reproduction remain poorly defined. Interestingly, as *Drosophila* eggs develop, nutrients accumulate in the germline in a coordinated stepwise manner. This stepwise accumulation, in conjunction with the advanced genetics available in *Drosophila*, provides an excellent system to investigate the roles of energy homeostasis during oocyte development and reproduction.

Using a combination of metabolomics, proteomics, and molecular genetics we have found that developing *Drosophila* follicles undergo a major metabolic transition after stage 10 that leads to massive glycogen accumulation. This transition leads to shift in the balance of glycolysis and gluconeogenesis that results in the build up of several glycolytic/gluconeogenesis intermediates as well as a subset of TCA intermediates. Intriguingly, this shift in carbohydrate metabolism is the result of germline mitochondria entering into a state of respiratory quiescence in the late stages of oogenesis. Mitochondria enter respiratory quiescence as a result of a dramatic remodeling of the electron transport chain (ETC). ETC remodeling and mitochondrial quiescence are triggered by a reduction in insulin/akt signaling, and mediated by glycogen synthase kinase 3 (GSK3). Using proteomic analysis of isolated germline mitochondria we have found that GSK3 induces respiratory quiescence by promoting a global shift in mitochondrial protein content. Intriguingly, we observed a similar glycogen and ETC remodeling in Xenopus oocytes, suggesting that remodeling the ETC and inducing carbohydrate storage are important and conserved aspects of the terminal stages of egg production. Collectively, our data indicates that GSK3-mediated ETC remodeling is a novel, but highly conserved, mechanism of mitochondrial regulation. Furthermore, these data provide valuable insights into the mechanistic links between metabolic syndrome and many reproductive disorders.

D103 The mitochondrial outer membrane protein MDI promotes local protein synthesis and mtDNA replication. *Y. Zhang*, Y. Chen, M. Gucek, H. Xu. NHLBI, Bethesda, MD.

Early embryonic development features rapid nuclear DNA replication cycles, but lacks mtDNA replication. To meet the high-energy demands of embryogenesis, mature oocytes are furnished with vast amounts of mitochondria and mtDNA. However, the cellular machinery driving massive mtDNA replication in ovaries remains unknown. Here we describe a *Drosophila* AKAP protein, MDI that recruits a translation stimulator, La-related protein (Larp), to the mitochondrial outer membrane in ovaries. The MDI-Larp complex promotes the synthesis of a subset of nuclear-encoded mitochondrial proteins by cytosolic ribosomes on the mitochondrial surface. MDI-Larp's targets include mtDNA replication factors, mitochondrial ribosomal proteins and electron-transport-chain subunits. Lack of MDI abolishes mtDNA replication in ovaries, which leads to mtDNA deficiency in mature eggs. Targeting Larp to the mitochondrial outer membrane independently of MDI restores local protein synthesis and rescues the phenotypes of *mdi* mutant flies. Our work suggests that a selective translational boost by the MDI-Larp complex on the outer mitochondrial membrane might be essential for mtDNA replication and mitochondrial biogenesis during oogenesis.

D104 Signaling through the G-protein-coupled receptor Rickets is important for polarity, detachment, and migration of the border cells in *Drosophila*. *Lauren ANLLO*, Trudi Schüpbach. PRINCETON UNIVERSITY, PRINCETON, NJ.

Cell migration plays crucial roles during development. An excellent model to study coordinated cell movements is provided by the migration of border cell clusters within a developing *Drosophila* egg chamber. In a mutagenesis screen, we isolated two alleles of the gene *rickets* (*rk*) – encoding a G-protein-coupled receptor. The *rk* alleles result in border cell migration defects in a significant fraction of egg chambers. In *rk* mutants, border cells are properly specified and express the marker Slbo. Yet, analysis of both fixed as well as live samples revealed that some single border cells lag behind the main border cell cluster during migration, or, in other cases, the entire border cell cluster can remain tethered to the anterior epithelium as it migrates. These defects are observed significantly more often in mosaic border cell clusters, than in full mutant clusters. Reduction of the Rk ligand, Bursicon, in the border cell cluster also resulted in migration defects, strongly suggesting that Rk signaling is utilized for communication within the border cell cluster itself. The mutant border cell clusters show defects in localization of the adhesion protein E-cadherin, and apical polarity proteins during migration. E-cadherin mislocalization occurs in mosaic clusters, but not full mutant clusters, correlating well with the *rk* border cell migration phenotype. Our work has identified a receptor with a previously unknown role in border cell migration that appears to regulate detachment and polarity of the border cell cluster coordinating processes within the cells of the cluster themselves.

D105 A mutation in *fat2* uncouples tissue elongation from global tissue rotation in *Drosophila*. *F. Aurich*, C. Dahmann. Technische Universität Dresden, Dresden, Germany.

Global tissue rotation was proposed as a morphogenetic mechanism controlling tissue elongation. In *Drosophila* ovaries, global tissue rotation of egg chambers coincides with egg chamber elongation. Egg chamber rotation was put forward to result in circumferential alignment of actin filaments and extracellular fibers. These fibers serve as molecular corsets to restrain growth of egg chambers perpendicular to the anteroposterior axis, thereby leading to the preferential egg chamber elongation along this axis. The atypical cadherin Fat2 is required for egg chamber elongation, rotation, and the circumferential alignment of actin filaments and extracellular fibers. The localization of Fat2 protein at the basal side of follicle cells is planar polarized. Fat2 protein is enriched on cell junctions oriented parallel to the long axis of the egg chamber. The functional relationship between Fat2 planar polarized localization, egg chamber rotation and egg chamber elongation, however, remained unclear.

Here we have generated a truncated form of Fat2 that lacks its entire intracellular region. *fat2* mutant egg chambers expressing this truncated protein display normal actin and extracellular fiber alignment and properly elongate. However, the localization of the truncated Fat2

protein is not planar polarized and these egg chambers fail to rotate. Our data for the first time uncouple Fat2 planar polarization and global tissue rotation from egg chamber elongation. Thus global tissue rotation, even though coinciding with tissue elongation, is not a necessary prerequisite for elongation.

D106 Steroid signaling in mature follicles is essential for *Drosophila* ovulation. *E. Knapp*[1], J. Sun[1,2]. 1) Physiology and Neurobiology, University of Connecticut, Storrs, CT; 2) Institute for Systems Genomics, University of Connecticut, Storrs, CT.

Steroid hormones ecdysone (E) and 20-hydroxyecdysone (20E) are not only critical for early development in *Drosophila*, but are also essential in adult physiology. Particularly, ecdysteroid signaling has been shown to regulate female specific metabolic state and multiple processes of oogenesis, thus functionally equivalent to estrogenic steroids in mammals. Unlike estrogenic steroids, mainly progesterone, that are essential for ovulation, it is unknown whether ecdysteroid signaling regulates *Drosophila* ovulation. Our recent work demonstrated that Drosophila ovulation, resembling mammalian ovulation, involves the degradation of posterior follicle cells, the rupture of mature oocytes into the oviduct, and the formation of corpus luteum by the residual follicle cells. In addition, this rupture process is induced by the octopamine (OA) signaling in mature follicle cells that elicits an increase in intracellular calcium to activate matrix metalloproteinase 2 (Mmp2) enzymatic activity. In this study, we demonstrated that ecdysteroid signaling is operating in mature follicle cells to control ovulation. Knocking down shade (shd), encoding the enzyme for converting E to 20E, in mature follicle cells inhibits OA-induced follicle rupture *ex vivo* and ovulation *in vivo*. These defects can be rescued by ectopic expression of Shd or exogenous 20E. In addition, disruption of the ecdysone receptor (EcR) function in mature follicle cells also causes similar ovulation defects. Furthermore, we showed that ecdysteroid signaling functions upstream of calcium rise to regulate Mmp2 activity. Our data strongly suggest that 20E production in follicle cells prior to ovulation is necessary to prime mature follicles to be responsive to neuronal ovulatory stimuli, thus providing mechanistic insights into steroid signaling in ovulation and further evidence that ovulation control is largely conserved between fly and mammals.

D107 Identification and characterization of an "insect epididymis". *T. L. Karr*[1], M. Rosenow[2], L. Benner[3], B. Oliver[3], S. Skerget[4]. 1) Drosophila Genomics and Genetic Resources, Kyoto Institute of Technology, Kyoto, JP; 2) Caris Life Sciences, Phoenix, AZ; 3) National Institutes of Health, NiDDK, Bethesda, MD; 4) Arizona State University, Tempe, AZ.

Sperm development is accompanied by post-testicular modification of sperm as they transit the epididymis- a process necessary for fertilization competency. However, unlike well-studied mammalian systems, less is known about what, if any, similar sperm modifications occur in in other taxonomic groups, including Drosophila. However, recent work on the Dmel sperm proteome (DmSP, ~1100 proteins) revealed that ~50% of genes encoding the DmSP were not significantly expressed in the testis suggesting they were inserted following spermatogenesis. RNA-seq analysis confirmed that these genes were significantly expressed in the terminal epithelium and seminal vesicle (further termed "TE/SV" tissue). This raised the intriguing possibility that these proteins are inserted into sperm after exit from the testis. Analysis of the seminal vesicle proteome revealed functional enrichment in protein secretion, translocation and targeting similar to those found in secretory cells and polarized epithelia of the mammalian epididymis suggesting a similar function in the TE/SV. To further explore tissue-specific origin of the DmSP we identified a subset of genes not overexpressed in the testis (FA "down") but present in significant protein levels (determined by spectral counting). From this list, three readily available CPTI protein-trap lines of the DmSP were identified and their cell- and tissue-specific expression patterns were monitored by epifluorescence microscopy. As predicted, all three proteins showed low level testis expression and high expression in the seminal vesicle and the terminal epithelium suggesting that sperm exiting the testis were modified during transit into, or residence within, the TE/SV. Taken together these results suggest that Drosophila sperm are extensively modified following exit from the testis during transit into, and storage within, the seminal vesicle in a manner akin to that observed in the mammalian epididymis. As such, an "insect epididymis" could provide a rich new experimental model system useful for the study of fertility and related reproductive processes in both arthropods and vertebrates.

D108 An autonomous requirement of the lysosomal nuclease DNaseII in a caspase-independent primordial germ cell death in the *Drosophila* embryo. *L. Tarayrah*, E. Arama. The Weizmann Institute of Science, Rehovot, IL.

Programmed cell death (PCD) is an intracellular genetic program that is activated by all dying metazoan cells. Apoptosis is the most abundant form of PCD and is manifested by the activation of cysteine proteases called caspases. However, activation of caspases does not always lead to apoptosis and emerging evidence suggests the existence caspase-independent alternative cell death (ACD) pathways. Considering that cancer cells have developed ways to evade apoptotic cell death, uncovering the molecular mechanisms underlying ACD pathways has tremendous therapeutic value. However, progress in this field has been slow primarily due to the lack of physiological paradigms of ACD. In this study, we characterize the non-apoptotic cell death of primordial germ cells (PGCs) in the *Drosophila* embryo. During the early stages of embryo development, germ-line progenitors known as pole cells originate at the posterior pole and must migrate to their somatic partners in order to form the embryonic gonad. Prior studies have demonstrated the involvement of guidance cues that drive PGC migration, while mismigrating germ cells that remain ectopic to the gonad are eliminated. These findings suggest the existence of a mechanism within the embryo that regulates germ cell survival, however little is known about the molecular details governing this elimination mechanism. Our results indicate that PGCs that do no coalesce into a gonad by stage 13 of embryo development undergo caspase-independent cell death that involves the activity of lysosomes. We also find that DNaseII, a lysosomal deoxyribonuclease, is required maternally and cell autonomously in PGCs to drive their elimination providing an excellent physiological system to investigate a caspase-independent cell death pathway *in vivo.* .

D109 Move or Die: Linking caspases and cell migration and invasion in *Drosophila*. *E. Arama*, A. Gorelick, R. Weiss, A. Florentin, L. Tarayrah. Weizmann Institute of Science, Rehovot, IL.

The initiator caspase Dronc and the effector caspase Drice are required for most of the developmental and stress-induced apoptosis in *Drosophila*, whereas the role of the minor effector caspase, Dcp-1, is largely dispensable. After irradiation-induced apoptosis, both Drice and Dcp-1 become activated in wing imaginal discs (WDs). Here we show that following ionizing irradiation, WD cells compromised for caspase activity change their morphology, form protrusions, loose E-cadherin expression, delaminate and migrate away from their original compartment in an invasive manner. We termed this process irradiation-induced cell migration (ICM), and it is reminiscent of the epithelial-to-mesenchymal transition (EMT). Interestingly, we revealed both autonomous and non-autonomous factors required for ICM induction. We performed a candidate RNAi screen for signaling pathways involved in ICM induction. We then isolated the migrating cells and subjected them to RNA-seq analysis. Here I will describe our recent findings about the pathways and mechanisms underlying this unexpected phenomenon.

D110 Lysosome activity controls nurse cell death non-autonomously. *Albert Mondragon*, Alla Yalonetskaya, Anthony Ortega, Yuanhang Zhang, Kim McCall. Boston University, Boston, MA.

One of the most prevalent forms of cell death in the human body is phagoptosis, where one cell kills and engulfs another cell that would otherwise continue living. The mechanism of phagoptosis is poorly understood. We have recently shown that ovarian nurse cells die by this form of cell death, providing a powerful model for phagoptosis. In the Drosophila ovary, there are 15 nurse cells that support the oocyte throughout development. In late stages of oogenesis the nurse cells dump their cytoplasmic contents into the oocyte, and are subsequently acidified and cleared by the surrounding stretch follicle cells. We have previously found that the engulfment machinery (Draper, Ced-12, etc.) acts through the JNK pathway within the stretch follicle cells to promote nurse cell death, but the exact mechanism remained elusive. Through genetic manipulation and live imaging, we have determined that lysosomal components actively kill the nurse cells. To understand the role of lysosomes more precisely, we cultured late stage egg chambers and live-imaged nurse cells throughout death with probes and GFP fusion proteins. LysoTracker staining shows a gradual increase in nurse cell acidification and clearance by surrounding follicle cells. A candidate RNAi screen of genes encoding lysosomal proteins identified several key lysosomal components such as V-ATPases and cathepsins, which are required non-autonomously for the acidification and clearance of nurse cells. Additional hits from the RNAi screen are being followed up by confocal imaging and live imaging of GFP-tagged proteins. Altogether, this work further characterizes this novel form of cell death and illustrates the importance of non-autonomous control over cell death. Our work also demonstrates an essential role for lysosomal machinery in actively killing neighboring cells.

D111 Programmed necrosis control germ cell homeostasis during Drosophila spermatogenesis. *B. Mollereau*[1], S. Vincent[1], S. Yacobi-Sharon[2], B. Gibert[3], P. Mehlen[3], J. Felten[1], G. Chatelain[1], M. Decoville[4], V. Girard[1], E. Arama[2], F. Napoletano[1,5]. 1) Ecole Normale Supérieure de Lyon, LBMC, CNRS, INSERM, Lyon, France; 2) Weizmann Institute of Science, Rehovot, Israel; 3) Centre de Cancérologie de Lyon, INSERM, France; 4) CNRS, Orléans, France; 5) Department of Life Sciences, University of Trieste, Italy.

Regulated necrosis occurs in pathologies such as cerebral stroke and myocardial infarction, however the underlying mechanisms and its physiological relevance remain unclear. Here, we report a role for p53 in regulating necrosis in *Drosophila* spermatogenesis. We found that *Drosophila* p53 is required for the programmed necrosis that occurs spontaneously in mitotic germ cells during spermatogenesis. Prevention of p53-dependent necrosis resulted in testicular hyperplasia, which was reversed by restoring necrosis in spermatogonia. *Drosophila* spermatogenesis will thus be useful models to identify inducers of necrosis to treat cancers that are refractory to apoptosis.

D112 Regulation of cell size by variant cell cycles. *T. L. Orr-Weaver*[1,2], J. Von Stetina[1], L. E. Frawley[1,2], Y. Unhavaithaya[1]. 1) Whitehead Institute, Cambridge, MA; 2) Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA.

The endocycle is a modified cell cycle consisting of solely S and G phases that is widely used in differentiated Drosophila tissues. DNA replication in the absence of mitosis and cell division increases ploidy and cell size. We find that in addition to the endocycle an alternate cell cycle, endomitosis, is utilized in the subperineurial glia (SPG) in the Drosophila brain. In this variant cycle, mitosis and nuclear division occur, but not cytokinesis. Thus cell number does not increase, and large multinucleate cells are produced. The SPG are the first identified endomitotic cells in Drosophila. The presence of both endocycling and endomitotic SPG raises the question of the function of each of the two cell cycle types. We previously showed that increased cell size of the SPG is crucial for their role in maintaining the blood-brain barrier as the neuronal mass of the nervous system increases during development. Therefore we analyzed the developmental regulation of the endocycle and endomitosis, and the contribution of each to SPG size and the blood-brain barrier. We found that during embryogenesis initially all SPG enter the endocycle, but between the first and second larval instar stages the majority of SPG in the brain switch to endomitosis. Notch signaling is required for SPG to remain in the endocycle. To evaluate the functional roles of endomitosis and the endocycle, we reduced Notch signaling and consequently increased the proportion of SPG undergoing endomitosis. Additionally, we generated brains in which all SPG are endocycling by reducing expression of the Cdc25 phosphatase, String. Either of these changes in the ratio of endocycling to endomitotic cells caused defects in the blood-brain barrier. Thus the proper proportion of endocycling and endomitotic SPG is essential, likely to ensure total cell size of the SPG tissue layer to maintain an envelope over the neuronal mass, as we observe that cell size increases disproportionally to ploidy with increasing nuclear number.

D113 An inhibitory mono-ubiquitylation of the *Drosophila* initiator caspase Dronc functions in both apoptotic and non-apoptotic pathways. *HE Kamber*[1*], M. Ditzel[2], P. Meier[3], A. Bergmann[1]. 1) Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, Worcester, MA, 01605, USA; 2) MRC Institute for Genetics and Molecular Medicine, The University of Edinburgh, Edinburgh, UK; 3) The Breast Cancer Now Toby Robins Research Centre, Institute of Cancer Research, London SW3 6JB, UK.

Apoptosis is an evolutionary conserved cell death mechanism, which requires activation of initiator and effector caspases. The *Drosophila* initiator caspase Dronc, the ortholog of mammalian Caspase-2 and Caspase-9, has an N-terminal CARD domain that recruits Dronc into the apoptosome for activation. In addition to its role in apoptosis, Dronc also has non-apoptotic functions such as compensatory proliferation and male genitalia rotation. Given the detrimental effect of caspases, their activity is tightly controlled. One control mechanism is ubiquitylation. However, the mechanistic details of ubiquitylation of Dronc are less clear. For example, monomeric inactive Dronc is subject to non-degradative ubiquitylation in living cells, while ubiquitylation of active apoptosome-bound Dronc triggers its proteolytic degradation in apoptotic cells. Here, we examined the role of non-degradative ubiquitylation of Dronc in living cells *in vivo*, i.e. in the context of a multi-cellular organism. Our *in vivo* data suggest that in living cells Dronc is mono-ubiquitylated on Lys78 (K78) in its CARD domain. This ubiquitylation prevents activation of Dronc in the apoptosome and protects cells from apoptosis. Furthermore, K78 ubiquitylation plays an inhibitory role for non-apoptotic functions of Dronc. We provide evidence that not all of the non-apoptotic functions of Dronc require its catalytic activity. In conclusion, we demonstrate a mechanism whereby Dronc's apoptotic and non-apoptotic activities can be kept silenced in a non-degradative manner through a single ubiquitylation event in living cells.

D114 The *Drosophila* TNF Eiger activates Dronc-dependent necrosis when apoptosis is blocked. *M. Li*, Y. Fan. School of Biosciences, University of Birmingham, Birmingham, United Kingdom.

Eiger (Egr), the homolog of the mammalian tumor-necrosis factor (TNF), is the ligand of the c-Jun N-terminal kinase (JNK) stress response signaling pathway in *Drosophila*. Although expression of Egr frequently leads to apoptosis, it has also been implicated in activation of non-apoptotic cell death. For example, expression of Egr under the control of the eye-specific driver GMR (*GMR>egr*) results in a small eye phenotype which cannot be suppressed by P35, an inhibitor of the effector caspases DrICE and Dcp-1. Paradoxically, it has been reported that *GMR>egr*-induced eye ablation phenotype can be partially rescued by defective apoptosis through reduction of pro-apoptotic gene expression or inhibition of the initiator caspase Dronc. It is therefore not yet clear whether Egr can induce both apoptotic and non-apoptotic cell death, and if so, how such processes are coordinated. Here, we show that *GMR>egr* primarily induces apoptosis starting from the 3rd instar larval stage. Loss-of-function analysis reveals that such induction of apoptosis is through the canonical pathway and mainly mediated by the pro-apoptotic gene *hid*. Intriguingly, when apoptosis is blocked by expression of P35 (*GMR>egr, p35*), necrosis, indicated by Propidium Iodide labeling and Electron Microscopy, is induced that counteracts apoptotic inhibition by P35 resulting small eyes at the end. Moreover, loss one copy of Dronc, which does not affect *GMR>egr*-induced apoptosis, can suppress *GMR>egr, p35*-induced eye ablation phenotype suggesting different levels of Dronc are required for Egr-induced apoptosis and, if apoptosis is blocked, necrosis. As the mammalian TNF can activate the extrinsic apoptosis pathway and, if apoptosis is defective, triggers necrosis, our study suggests such a process is more conserved in *Drosophila* than previously thought.

D115 Enhancer of Polycomb represses transcription of Cyclin B during male germ cell differentiation. *L. Feng*, X. Chen. Johns Hopkins University, Baltimore, MD.

Adult stem cells modulate balance between proliferation, differentiation and cell death to maintain tissue homeostasis. We use *Drosophila* testes to study how germ cells transit from proliferation to differentiation, and repair DNA damage to prevent cell death. In testes, Cyclin B (CycB) accumulates in proliferating germ cells, decreases upon completion of mitosis, and regain expression just before meiotic G2/M transition. Transcriptional activation and translational repression of CycB in meiosis are well studied. But how CycB is downregulated after mitosis and if such downregulation is crucial remain unknown. Here we found chromatin factor E(Pc) transcriptionally represses CycB by modulating H4 acetylation and reduced CycB is essential for germ cell differentiation. Moreover, E(Pc) depletion causes accumulated DNA double strand breaks and severe germ cell death. In addition, lacking activity of Tip60 histone acetyltransferase (HAT) leads to similar defects. Taken together, acetylation plays crucial roles in maintaining tissue homeostasis though transcriptional regulation and DNA damage repair.

D116 Towards a Genetic Understanding of Behavior Evolution: An Ion-channel Gene Causes Natural Courtship Song Variation in *Drosophila*. *Yun Ding*[1], Augusto Berrocal[1,2], Tomoko Morita[1], Kit Longden[1], David Stern[1]. 1) Janelia Research Campus, HHMI, Ashburn, VA; 2) UC Berkeley, Berkeley, CA.

Animal species display enormous variation for innate behaviors, but little is known about how genomes evolve to generate this diversity. The features of ***Drosophila*** courtship song are easy to quantify and vary widely within and between species, making song an excellent system for genetic studies. We developed a mapping strategy that combined traditional mapping protocol with high-resolution recombinant mapping using engineered visible markers via CRISPR/Cas9 genome editing. This approach allowed us to map the sine song frequency difference between two wild isolates, ***Drosophila simulans 5*** and ***D. mauritiana 29***, to a 966 bp region within the ***slowpoke*** locus, which encodes a calcium-activated potassium channel. We confirmed that ***slowpoke*** is the causal locus using a reciprocal hemizygosity test, and further narrowed down the casual mutation to an intronic insertion of retrotransposon that occurred within ***D. simulans*** populations. Difference in exon usage was observed around the retrotranspon insertion site. Like many ion-channel genes, ***slowpoke*** is expressed widely in the nervous system and influences many behaviors. The ***slowpoke*** mutants sing very little song with disrupted features. However, the naturally evolved ***slowpoke*** alleles cause significant variation in only one component of courtship song, showing that a highly pleiotropic ion channel gene can

evolve to generate a specific new behavior pattern through changes in gene regulation. Currently, we are trying to confirm the retrotranspon insertion as the causal mutation by targeted deletion, and identify the molecular mechanism of how the retrotranspon insertion has fine-tuned the *slowpoke* function.

D117 A delicate balance of mating preference in *Drosophila melanogaster*. *Akihiko Yamamoto*[1,2], Michael Magwire[1,2,3], Lauren Dembeck[1,2,4], Mary Anna Carbone[1,2], Trudy Mackay[1,2]. 1) Biological Sciences, North Carolina State University, Raleigh, NC; 2) W M Keck Center for Behavioral Biology , North Carolina State University, Raleigh, NC; 3) Syngenta Biotechnology, Durham, NC; 4) Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan.

Incipient speciation between stocks of Sub-Saharan Africa and cosmopolitan populations in *D. melanogaster* has been reported for decades but genes involved in their mating preference are elusive because prevalence of inversions in African populations hampers detailed genetic analyses. We employed a new strategy to explore the variations in a cosmopolitan population and confirmed 42 genes which affected significantly on the copulation success of cosmopolitan males to Zimbabwean Z30 females. Surprisingly, most of the RNAi mediated disruptions of these genes enhanced the male copulation success and a weak driver did so rather than the strong ones. Using mutants affecting female's sensory systems and surgical removal of female antennae, we demonstrated that the effects of gene-disruption on cosmopolitan males were perceived by *Ir8a*/*Ir25a* mediated olfaction. We further specified male oenocytes as the target of these genes and most of methyl branching cuticular hydrocarbons were reduced in the gene-disrupted males.

D118 Mechanism of hybrid incompatibility between two subspecies of *Drosophila pseudoobscura*. *C. Large*, N. Phadnis. University of Utah, Salt Lake City, UT.

Understanding the genetic and molecular basis of hybrid incompatibilities is a long-standing and central problem in evolutionary genetics. Despite decades of studies, we understand little about the genetic basis of hybrid sterility and even more lacking is our understanding of the molecular basis of hybrid dysfunction. The Bogota and USA subspecies of *Drosophila pseudoobscura* provides a paradigmatic case of the earliest stages of speciation. Previously, we have shown that a single gene, *Overdrive* (*Ovd*) is essential for both hybrid sterility and segregation distortion in hybrid F1 males between these populations. Little is known, however, about the normal function of *Ovd* within species and its molecular role in causing hybrid sterility between species.

To understand the molecular basis of hybrid sterility, we performed a comprehensive cytological characterization of spermatogenesis in *D. pseudoobscura* hybrid F1 males. Our results show that the primary defect in hybrid males is a failure to compact DNA after the last meiotic division. This failure to properly condense DNA leads to an arrest in sperm development later in spermiogenesis, leading to little to no functional sperm. We further employed CRISPR-Cas9 based methods to GFP tag *Ovd* at its native locus in *D. melanogaster*. Our studies on the localization patterns of *Ovd* in the developing male germline in congruence with the hybrid defect suggests that *Ovd* has a direct effect on chromatin state during spermatogenesis that leads to hybrid incompatibility between these new subspecies. Together, these findings are providing the first insights into the molecular function of *Ovd* and highlight the role of chromatin state dynamics in the evolution of reproductive barriers between species.

D119 Recurrent changes to *pdm3* drive convergent evolution of female-limited polymorphism in the *Drosophila montium* subgroup. *Emily K. Delaney*[1], Amir Yassin[2], Adam Reddiex[3], Héloïse Bastide[2], Thaddeus Seher[1], Nicholas Appleton[3], Justin Lack[2], Jean R. David[4], Stephen F. Chenoweth[3], John E. Pool[2], Artyom Kopp[1]. 1) University of California-Davis, Davis, CA; 2) University of Wisconsin-Madison, Madison, WI; 3) University of Queensland, St. Lucia, Australia; 4) Université Paris Sud, Gif-sur-Yvette, France.

In spite of a shared genome, males and females can have dramatically different phenotypic traits. Some sex-specific traits are also polymorphic, so that different genotypes are expressed as two or more phenotypes in one sex but not the other. Such sex-limited polymorphisms offer an opportunity to reconstruct the origin of molecular mechanisms limiting trait expression to a single sex. The *Drosophila montium* subgroup, comprised of 76 species, harbors diverse abdominal pigmentation: some species are sexually monomorphic, others are dimorphic, and many species have female-limited color dimorphism (FLCD), where females are either light or dark but males are always the same color. Thus, sexually dimorphic and sexually monomorphic genotypes can either segregate within a single species or be fixed between closely related species, making this group an ideal model for reconstructing evolutionary transitions between sex-specific and sexually monomorphic development. We mapped FLCD in four distantly related species from the *Drosophila montium* subgroup and found that it mapped to the same locus, the POU domain transcription factor (*pdm3*), in all cases. In *D. serrata*, we pinpointed a small region within the first intron of *pdm3* that differs in size and sequence between color morphs. The dark allele contains putative binding sites for *dsx,* the transcriptional effector of the *Drosophila* sex determination pathway, and the HOX gene *Abd-B,* which controls the development of posterior abdominal segments. The region spanning this complex structural variant acts as an abdominal enhancer in transgenic reporter assays, suggesting that sex-limited polymorphism is due to intraspecific segregation of *cis*-regulatory variation in *pdm3*. In *D. kikkawai, D. leontia,* and *D. burlai*, the structural polymorphism observed in *D. serrata* is not present, and female pigmentation is associated with different non-coding variants upstream of *pdm3*. These results suggest that FLCD has originated multiple times through regulatory changes in the same locus.

D120 Evolution of the Sex Peptide Network: Lineage-specific adaptive evolution and gene duplication. *Meaghan McGeary*, Geoffrey Findlay. College of the Holy Cross, Worcester, MA.

The sex peptide (SP) network consists of at least 13 proteins from males and females that interact to control female behavior and physiology after mating. In *Drosophila melanogaster*, these changes can last for several days and include increased egg production, efficient sperm storage, and a decrease in female receptivity to remating. However, other *Drosophila* species show dramatically different reproductive phenotypes, including faster female remating and differences in sperm size and sperm storage patterns. We thus investigated the molecular evolution of the SP network proteins across the *Drosophila* genus. After bioinformatically identifying SP network proteins in up to 22 species, we used PAML to test whether any proteins had sites that had evolved under recurrent positive selection and to test for adaptive evolution on specific phylogenetic branches that correlate with major changes in reproductive phenotypes. While only 2 of 11 tested genes showed evidence of recurrent positive selection across the phylogeny, we identified bursts of adaptive evolution on lineages correlating with phenotypic changes. For example, the lineage leading to the melanogaster species group showed evidence of adaptive evolution on three genes. This lineage corresponds to a change in the expression level of the SP receptor in the female reproductive tract and to two gene duplication events of the network member, seminase. We then investigated the importance of gene duplication in the network's evolution by studying seminase in further detail. Three copies of this gene are present in the melanogaster group of *Drosophila*, while only a single copy is found in other species. PAML analysis showed no evidence for adaptive evolution of seminase following duplication, and gene expression analysis in seven species showed that seminase and both paralogs are expressed specifically in males. Taken together, these results paint a dynamic picture of SP network evolution, with different lineages and genes having experienced different selective pressures.

D121 Investigating the female's role in sperm competition in *Drosophila melanogaster*. *S. White*, J. Sitnik, C. Chow, A. Clark, M. Wolfner. Cornell University, Ithaca, NY.

The formation of a fertilized egg involves many interactions between males, females, and their gametes. This is even more complex in cases of multiple mating, as in *Drosophila melanogaster,* as the presence of ejaculates from multiple males presents the opportunity for sperm competition to occur. Male-derived seminal fluid proteins are known to influence sperm competition outcomes, in addition to other post-mating effects. While studies have shown that female genotype is also important for sperm competition outcome, the mechanisms underlying the female's contribution to the success of a particular male's sperm are less understood.

A previous GWAS screen based on sperm competition phenotypes across natural isolates of *D. melanogaster* identified candidate genes for the female's contribution to sperm competition. Interestingly, about half of the top 33 genes have predicted neurological function, pointing to an active role for the female in sperm use and preference. We performed ubiquitous or tissue-targeted local RNAi knockdowns to assess the impact of decreased expression of these genes on sperm competition. Females were scored for sperm competition effects using progeny-phenotype assays, and for reproductive processes including fecundity, fertility, and resistance to remating. Of 29 genes tested, knockdown of 9 affected sperm competition outcomes. Genes whose knockdowns in females affect sperm competition outcomes will be further characterized for their effects on sperm storage, retention and release, and on sperm competition, by direct examination of sperm dynamics within the female, for the neurons (or other tissues) through which these genes exert their effects, and for the roles of the specific alleles found in the wild-derived genomes. Collectively, results from these experiments will provide a clearer picture as to how females are influencing sperm competition outcomes.

We thank the NIH for funding (R01-HD059060) and HHMI for a Gilliam Fellowship to SLW.

D122 Missing variation revealed by deep sequencing of individuals in a population of *D. simulans*. *S. A. Signor*[1], Felicia New[2], Lauren McIntyre[2], Sergey Nuzhdin[1]. 1) University of Southern California, Los Angeles, CA; 2) University of Florida, Gainesville , FL.

The observed heritable variation in complex phenotypes can only partially be explained by mutation-selection balance. Directional selection is expected to remove most additive variation, so possible hypotheses for its maintenance include balancing selection, inefficient selection, or failure of populations to reach equilibrium . Looking at patterns of polymorphisms to corroborate these hypotheses mostly negative values of Tajima's D have been observed in *Drosophila* (indicating selective sweeps or rapid population expansion). Here, we have established a panel of 170 nearly homozygous *D. simulans* genotypes from a single stable population and found that D is strongly biased towards positive values in the regions of normal recombination. We rule out demography, admixture, and balancing selection based on the patterns of linkage disequilibrium and Tajima's D. Using recently developed statistical tests based on a measure of haplotype homozygosity (H12) we were able to detect numerous soft and hard sweeps and distinguish between them (H2/H1). We found evidence for abundant incomplete soft sweeps, including that they are much more numerous than in comparable populations of *D. melanogaster*. We hypothesize that transient selective sweeps might be responsible for the bulk of phenotypic variation in complex traits. This is a potential explanation for the excess of variation observed in most traits compared to theoretical expectations.

D123 Mutational patterns in Drosophila melanogaster. *Zoe June Assaf*, Dmitri Petrov. Stanford University, Stanford, CA.

It is critical to have accurate estimates of mutational rates and biases in order to successfully perform many population genetic analyses. Yet, because a substantial fraction of new mutations are deleterious, our ability to detect de novo events is severely limited. Fortunately, the scientific community studying Drosophila melanogaster has generated a large body of sequence data that is publicly available. We leverage these resources, in combination with additional strains sequenced in our lab, to investigate mutational patterns in Drosophila melanogaster using two different strategies.

The first strategy is via mutational accumulation, an experiment that allows new mutations to accrue in the genomes of laboratory strains,

and which has now been conducted three independent times. The two previously published experiments used the inbreeding method of mutation accumulation (Keightley et. al. 2009, Schrider et. al. 2013) and to this we are contributing a novel data set that was generated using an alternative experimental method, one which additionally permits recessive strongly deleterious mutations to accumulate. We find a significantly higher mutation rate than previously reported, suggesting that strongly deleterious recessive mutations are an important part of the mutational spectrum. In other metrics the three experiments have very similar patterns, and thus we merge these three resources and give estimates for a variety of fundamental mutational biases.

Mutation accumulation studies are excellent for measuring mutation rates, yet our ability to characterize other more subtle mutational patterns is still limited by the small number of events which occur during these experiments. To address this, we combine the individually sequenced strains made available by the Drosophila Genome Nexus (which includes lines from DGRP, DPGP, and DSPR) (Lack et. al. 2015), with newly generated sequence data using pooled individuals from natural populations, which collectively represent >17,000X coverage of >5,000 strains. These data sets allow the detection of extremely rare polymorphisms, which we further validated with resequencing. Extremely rare variants are a class of sites enriched for new deleterious mutations, because at low frequencies their dynamics are dominated by stochastic rather than selective forces. We show that the these extremely rare variants in fact approach the de novo spectrum as revealed by mutation accumulation experiments, and use this high quality set of rare variants to measure additional mutational patterns.

D124 Cell fate transformations in sine oculis eye-specific LOF mutants obscures direct regulatory interactions within the retinal determination network. *B. Weasner*, J. Kumar. Indiana University, Bloomington, IN.

The *sine oculis* (*so*) and *eyes absent* (*eya*) genes are required for retinal development as mutations in either gene leads to the complete absence of the adult retina. These proteins are known to form a biochemical complex which functions as a bipartite transcription factor to activate targets necessary for eye development. Examination of null clones of either gene shows a corresponding absence of the partner protein. Together these data support a model in which *so* and *eya* regulate each other's expression. The no-eye phenotype that characterizes the eya^2 mutant is caused by the deletion of an eye specific enhancer element. Prior studies have concluded that this enhancer is the main cis-regulatory element controlling the expression of *eya* during eye specification. The enhancer contains a So binding site thus *eya* expression could be initiated and maintained by the So-Eya complex itself. Several additional So binding sites are present within the locus and a recent ChIP-seq study indicated the presence of several So peaks. We sought to identify additional cis-regulatory elements that might be responsive to the So-Eya complex but whose activity might be masked in the eya^2 mutant. To this end we expressed a SoVP16 chimeric molecule in eya^2 mutants and found it capable of restoring some Eya protein and partially rescuing the no-eye phenotype implying the presence of additional So responsive enhancers. We have identified multiple new cis-regulatory elements that are responsible for robust eya expression in the retina. Surprisingly, these elements are still activated in *so* mutants suggesting they are not regulated by So. This led us to more thoroughly examine Eya expression in *so* mutants. We find that the loss of Eya protein corresponds to the temporal de-repression of non-ocular genes within the eye field. We have previously shown that this de-repression ultimately results in a cell fate switch from retinal progenitor to head epidermis. Concurrently, a wave of cell death in the eye field clears retinal progenitors which, for reasons unknown, are incapable of undergoing the cell fate transformation. These two events lead to a progressive loss of Eya protein. Our examination of *so* mutant clones indicates that those lacking Eya protein expression ultimately differentiate into head capsule. As expected those clones which have not undergone a cell fate switch still contain Eya protein further supporting our model that *so* is not directly regulating *eya* expression in the developing retina. Finally, our data suggests that caution should be exhibited when using RD mutants to interpret regulatory interactions among RD network members as cell fate transformations can lead to false positive interactions.

D125 Genome-wide analyses of Hox target genes in *Drosophila melanogaster*. *N. P. Singh*[1], Bony De Kumar[1], Cyber Cynthia[1], Alexander Stark[2], Julia Zeitlinger[1], Kausik Si[1], Robb Krumlauf[1]. 1) Stowers institute for Medical research, Kansas City, MO; 2) Research Institute of Molecular Pathology, Vienna, Austria.

Hox genes regulate the antero-posterior (AP) body axis in all Bilaterians. Hox genes encode transcription factors which regulate expression of downstream target genes important for morphogenesis during early development. While the conserved role of these proteins is well established little is known about the nature of the downstream targets of Hox genes. Another challenge is that Hox proteins have very similar protein sequences, which makes it difficult to understand the basis for their individual specificities and functional activities. Studies in our group using epitope-tagged versions of Hox proteins and programmed differentiation of mouse ES cells have enabled us to examine binding regions and downstream targets of mammalian Hox proteins on a genome wide basis through ChIP-seq approaches. This work has uncovered both novel and overlapping sets of downstream target genes for Hox proteins. Computational analyses of these mammalian Hox binding sites and co-associated binding motifs is also helping to uncover roles for partners (factors and complexes) and co-factors that may serve to potentiate the activity and regulatory roles of the Hox proteins. The function of Hox genes in AP patterning is conserved from arthropods to humans. Hence, my research objective has been to compare and contrast genome wide binding properties of mouse and *Drosophila* Hox proteins to understand the general principles and molecular mechanisms underlying Hox protein binding at target loci. Towards this goal, I used specific antibodies against Drosophila Hox proteins and a fly stock having an epitope tagged version of Ubx. Using ChIP-seq and ChIP-nexus I have mapped the downstream targets of Antp, Ubx, Abd-A and Abd-B in fly genome in 4-16 hr embryos. Analysis of the target genes is revealing distinct subsets of common and different target regions. Based on finding in our mammalian Hox studies, we have also generated specific antibodies against the Drosophila REST protein (Charltan-Chn) and Mediator complex subunit Med19and mapped their downstream target genes. Our initial results suggest that Mediator, REST and Hox proteins share a large number of common targets in *Drosophila* genome. Genetic and biochemical experiments further indicate genetic and physical interactions between Hox, Chn and Mediator complexes. This data

implies that regulation of Hox target genes may involve input from Chn/REST and Mediator complexs in both *Drosophila* and mammals. We are now doing locus specific experiments to validate and understand more precisely the mechanisms of these interactions in gene regulation.

D126 Manipulating fate with light – an optogenetics tool to understand Bcd function. *A. Huang*[1], C. Amourda[1], T. E. Saunders[1,2,3]. 1) Mechanobiology Institute, Singapore; 2) Department of Biological Sciences, NUS, Singapore; 3) IMCB, A-star, Singapore.

At the onset of embryogenesis, the segmentation gene network translates maternal inputs into more complex body patterns. Quantitative studies using *Drosophila melanogaster* embryo as a model have characterized the temporal evolution of the maternal Bicoid (Bcd) gradient as well as downstream hierarchical genes. Nevertheless, how individual cells interpret a dynamic Bcd gradient at different time windows remains elusive. To better understand the temporal readout of Bcd, we developed an optogenetics tool to turn off Bcd transcription activity with blue-light (488nm) illumination while the activity is restored minutes after switching back to dark. We used the *hunchback* (hb)-MS2 reporter assay to confirm that 488nm illumination immediately abolishes the Bcd-dependent transcription of Hb in the anterior region of the embryo while leaving the posterior Bcd-independent domain intact. By inactivating Bcd activity at different time-windows during the blastoderm stage, we found that Bcd activity during both nuclear cycle (NC) 14 and NC 13 is essential for embryonic viability. Interestingly, although inactivation of Bcd during NC10-12 does not cause embryonic lethality, it results in an anterior shift of the downstream gap genes and pair-rule genes, resembling that of 1-copy Bcd embryos. This suggests that, at early stages, Bcd may be playing a role in presetting the competency of the nuclei to interpret Bcd in the later stages potentially by occupying DNA binding sites and/or remodeling the chromosomal conformation. Furthermore, we analyzed the cuticle patterns of embryos exposed to different time windows of Bcd-inactivation. Even during fast phase of cellularization Bcd inactivation results in embryo lethality, due to subtle defects at the most anterior region (e.g. mouth hook, cirri). Prolonged inactivation of Bcd (throughout cellularization or even earlier) impedes the formation of more posterior structures. This suggests that the more anterior in space the structure is, the later in time cell fate is determined. Last, by combining our optogenetics tool with light-sheet microscopy imaging, we were able to inactivate Bcd activity in only one lateral half of the embryo. We observed that the cephalic furrow formation was blocked at the illuminated side of the embryo, while the tissue invagination occurs normally in the non-illuminated side. As the mechanism of cephalic furrow formation has yet to be understood, our approach has the potential to elucidate the property of this morphological movement and identify essential molecular players. Our future work will focus on understanding the link between the initial Bcd-dependent cell fate determinations and morphogenesis during later embryonic development.

D127 Regulation of Dpp signaling by O-linked glycosylation. *M. J. Moulton*[1], G. B. Humphreys[2], A. Letsou[1]. 1) University of Utah, Salt Lake City, UT; 2) Pennsylvania State University, University Park, PA.

Animal embryogenesis requires input from diverse signaling pathways to coordinate proper placement and organization of body structures, tissues, and organs. Activation and deactivation of signaling pathways at the right time and place is essential for embryogenesis, with defects in signaling often leading to inborn errors of development. A molecular and biochemical understanding of signal transduction pathways is important for proper diagnosis and treatment of congenital developmental abnormalities. Our lab uses the fruit fly, *Drosophila melanogaster*, to understand developmental defects caused by alterations in Decapentaplegic (Dpp) signaling. Dpp is a homologue of vertebrate BMP2/4 and is a member of the TGF-β family of cytokines. In the fly, both losses and gains of Dpp signaling result in embryonic lethality with associated defects in dorso-ventral patterns and structures.

While we have previously reported that loss of the *mummy* (*mmy*)-encoded UDP-*N*-acetylglucosamine diphosphorylase results in ectopic Dpp signaling and embryonic lethality, here we provide mechanistic insight into the specific role of GlcNAc in Dpp signal antagonism. Our studies have their foundation in our discovery of *super sex combs* (*sxc*), the Drosophila O-GlcNAc transferase, in an RNAi screen for glycosyltransferases that share loss-of-function phenotypes with *mmy*. Here we show that, like Mmy, Sxc is a Dpp signaling antagonist that functions to restrict Dpp signal transduction in the *Drosophila* embryonic epidermis. Loss of *sxc* results in ectopic Dpp phenotypes, including an expanded pMad domain and a loss of larval ventral denticle belts. Notably, these markers of ectopic Dpp signaling persist in the absence of the Dpp Type I receptor Thickveins (Tkv), but require Saxophone (Sax). Taken together, our data point to Sax as a potent Dpp signal transducer in the embryonic epidermis, with the capacity to activate Mad well beyond the domain normally specified by Tkv. We speculate that epidermal Tkv and Sax function as short- and long-range signaling receptors, respectively, and that Sxc-mediated inhibition of Sax limits epidermal Dpp signaling to Tkv. Our studies are the first to: 1) demonstrate a role for Sxc in embryogenesis, and 2) provide mechanistic insight into the role of GlcNAc in modulating responses to Dpp.

D128 A Transcription Factor code controlling serial specification of muscle identities in *Drosophila* . *A. J. M. Vincent*, Laurence Dubois, Jean-Louis Frendo, Hélène Chanut-Delalande, Michèle Crozatier. CNRS/University Toulouse 3, Toulouse, FR.

Each Drosophila body wall muscle is seeded by one Founder Cell (FC) issued from division of a Progenitor Cell (PC). Each PC is selected from an equivalence group of myoblasts, called promuscular cluster (PMC), at a precise position within the somatic mesoderm. Muscle identity – i.e, orientation, size, shape and final skeletal attachment sites - reflects the expression by each PC and FC of a specific combination of identity Transcription Factors (iTFs). Our previous observation of the serial emergence of several PCs at the same position raised the question of how developmental time controlled the muscle iTF code. Through a systematic chromosomal deficiency screen, and focusing on the 6 dorso-lateral muscles, all issued from the same PMC, we identified new roles of Anterior Open (Aop), ETS domain lacking (Edl), Eyes absent (Eya), No Ocelli (Noc), and Sine oculis (So), in serial specification of muscle identities. The muscle phenotypes and windows of transcription of these, and several other transcription factors such as *collier/knot* (*col/kn*), and *slouch/S59*, in wild type and mutant embryos, as determined by using intronic probes, revealed a cascade of regulations integrating temporal with positional information. This includes lineage-specific feed-forward

or incoherent loops, and sequential use of alternative transcription start sites. Together, our data provide a dynamic view of the transcriptional control of muscle identity in *Drosophila* and an extended framework for studying interactions between general myogenic factors such as Nautilus/Myo-D and iTFs in evolutionary diversification of body wall muscles.

D129 Tracking morphogens down: Uncovering the Dpp morphogen gradient. *P. Sánchez Bosch*[1], R. Yagi[2], K. Basler[1]. 1) University of Zurich, Zurich, CH; 2) ETH Zurich, Zurich, CH.

Morphogens are secreted proteins that regulate key processes during development, such as cell proliferation, growth and patterning. They are produced from a localized source and somehow establish a concentration gradient. Decapentaplegic (Dpp), the *Drosophila* BMP, is expressed at the anterior-posterior (AP) boundary of the wing imaginal disc, generating an AP morphogenetic gradient that regulates the expression of key genes involved in the wing development. Despite the broad knowledge regarding its function, the mechanism by which the Dpp gradient is established and how it synchronizes proliferation and patterning has been under debate for years.

In order to analyze the behavior of Dpp and its effects in development, we have modified the endogenous *dpp* locus by CRISPR/Cas9, replacing the first coding exon by a transgenic dpp tandem. This gene tandem can be manipulated via FLP/FRT recombination. With the help of this system, we studied the behavior of the Dpp gradient, tracking the intracellular and extracellular Dpp populations.

We have imaged and measured Dpp gradient formation, protein degradation and other parameters involved in the movement of Dpp, in an effort to model the gradient dynamics. Our results show a fast protein turnover in the producing cells, as well as a high retention of the extracellular Dpp population. Our current data suggest that the gradient is generated by restricted Dpp diffusion and oppose to a free diffusion model, where fast protein degradation is required.

D130 Flies have 11 abdominal segments (as suggested by the bithorax complex). *Welcome Bender*[1], Sarah Bowman[2], Heber Domingues[1], Robert Kingston[2]. 1) Harvard Medical Sch, Boston, MA; 2) Mass. Genereal Hospital, Boston, MA.

Drosophila larvae have 8 obvious abdominal segments, and rudiments of the 9th and 10th abdominal segments have been described, based on ENGRAILED expression patterns in early embryos. The bithorax complex has a series of regulatory domains, one for each parasegment, from PS5 (~3rd thoracic segment) through the posterior abdominal segments. The domains were first described by E. B. Lewis, based on mapping of mutations affecting different segments. These domains have been confirmed by enhancer trap expression patterns, by parasegment-specific discontinuities in H3K27 methylation patterns, and by the coincidence of most domain borders with binding sites for CTCF. We have used CRISPR-mediated gene conversion to introduce a Gal4 reporter into putative parasegmental domains towards the distal end of the bithorax complex. We see distinctive expression patterns, especially in the CNS, corresponding to domains for PS12-16 (~7th through 11th abdominal segments). We are currently working to isolate nuclei from each of these parasegments, in order to confirm these domains with H3K27me3 maps.

D131 Spatial patterning of the *Drosophila* ventral epithelium is important for proper tissue shape. *Natalie Heer*, Adam Martin. MIT, Cambridge, MA.

During development two-dimensional epithelial sheets fold into three-dimensional structures. In many cases, such as the vertebrate neural tube and *Drosophila* gastrulation, epithelial cells constrict their apical surfaces, which coupled with a lack of constriction, or even expansion, of the basal domain is thought to cause epithelial folding. In *Drosophila* gastrulation, the ventral epithelium, or presumptive mesoderm, folds in response to the expression of two embryonic transcription factors, Twist and Snail. Here, we use quantitative microscopy to measure the pattern of gene activation, signaling, myosin activation, and cell shape in the ventral mesoderm. We used *in situ* hybridization to measure the distribution of Twist mRNA before and during tissue invagination. Next, we measured downstream signaling components, including T48 and DRhoGEF2, using fluorescent fusion proteins under the control of the endogenous promoters. In addition, we have measured the kinetics of non-muscle myosin II accumulation per cell along the dorsal-ventral axis within the ventral region. We have identified multiple mutations in the pathway downstream of Twist that lead to phenotypically similar defects in furrow morphology, some of which prevent invagination. We are testing whether these mutants disrupt the normal spatial pattern of contractility across the ventral mesoderm.

D132 An actomyosin-Arf-GEF negative feedback loop for tissue plasticity. *J.John. West*[1], T. Zulueta-Coarasa[1], R. Fernandez-Gonzalez[1,2], T. J. C. Harris[1]. 1) University of Toronto, Toronto, Ontario, CA; 2) The Hospital for Sick Children, Toronto, Ontario, CA.

Actomyosin contractility is critical for organizing various forms of morphogenesis. In some cases, high levels of contractility are important for driving cell shape changes, while in other cases, contractility must be maintained at moderate levels that help guide morphogenetic processes. The mechanisms that induce contractility are well characterized, however, it is not clear how actomyosin networks are counteracted to maintain moderate levels of contractility. Recently, the Arf-GEF Steppke was shown to antagonize actomyosin contractility during cleavage of the early *Drosophila* embryo. Therefore, we hypothesized that Steppke plays a similar role during later stages of embryogenesis as the ectoderm undergoes various forms of actomyosin driven morphogenesis. We report that Steppke is enriched in areas of high actomyosin contractility throughout embryonic development, including cytokinetic rings and adherens junctions. *step* zygotic mutant embryos fail head involution, and display a disorganized epithelium beginning at germ band retraction. In contrast to the wildtype tissue in which cells become elongated and aligned along the dorsal-ventral axis. Expression of a Steppke construct can rescue these defects, and its GEF activity is required for normal tissue structure. *step* mutants display abnormal local increases of actin and myosin levels, as well as increased tissue tension in the ectoderm. Thus, Steppke seems to act locally to antagonize actomyosin activity to promote the orderly spreading of an epithelial sheet. Strikingly, actomyosin activity appears responsible for locally recruiting Step since loss or gain of actomyosin function results in decreased or

increased Steppke levels respectively. Taken together, these data suggest a negative feedback loop in which Step is recruited to areas of high actomyosin activity, and antagonizes this activity for tissue plasticity.

D133 E-Cadherin decrease transmits proliferative-dependent forces via actin-myosin flows. *D. Pinheiro*[1,2], E. Hannezo[3], S. Herstzerg[1,2,4], I. Gaugue[1,2], M. Balakeriva[1,2], Z. Wang[1,2], S. Rigaud[1,2], O. Markova[1,2], Y. Bellaiche[1,2]. 1) Institut Curie, Paris, FR; 2) Sorbonne Universités, UPMC, France, Fr; 3) Cavendish Laboratory, Department of Physics, Cambridge, UK; 4) The Francis Crick Institute, Mill Hill Laboratory, London, UK.

During epithelial cytokinesis, the remodelling of adhesive cell-cell contacts between the dividing cell and its neighbours has profound roles in the integrity, arrangement and morphogenesis of proliferative tissues. This remodelling is powered by an interplay between contractile ring cosntriction in the dividing cell and the dynamics of non-muscle Myosin II (MyoII) in the interphasic neighbouring cells. Here we explore how the dividing cell regulates MyoII dynamics in its neighbours. We found that pulling forces, resulting from contractile ring constriction, lead to MyoII accumulation in the neighbouring cells independently of the classical Vinculin-mediated mechano-transduction pathway. We established that the contractile ring pulling forces promote local junction elongation, resulting in a decrease of E-Cadherin concentration at the ingressing ***adherens*** junction (AJ). In turn, the local reduction of E-Cadherin concentration and the contractility of the neighbouring cells promote a self-organized outward flow of F-Actin and MyoII, ultimately leading to their accumulation at the base of the ingressing AJ. Mechano-sensing has been extensively studied in the context of AJ strengthening to stabilize adhesive cell-cell contacts, we propose an additional mechano-sensing mechanism able to coordinate actin-myosin dynamics between epithelial cells and to sustain AJ remodelling in response to mechanical force.

D134 A gradient of Rac activity determines protrusion form and position in a 3-dimensional epithelial sheet. *M. Georgiou*, A. Couto, N. Mack, L. Favia. University of Nottingham, Nottingham, Nottinghamshire, GB.

Epithelial sheets exhibit several defining characteristics, including mechanically strong cell-cell junctions and a coordinated cell polarity, which maintain epithelial integrity and impart correct cell shape and tissue organization. Key to the acquisition of these characteristics is the intimate interplay between cell-cell adhesion, polarity proteins and regulators of the actin cytoskeleton.

By combining genetic and cell biological analyses we show that epithelial cells within the fly dorsal thorax possess distinct classes of dynamic protrusion along their apical-basal axis. Cells possess apical microvilli, lateral sheet-like protrusions at an intermediate level, and filopodia and lamellipodia at the base of the cell. Using in vivo inducible constructs that can sense or modify Rac activity, we demonstrate an apicobasal gradient of Rac activity that is required to correctly form and position these distinct classes of protrusion along the apicobasal axis of the cell. Apicobasal polarity is required to form this gradient, and we show that we can modify the Rac activity gradient in genetic mutants for specific polarity proteins, with consequent changes in protrusion form and position. We additionally show, using photo-activatable Rac constructs, that it is the level of Rac activity that determines protrusion form, with high levels of Rac activity required to form filopodia and lower levels required to form lamellipodia. Following exposure to pulses of laser light, thereby increasing Rac activity in photo-activatable-Rac expressing cells, we are able to convert lamellipodial protrusions to filopodial protrusions in the living animal.

It has long been known that polarity proteins are essential in maintaining epithelial cell shape, with loss of apicobasal polarity being a prerequisite for epithelial-to-mesenchymal transitions, and abnormal cell invasiveness. Our data provide a mechanism by which polarity proteins can influence Rac activity and the actin cytoskeleton, thereby ensuring the maintenance of epithelial cell shape, and consequently the architecture of the epithelium as a whole.

D135 A STRIPAK-like complex regulates axonal transport of autophagosomes and dense core vesicles by modulating PP2A activity. *A. L. Neisch*, T. P. Neufeld, T. S. Hays. University of Minnesota, Minneapolis, MN.

Axonal transport is essential for neuronal function and survival, and dysregulation of this transport is associated with neurodegenerative disorders. Axonal transport is precisely regulated by adaptor and scaffolding protein complexes that mediate the linkage and transport of cargoes by kinesin and dynein motor proteins. In an RNAi screen for autophagosome transport defects we identified Connector of kinase to AP-1 (CKA), a scaffolding protein with a previously uncharacterized role in axonal transport. CKA is a regulatory subunit of the phosphatase PP2A and a scaffolding protein for the Striatin-interacting phosphatase and kinase (STRIPAK) complex. We find that CKA, together with the core STRIPAK components Mob4 and Strip, regulates retrograde axonal transport of autophagosomes and dense cores vesicles. Additionally we show that CKA is in a complex with dynein, suggesting that a STRIPAK-like complex may regulate dynein activity. Our results reveal that CKA also interacts directly with the autophagosomal membrane protein Atg8, potentially providing a linkage between dynein and its autophagosome cargo. Furthermore, we show that CKA's ability to bind and regulate PP2A is required for dense core vesicle transport. Decreased PP2A activity is linked to neurodegenerative disease, and we find that dysregulation of PP2A activity by CKA disrupts axonal transport, which may contribute to disease progression. These findings demonstrate that a STRIPAK-like complex functions to regulate the transport of a subset of organelles important for both protein turnover and neuronal signaling, two processes essential for neuronal survival.

D136 Centrosomal proteins are required for autophagy to maintain neural homeostasis. *Y. Zheng*, O. Cabrera, J. Chen, B. Dietrick, R. Buchwalter, L. Kao, T. Megraw. Florida State University, Tallahassee, FL.

The centrosome is the major microtubule-organizing center in animals. Mutations in centrosomal genes cause primary microcephaly (MCPH), a neurological disorder characterized by smaller brain size due to insufficient neurogenesis, highlighting the importance of centrosome proteins in neural development and in maintaining neural health. Yet the etiological basis for MCPH and the role of centrosome proteins in its pathogenesis remains unclear. To explore the etiological basis of MCPH, we performed 2-D difference gel electrophoresis in combination with mass spectrometry to identify molecular signatures of MCPH mutant brains in Drosophila. Proteomic analysis led us to discover a novel role of

centrosomal proteins-regulation of autophagy. Autophagy is a major means by which cells clear misfolded or aggregated proteins and damaged organelles that contribute to the decline of neuronal health. We show that mutations in two essential centrosome proteins in Drosophila, *centrosomin (cnn)* and *sas-4*, impair starvation-induced autophagy and accumulate dramatically more protein aggregates. Consistently, *cnn* mutant larvae and flies are much more sensitive to starvation and have poor locomotor function compared to wild-type. Transmission electron microscopy analysis showed that Cnn regulates early steps in the assembly of autophagosomes, the double membrane structures that deliver cargos for lysosomal degradation. Mechanistically, our preliminary data showed that Cnn could act through a novel partner protein, an E3 ubiquitin ligase, to regulate autophagy. Overexpression of this E3 ligase induces autophagy, reduces cell size and causes tissue degeneration dependent on its E3 ligase activity. These findings reveal a novel role of centrosomal proteins and implicate defective autophagy as a novel function for centrosome proteins and a novel etiological basis for MCPH.

D137 Centrosome-pole cohesion requires Abnormal Spindle and Calmodulin to ensure proper centrosome inheritance in neural stem cells but is dispensable for brain size. *T. Schoborg*, A. Zajac, C. Fagerstrom, R. X. Guillen, N. M. Rusan. Naitonal Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD.

The interaction between microtubule organizing centers (MTOCs) and spindle poles is critical for the establishment and maintenance of the mitotic apparatus in many organisms. However, our understanding of the dynamics of this relationship and the potential implications for tissue homeostasis remains largely unexplored. Here we report that the microcephaly-associated protein, Abnormal Spindle (Asp), plays a key role in maintaining centrosome-pole attachments and pole focusing in *Drosophila* neural stem cells. Complete loss of function mutations in *asp* cause centrosome detachment from poles shortly after metaphase, leading to free-ranging centrosomes that randomly move around the cell until anaphase onset. As a consequence, centrosome inheritance is randomized, with neural stem cells either losing their centrosome or retaining both following asymmetric division. Furthermore, we show that Asp's spindle function is dependent on the calcium-sensing protein Calmodulin (CaM). Both proteins colocalize on spindles and dynamically move towards spindle poles with similar velocities, suggesting that they form a complex with CaM acting as a regulator of Asp. Our direct binding assay and structure-function analysis of Asp support this hypothesis. However, the Asp-CaM interaction is dispensable for head and brain size, and the spindle defects observed in neural stem cells of *asp* mutants do not correlate with microcephaly phenotypes. Instead, the ability of Asp to suppress microcephaly is conferred by an unknown domain in the N-terminus of the protein through a mechanism that is currently under investigation.

D138 Local Adaptation and the Establishment of Inversions in Natural Populations of *Drosophila pseudoobscura* Through the Indirect Effects of Suppressed Recombination. *Zachary Fuller*[1], Gwilym Haynes[1], Stephen Richards[2], Stephen Schaeffer[1]. 1) Penn State, University Park, PA; 2) Baylor College of Medicine, Houston, TX.

Despite the expected homogenizing effects of extensive gene flow, the third chromosome of *Drosophila pseudoobscura* is polymorphic for > 30 gene arrangements that are distributed over a stable gradient in frequency across the American Southwest. Furthermore, no chromosome arrangement has been detected at complete fixation in any ecological niche and heterokaryotypes can form at appreciable frequencies. Inversions may become established in populations due to (1) direct effects of the inversion mutation, (2) indirect effects of suppressed recombination where different arrangements capture adaptive alleles and maintain associations of multiple selected genes, (3) the capture of a single sweeping adaptive allele, or (4) random genetic drift. We tested hypotheses regarding the establishment of inversions by sequencing the complete genomes of 54 *D. pseudoobscura* strains that include multiple copies of six common arrangements. Additionally, we quantified gene expression across three life stages and tested for differential gene expression between arrangement homokaryotypes and heterokaryotypes. We find evidence for high levels of genetic differentiation across inverted regions of the third chromosome and observe a significant overabundance of derived allele and amino acid frequency changes. The number of selected genes within an arrangement is positively correlated to the size of the inversion mutation, which is consistent with models of local adaptation that find that the size of an inversion is proportional to the number of genes contributing to local adaptation. Inverted regions also harbor significantly different patterns of gene transcription between homokaryotypes, yet in heterokaryotypes we find prevalent additive interactions of gene copies derived from different chromosome arrangements. Genes involved in odorant and sensory perception pathways are significantly enriched for both differential expression and derived amino acid changes. Our results provide evidence that inversions have become established in *D. pseudoobscura* through the indirect effects of suppressed recombination that have maintained genetic and transcriptional differences across arrangements. We further expand upon existing theory and propose a multi-locus model to explain the existence of a stable gradient as a result of local adaptation across a heterogeneous environment.

D139 *Cis*-regulatory basis of expression divergence between recent gene duplicates. *K. Tanaka*[1], L. Baudouin-Gonzalez[1], M. Santos[1], É. Sucena[1,2]. 1) Instituto Gulbenkian de Ciência, Oeiras, PT; 2) Faculdade de Ciências da Universidade de Lisboa, Lisbon, PT.

Gene duplication plays a major role in evolution of novel gene functions as it provides a material basis for selection to act upon. How *cis*-regulatory landscapes are altered after duplication events to contribute to expression divergence is not well understood. Here we address this question by studying a recent duplicate pair originating at the base of drosophilids. The *Drosophila* Ly6 genes *CG9336* and *CG9338* arose through the tandem duplication of a single ortholog still present outside Drosophilidae. Despite their recent origin, the paralogs in *D. melanogaster* have diverged in embryonic tissue-specificities from each other as well as from the unduplicated ortholog. While retaining the ancestral expression in the glia, both genes acquired a novel expression in the embryonic heart and *CG9338* alone in the hemocytes. Interestingly, these two novel expression domains are only present in subsets of *Drosophila* species examined, suggesting that their acquisitions may have been lineage-specific.

To elucidate the *cis*-regulatory basis of expression divergence, we first mapped the enhancer activities of the duplicated locus in *D. melanogaster*. We identified two enhancers driving the conserved glial expression, one located in the 3' intergenic region of *CG9336* and the other in the second intron of *CG9338*. Interestingly, the two fragments also showed activities in the heart and the hemocytes, respectively. Homologous regions from four additional *Drosophila* species all showed the conserved glial activity. However, they had a variable capacity to drive heart and hemocyte expression matching the species-specific mRNA expression. These results suggest that the pre-exiting glial enhancers inherited by each duplicate were differentially modified to acquire novel tissue-specific activities. Our study delineates a possible trajectory of enhancer evolution at a duplicated locus, which culminated in transcriptional divergence between the paralogs.

D140 Beyond the tip of the iceberg: New *Drosophila* reference genomes reveal novel structural variants. *M. Chakraborty*, Anthony Long, J. J. Emerson. University of California, Irvine, CA.

Identifying functionally important sequence variants in a genome is a key step in studying phenotypic evolution and uncovering disease causing mutations. Variation that copies, deletes, or rearranges chromosome segments has been shown both to be ubiquitous and phenotypically important. However, our ability to study such structural variation is hobbled by incomplete and fragmented genomes assembled from short reads. Recent advances in long read sequencing technology makes studying copy number variation (CNV) and transposable elements (TE) far easier, revealing a vast reservoir of genetic variation that was previously invisible. Here we report a new assembly and SV analysis of a *Drosophila melanogaster* strain, called A4, using PacBio long reads. The A4 genome is accurate, complete, and exceeds sequence contiguity of even the *D. melanogaster* reference genome assembly. Using this high quality genome, we discovered previously unknown gene sequences, like *Mitf* on chromosome 4, in the heterochromatic regions. Additionally, comparative genomics between the A4 and the reference genome ISO1 revealed a large number of CNVs, 40% of which were invisible to previous methods relying on PE Illumina data. Nearly 20% of the A4 genome is made up of TEs, with hundreds of new insertions distributed non-uniformly across the genome. In order to characterize the population genetics and phenotypic consequences of structural variation, we chose to sequence the 15 Drosophila Synthetic Population Resource (DSPR) founder strains (www.flyrils.org). We have generated a list of genetic variants, including both SNP and SV from the platinum grade DSPR assemblies. These data provide a comprehensive platform for understanding evolution and the phenotypic consequences of structural variation.

D141 Functional and evolutionary consequences of epigenetically silenced transposable elements in euchromatin. *Grace Yuh Chwen Lee*[1], Gary Karpen[1,2]. 1) Lawrence Berkeley National Lab, Berkeley, CA; 2) University of California, Berkeley, CA.

Transposable elements (TEs) are genome parasites that can increase their copy number at the expense of host fitness. Natural selection against deleterious TE insertions is a potent evolutionary mechanism for counterbalancing the constant increase in TE copy number. There is a rich understanding of the deleterious effects of TEs that are mediated by physical disruption of DNA, such as insertions of TEs into functional elements. Instead, we focus on the largely unexplored *epigenetic* consequences of TE insertions. In *Drosophila,* euchromatic TEs can be epigenetically silenced via small RNA-dependent enrichment of heterochromatic marks, which can spread to and influence the function of adjacent sequences. We hypothesized that this spread of heterochromatic marks from euchromatic TEs has deleterious functional impacts and leads to selection against individual TEs, both of which were supported by our previous genomic study. We further investigated the evolutionary consequences of TE's epigenetic effects in natural populations using wildtype *D. melanogaster* strains and the closely related *D. simulans.* In these wild-derived genomes, we also observe enrichment of heterochromatic marks in sequences adjacent to TEs, suggesting that the epigenetic effects of TEs are a general phenomenon and can have a significant role in the evolutionary dynamics of TEs in natural populations. The vastly different positions of TE insertion sites between the genomes of wildtype strains allowed for unprecedented quantification of the epigenetic effects of TEs. More than half of the TE insertions show enrichment for silencing marks in adjacent sequences. This spreading of silencing marks from TEs can extend up to 20kb (average 4kb) and lead to maximally a 2.5 fold (average 48%) increase in heterochromatic mark enrichment, demonstrating the pervasive epigenetic effects of TEs in host genomes. Importantly, TE families that show more extensive spread of heterochromatic marks have lower population frequencies, which again supports our evolutionary hypothesis. Interestingly, TE families that show significant spread of heterochromatic marks are enriched for TE families displaying recent horizontal transfer between *Drosophila* species, as well as those targeted by the endo-siRNA pathway. Furthermore, the type of TEs that show the strongest epigenetic effects is different between *D. melanogaster* (LTR TEs) and *D. simulans* (TIR TEs), which echoes the well-known differences in the predominant type of TEs in these two host genomes (LTR in *D. melanogaster* and TIR in *D. simulans*). We will report on ongoing investigation aimed at identifying the functional causes and evolutionary implications of these observations.

D142 Co-evolution within the nuclear branch of the *Drosophila* piRNA pathway. *S. Parhad*, S. Tu, B. Koppetsch, Z. Weng, W. Theurkauf. Univ Massachusetts Med Sch, Worcester, MA.

piRNA pathway protects the genome from transposons. Many piRNA pathway genes are rapidly evolving, consistent with a host-pathogen arms race between the pathway and transposons. To directly assay for the functional consequences of piRNA pathway protein evolution, we rescued null mutation in *D. melanogaster rhino (rhi)* gene with *rhi* from the sibling species *D. simulans.* The *rhino* gene encodes a rapidly evolving HP1 homolog that associates with piRNA producing loci termed clusters, and has an essential function in piRNA biogenesis and transposon silencing. Expression of *sim-rhino* fails to complement null *D. melanogaster rhi* mutants, while expression of *mel-rhino* completely restores fertility. Small RNA sequencing and RNA-seq analyses show that *sim-rhi* is equivalent to a null allele. Rhino is composed of chromo, hinge and shadow domains. We therefore swapped individual *D. simulans* domains into a *D. melanogaster*-Rhino backbone and assayed for transgenic rescue of fertility, transposon silencing, and piRNA production. Hybrid proteins carrying the *D. sim* shadow domain are equivalent to

a null allele, and mass spec analysis of proteins associated with full length and domain substitution hybrids indicate that the *D. sim* shadow domain fails to interact with Deadlock, which form a complex with Rhino and recruits additional components of the nuclear piRNA precursor processing machinery. These observations indicate that Rhino and Deadlock are co-evolving, which has generated a species-specific interface, which may be the target for transposon encoded inhibitor that drives an arms race between the piRNA pathway and mobile elements.

D143 Lineage-specific rapid gains of satellite DNA in Drosophila. *K. H. C. Wei*, A. G. Clark, D. A. Barbash. Cornell, Ithaca, NY.

Tandemly repeating DNA elements known as satellite DNA occupy significant portions of Drosophila genomes. As they are prone to cause genomic instability through non-allelic exchange, they are predominantly sequestered in the repressive heterochromatin near the centromeres and telomeres, as well as on the Y where they accumulate as the chromosome degenerates. Interestingly, the types and abundances of satellites vary dramatically between closely related species, suggesting that they are turning-over at high rates. Yet they are often ignored in genome assemblies due to low-sequence complexity and repetitiveness, leading to poor understanding of the evolutionary dynamics underlying the observed rapid changes. Here, we identify and quantify simple satellites using the program k-Seek on whole genome sequences of nine Drosophila species, spanning 40 million years of evolution. We find that very few satellites are shared across species, consistent with the expectation of rapid turn-over. However, when we determined the gains and losses of satellite DNA along the Drosophila phylogeny based on parsimony, we found that most of the interspecific differences are due to lineage-specific gains of satellites; loss events, in comparison, are extremely rare. The rates of gain also differ greatly among lineages, as some species appear to have acquired few to no satellites, while others, including those in the *melanogaster* complex, have accumulated large numbers in a relatively short time. To separately test for satellite age, we identified heterogeneity in satellites caused by mutations and found that older satellites indeed have significantly more indels than younger satellites. In addition, we identified Y-linked satellites via enrichment in males, and found that some Ys are, unexpectedly, satellite-poor, such as the young neo-Ys in the species of the *obscura* group. Our results indicate that within Drosophila species, the vast differences in satellite DNA are predominantly driven by rapid accumulations in only some species.

D144 Repetitious elements drive silencing in the *Drosophila melanogaster* genome through heterochromatin formation. *Sarah C. R. Elgin*[1], Michael Lee[1], Tingting Gu[2], Pui Pik Law[3], Richard Festenstein[3], Elena Gracheva[1]. 1) Washington University, St Louis, MO; 2) Nanjing Agricultural University, Nanjing, China; 3) Imperial College, London, UK.

Within eukaryotic genomes, repetitious sequences are often silenced. Within the *Drosophila melanogaster* fourth chromosome, the DNA transposon remnant *1360* is an effective target, promoting silencing of an adjacent *hsp70*-driven *white* gene; this results in a Position Effect Variegation (PEV) phenotype. However, outside of the fourth chromosome, PEV is only observed when the reporter construct *P*{*1360, hsp70-white*} is present within or close to a heterochromatic domain (Haynes et al 2006 Curr Biol 16: 2222-7). At the 1198 "landing pad" site at the base of chromosome arm 2L, a copy of either *1360* or *Invader4* (a retrotransposon remnant) can switch a euchromatic region to a heterochromatic one, with increased HP1a and H3K9me2; here the variegating *hsp70-white* shows a ~2-fold decrease in expression (Sentmanat & Elgin, PNAS 109:14104-9). Recently we observed that 256 copies of a 36 bp *lacO* fragment induces even stronger PEV, an ~8-fold decrease in expression of the *hsp70-white* reporter at this site. Surprisingly, *lacO* mediated silencing shows an inverse temperature effect, with a loss of silencing at lower temperatures (G. Reuter; EG). A fragment with 310 copies of the GAA triplet repeat in the 1198 site similarly results in eight-fold silencing. All three cases exhibit a loss of silencing on introduction of HP1a mutations, confirming that heterochromatin assembly is required. While mutations in the H3K9 methyl transferase *Su(var)3-9* are dominant suppressors of *1360*- and GAA_{310}-induced silencing, this is not the case for *lacO*-induced silencing at 25°C. We find that *lacO*-induced silencing, the PEV observed in the w^{m4} line, and the PEV phenotype of a reporter in the pericentric heterochromatin (118E-10 line), are all sensitive to nicotinamide, an HDAC inhibitor, supporting a general role for histone deacetylation in heterochromatin formation in Drosophila. The heterochromatin-based mechanism for silencing the tandem *lacO* repeats apparently differs in some important aspects from that used to silence TEs, while the silencing induced by GAA_{310} mimics that seen at TEs and in pericentric heterochromatin generally. Supported by NIH GM068388, NSF MCB-1243724, & NSF MCB-1517266.

D145 Establishment and maintenance of heritable patterns of chromatin structure during early embryogenesis. *S. A. Blythe*, E. F. Wieschaus. Princeton University, Princeton, NJ.

Throughout the life cycle, the functional specializations of cells and tissues are directly reflected in the patterns of chromatin structure. During embryogenesis, genetic loci involved in cell fate specification and differentiation –committed decisions that restrict a cell's developmental potential– must be packaged in such a way that they can ultimately respond to precise spatial and temporal developmental cues. Furthermore, a mechanism must exist for faithfully replicating such chromatin states in daughter cells following mitosis. We have measured by ATAC-seq how the initial patterns of chromatin structure are established and maintained during early *Drosophila* embryogenesis with three-minute time resolution for the three cell cycles preceding the midblastula transition (MBT). During this period, the embryo undergoes large-scale zygotic genome activation, and initiates the process of pattern formation. However, because such embryos are also simultaneously engaged in mitotic nuclear amplification, one longstanding question has been to what extent stable chromatin structure could be established in the context of an extremely rapid cell cycle. We find that extensive heritable chromatin structure can be established under these conditions. Over this period, thousands of genomic loci acquire chromatin accessibility at specific times, in response to different biological timing mechanisms. Enhancers, for example, are accessible from very early in development, whereas the majority of promoters acquire accessibility at or around the MBT. The majority of these promoters gain accessibility in response to the biological timer that measures the nucleo-cytoplasmic ratio and controls the onset of the MBT. In addition, we observe that once established, these patterns of accessibility

are maintained in metaphase chromatin, suggesting that the major mechanism for epigenetic inheritance of embryonic chromatin structure relies on acquisition of mitotic stability or chromatin "bookmarking".

D146 Deciphering double strand break repair in heterochromatin and euchromatin using an *in vivo* Drosophila model. *Aniek Janssen*[1], Gregory Breuer[2], Eva Brinkman[3], Annelot van der Meulen[1], Sean Borden[1], Timothy Lee[1], Bas van Steensel[3], Ranjit Bindra[2], Jeannine LaRocque[4], Gary Karpen[1,5]. 1) Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA; 2) Departments of Therapeutic Radiology and Experimental Pathology, Yale School of Medicine, New Haven, Connecticut, USA; 3) Division of Gene Regulation, Netherlands Cancer Institute, Amsterdam, the Netherlands; 4) Department of Human Science, School of Nursing & Health Studies, Georgetown University, Washington DC, USA; 5) Department of Molecular and Cell Biology, UC Berkeley, Berkeley, California, USA.

Repair of DNA double strand breaks (DSBs) must be properly orchestrated within the diverse nuclear chromatin regions in order to maintain genome stability. The choice between the two main DSB repair pathways: non-homologous end joining (NHEJ) and homologous recombination (HR), is dictated by the cell cycle as well as chromatin context. Constitutive heterochromatin, which forms a dense, generally transcriptionally silent structure near centromeres and telomeres, is essential for maintaining genome stability and is enriched for repetitive DNA sequences. The compact nature of this chromatin structure and the presence of many repetitive sequences make heterochromatin potentially vulnerable to erroneous DSB repair.

Several studies have revealed that heterochromatic regions display specialized temporal and spatial responses to DNA damage that differ from repair in euchromatin. Irradiation (IR)-induced damage has been shown to result in relaxation of the heterochromatin domain and relocalization of resected DSBs to outside the heterochromatin domain, where HR is thought to be the main mode of repair.

Here, we have developed an *in vivo* single DSB system specifically targeted to heterochromatic as well as euchromatic loci. We find, using live imaging of larval discs, that single DSBs can recapitulate the heterochromatin-specific spatial and temporal DSB dynamics previously seen with IR-induced breaks in cell culture.

Importantly, we identify a prominent role for both the NHEJ and HR pathways in heterochromatic DSB repair and find that the homologous chromosome can be used as a template for HR repair in both eu- and heterochromatin.

These data suggest that, although DSBs in heterochromatin and euchromatin are subject to diverse spatiotemporal dynamics, repair pathway choice is ultimately highly similar in these two distinct chromatin regions.

D147 The Drosophila Y chromosome acts as a heterochromatin sink and contributes to sex-specific aging. *Emily Brown*, Doris Bachtrog. University of California, Berkeley, Berkeley, CA.

The Drosophila Y chromosome is gene poor and primarily consists of epigenetically silenced repetitive DNA, but nonetheless influences expression of thousands of genes genome-wide, possibly by sequestering heterochromatin machinery away from other positions in the genome. To directly test the influence of the Y chromosome on heterochromatin genome-wide, we assayed the genomic distribution of histone marks associated with constitutive heterochromatin (H3K9me2 and H3K9me3) in flies with varying sex chromosome complements (XO, XY, and XYY males, and XX and XXY females). We find that the number of Y chromosomes strongly influences the genome-wide enrichment patterns of repressive chromatin marks. Highly repetitive regions are enriched for heterochromatin marks in wild-type males and females, and even more so in XO males. In contrast, additional Y chromosomes in XXY females and XYY males dilute repressive marks away from normally silenced, repeat-rich regions, which is accompanied by a de-repression of Y-linked repeats. This suggests that the Y chromosome can sequester heterochromatic factors, thus acting as a heterochromatin sink.

We also investigated the role of the Y chromosome and heterochromatin in sex-specific aging. In most XY taxa, males have a shorter average lifespan than females, and accumulating evidence suggests that chromatin mis-regulation, and particularly loss of heterochromatin associated with aberrant expression of repetitive elements, contributes to organismal aging. To investigate whether the Y chromosome's role as a heterochromatin sink could contribute to the shorter male lifespan, we collected lifepan and RNA-seq data from XY, XO, and XYY males, and XX and XXY females. Our results suggest that the Y chromosome contributes to a shorter lifespan, as individuals with a Y chromosome, independent of sex, have a shorter lifespan. Additionally, repetitive elements show greater mis-regulation during aging in individuals with a Y chromosome, especially Y-linked repeats, suggesting that the Y chromosome's influence on heterochromatin may contribute to a shorter average lifespan in males.

D148 Progenitor expansion and competence are controlled by Lsd1, PRC2 and non-coding RNAs. *M. Lee*[1,2], A. C. Spradling[1,2]. 1) Carnegie Institution of Washington, Baltimore, MD; 2) HHMI.

Progenitors must expand sufficiently to achieve final tissue size, while retaining chromatin flexible enough to produce multiple cell types yet receptive to external signals. The Drosophila follicle cell stem cell (FSC) generates daughters that divide nine times to generate a large progenitor pool ideally suited for studying progenitor differentiation. Previously, we showed that antagonistic actions between lysine-specific demethylase 1 (Lsd1) and Trithorax (TRX) methyltransferase dynamically modulate methylation levels at histone lysine 4 (H3K4) to maintain plastic epigenetic states in early progenitors that are crucial for proliferation and to time differentiation (Lee and Spradling (2014) Genes Dev . 28, 2729). Here, we further report a coordinated interaction between an Lsd1 complex and Polycomb Repressive Complex 2 (PRC2) that modifies chromatin states to time progenitor differentiation. All three PRC2 core components (i.e., E(z), ESC and Su(z)12) regulate epigenetic plasticity and modulate progenitor competence during follicle progenitor differentiation. Moreover, our evidence suggests that the Lsd1 complex and PRC2 are coordinated by noncoding RNAs. These results argue that poised chromatin states are dynamically modulated by regulating methylation levels at H3K4 and H3K27 to control cell proliferation and competence during progenitor differentiation.

D149 A somatic piRNA pathway in the *Drosophila* fat body ensures metabolic homeostasis and normal lifespan. *S. L. Helfand*, B. C. Jones, J. G. Wood, C. Chang, A. Tam, M. J. Franklin, E. Siegel. Brown Univ, Providence, RI.

Transposable elements (TEs) are mobile sequences of DNA that parasitize the genomes of their hosts. Due to their replicative ability, TEs account for large portions of their host genomes including nearly 30% of the fly genome and 45% of the human genome. To combat the invasion and expansion of TEs, small RNA (smRNA) silencing pathways have evolved to suppress TEs across species from plants to humans. Such pathways are essential in maintaining genomic integrity by preventing TE transposition that could disrupt normal gene function in otherwise healthy tissues. The Piwi-interacting (piRNA) pathway, classically associated with gonadal tissues, employs 23-29nt smRNAs complexed with argonaute proteins to actively suppress TEs in the germline of animals. However, recent evidence has begun to suggest that components of this pathway may in fact be present in somatic tissues of multiple species including flies, mice, monkeys, and humans. Here we provide evidence of a functional somatic piRNA pathway in the adult *Drosophila* abdominal fat body including the presence of the piRNA effector protein Piwi and canonical 23-29nt long piRNAs that map to TEs and piRNA clusters known to produce TE piRNAs. We also observed fat body genic piRNAs that map to 3'UTRs, another known source of piRNAs in the gonads. *piwi* mutants exhibit depletion of both TE- and 3'UTR-mapping piRNAs, increased TE transcript levels, TE transposition in the fat body, altered metabolic gene expression, and reduced lipid and glycogen reserves. Finally, *piwi* mutants are also sensitive to starvation, immunologically compromised, and have shortened lifespans, all hallmarks of compromised tissue function in the fat body. These findings demonstrate the presence of a functional non-gonadal somatic piRNA pathway in the adult fat body that impacts normal metabolism, immunological function, and overall organismal health.

D150 The sexual identity of adult intestinal stem cells controls organ size and plasticity. *B. Hudry*, S. Khadayate, I. Miguel-Aliaga. Imperial College London, London, GB.

Male and female animals often differ in their metabolism and physiology. Genetic sex determination factors are known to play a key role during development in the formation of reproductive organs and secondary sexual characteristics, which could indirectly lead to physiological differences between the sexes. However, less is known about the maintenance and possible roles of sex determination mechanisms in fully developed organs. We have used the *Drosophila melanogaster* intestine to investigate the nature and significance of intrinsic sex determination pathways in an adult somatic organ *in vivo*. We find that the adult intestinal epithelium displays extensive sex differences in expression of genes with roles in growth and metabolism. We have focused first on one cell subpopulation, the intestinal stem cells (ISCs), to characterize the molecular mechanisms involved, and to establish their functional significance. Cell-specific reversals of the sexual identity of adult ISCs uncovers that this identity has a key role in controlling organ size, reproductive plasticity and response to tumorigenic insults. Unlike previous examples of sexually dimorphic somatic stem cell activity, the sex differences in ISC behaviour arise from intrinsic mechanisms that control cell cycle duration and involve a new *doublesex*- and *fruitless*-independent branch of the sex differentiation pathway downstream of *transformer*. Together, these findings indicate that the plasticity of an adult somatic organ is reversibly controlled by its sexual identity, imparted by a new mechanism, which may be active in more tissues than previously recognized. More recently, we have extended our characterization of these sex differences to cell types other than somatic stem cells. We find that the sexual identity of different - but lineage-related - cell types in the adult fly is maintained through different mechanisms, and are currently exploring the possible metabolic significance of such mosaicism.

D151 Mechanisms underlying sexually dimorphic growth. *Annick Sawala*, Alex P. Gould. The Francis Crick Institute, Mill Hill Laboratory, London, United Kingdom.

Sexual size dimorphism (SSD) is widespread throughout the animal kingdom. In *Drosophila*, sex differences in body size arise during larval development and result in females that are 20-40% larger than males. Signalling mechanisms that regulate larval growth and final body size have been identified, but how they are differentially regulated in males and females to produce SSD is not well understood. A recent study reported that the sex determination gene *transformer* (*tra*) acts in the fat body to promote female-specific body growth (Rideout et al. 2015, PLoS Genetics). Here we show that *Sex-lethal* (*Sxl*), an upstream gene in the sex determination pathway, functions in the nervous system to upregulate female body growth in a non-autonomous manner. Nervous system-specific knockdown of Sxl in females is sufficient to convert their body to a male size, completely abrogating SSD. Conversely, female body size in *sxl* mutants can be rescued by expressing Sxl in only the nervous system. We find that Sxl functions specifically in peptidergic and GABAergic neurons to promote body growth in females. Current research focuses on mapping the Sxl neural circuitry regulating female-specific growth.

D152 Body weight dependent autophagy induction mediates metamorphic timing control under nutrient restriction in Drosophila. *Xueyang Pan*[1,2], Michael O'Connor[1]. 1) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN; 2) Molecular, Cellular, Developmental Biology and Genetics Program, University of Minnesota, Minneapolis, MN.

In Drosophila, the timing of post-embryonic developmental transitions are finely correlated with body growth, and also respond to environmental variants such as food availability. During L3 stage, fasting condition causes arrest of metamorphic transition when larvae do not reach a body weight threshold (critical weight), however, beyond the threshold metamorphosis is no longer postponed by starvation. It is well known that insulin/TOR signaling couples nutrient condition and metamorphic timing in prothoracic gland (PG), the larva endocrine organ making the molting hormone ecdysone. However, how larva body weight poses impact on metamorphic timing control, especially during nutrient deficiency, is not clearly understood.

In this study we find that macroautophagy (hearafter autophagy), a degradative cellular process found in various larva tissues, functions in PG as a body weight sensitive controller of metamorphosis during nutrient restriction (NR). We firstly detected autophagy induction in PG during

NR, which tightly requires Atg genes and can be suppressed by insulin/TOR pathway. Intriguingly, NR-induced autophagy is only dynamic in early L3 stage but is gradually muted as larvae grow heavier. Upon autophagy suppression early L3 NR causes undersized larvae to pupariate precociously, leading to pupal lethality, whereas over-induction of autophagy causes developmental delay even in well fed larvae. Beyond confirming autophagy as a gatekeeper of metamorphosis, we further find that Anaplastic Lymphoma Kinase (Alk), a receptor tyrosine kinase, works as autophagy suppressor during late L3 NR. The Alk expression in PG keeps marginal until early L3 stage and boosts as larvae grow over the critical weight, indicating its functioning time window. Expression of constitutively active Alk in PG prevents NR-induced autophagy in early L3 stage, while knocking down Alk leaves autophagy markedly stimulated during late L3 NR. Besides autophagy, Alk activation larvae also phenocopy insulin pathway activation larvae on developmental timing and final body size, suggesting that Alk receptor may lead to similar signaling pathways to insulin receptor and thus compensate the loss of insulin signaling in PG during late L3 NR. In all, our findings uncover a novel view on how developmental timing is controlled in accordance with body growth.

D153 A SANT-like domain-containing protein regulates lipid droplet size. *X. Huang*, Y. Yao, X. Li, Z. Liu. IGDB, CAS, Beijing, Beijing, CN.

Lipid droplets are the main lipid storage sites in cells. The size of LDs varies greatly under different physiological conditions. Despite many studies reporting factors important for lipid storage, the underlying mechanisms of LD size regulation are still not fully understood. Through a large-scale overexpression screen in the *Drosophila* larval fat body, we found that a SANT-like domain-containing protein is critical for LDs size regulation. Overexpressing this protein leads to enlarged LDs, while mutations have small LDs. Proteins with SANT-like domain have been previously linked to chromatin remodeling. Here we provide multiple evidences suggest that this SANT-like domain-containing protein may links to NURF chromatin remodeling complex via interacting with PZG. Therefore, our work provides a new insight of lipid droplet size regulation.

D154 Identification and Characterization of a Novel Gene that Regulates Mitochondrial DNA Replication. *J. Tang*, Y. Zhang, H. Xu. National Institute of Health, bethesda, MD.

Mitochondrial DNA (mtDNA) replication is essential for mitochondrial function in response to developmental and physiological demand. Impaired mtDNA replication leads to developmental defects, aging and diseases. To identify novel genes involved in the regulation of mtDNA replication, we conducted an in vivo RNAi screen in *Drosophila* and identified a novel gene, EmptyHouse. We found that the protein encoded by EmptyHouse is associated with the mitochondrial inner membrane. EmptyHouse mutant has severe developmental delay and is lethal during pupa and adult stages. Genetic analysis indicates that this gene has an essential role in mtDNA replication. Loss of this gene results in impaired mitochondrial oxidative phosphorylation activity. The human homolog, WBSCR16, is one of the genes that flanking the Williams-Beuren syndrome (WBS) commonly deleted region. The function of WBSCR16 in human is completely unknown. We expressed WBSCR16 in human tissue culture cells and found it expressed in mitochondria. Knocking down WBSCR16 expression in human tissue culture significantly slows down the mtDNA replication rate. Further characterization of EmptyHouse mutant and its human homolog may provide insight into the mechanism of regulation of mtDNA replication during development and the pathogenesis of diseases.

D155 Circadian mutants lacking either *period* or *timeless* have an extended longevity phenotype due to altered mitochondrial function. *M. M. Shirasu-Hiza*, M. J. Ulgherait, C. R. Wayne, A. Chen, S. F. McAllister, M. A. Oliva. Columbia University Medical Center, New York, NY.

We found that arrhythmic mutants lacking the critical circadian regulators *period* or *timeless* exhibit extended lifespan relative to controls. Circadian rhythm is known to affect a wide range of vertebrate physiologies, including many known to impact aging and lifespan. We set out to ask if loss of circadian regulation alters longevity. Unexpectedly, we found that male *period* and *timeless* mutants are long-lived relative to controls. Focusing on male *per* mutants, we found that they do not achieve longevity by mimicking diet restriction: to the contrary, *per* mutants eat more than controls and lack changes in metabolism and signaling pathways typically associated with diet restriction and longevity. While *per* mutants appear to age more slowly than controls, as judged by intestinal and muscle health, their extended longevity is also independent of microbiota and activity. Our results suggest instead that altered mitochondrial function leads to longevity in these mutants. We observed changes in *per* mutants' mitochondrial morphology and DNA copy number that correlate with extended longevity; genetic experiments suggest that their longevity is not due to mitophagy but due to mitohormesis. We are currently investigating specific, circadian-regulated changes in mitochondrial function that underlie *per* mutants' longevity phenotype.

D156 Histidine metabolism perturbations inhibit neural tumours dependent on Myc-mediated dedifferentiation. *F. Froldi*[1], M. Szuperák[1,3], P. Pachnis[2], O. Costas[1], T. Fernando[2], A. P. Gould[2], C. Y. Cheng[1]. 1) Peter MacCallum Cancer Centre, Melbourne, Victoria, AU; 2) The Francis Crick Institute, London, UK; 3) University of Pennsylvania, Philadelphia, PA.

Metabolic rewiring is a hallmark of cancer and it is becoming increasingly clear that metabolic adaptations in tumours are not only driven by proliferative demands, but are highly context-dependent. In this study, we explore how the genetic profile of tumours determines how these respond to perturbations in amino acid metabolism. Using a combination of dietary manipulations and genetics, we show that histidine deprivation or knockdown of histidine metabolic enzymes inhibits the growth of neural tumours induced by *nerfin-1* loss of function. *nerfin-1* encodes for a zinc-finger transcription factor required to maintain neurons in a differentiated state. Upon its loss, post-mitotic neurons dedifferentiate and acquire a stem cell fate in a step-wise process that relies on Myc-dependent cellular growth. Manipulations of histidine metabolism inhibit *nerfin-1* tumour growth by reducing Myc expression and nucleolar volume, and decreasing the overall rate of dedifferentiation. Interestingly, neural tumours caused by Notch hyperactivation, which are the result of a Myc-dependent reversion of

intermediate neural progenitors to stem cells also show a similar sensitivity to histidine metabolism inhibition. In contrast, none of these perturbations have major effects upon the growth of normal neural stem cells, epithelial tumours or *prospero* mutant neural tumours. This study shows that the sensitivity of tumours to dietary manipulations depends on the tumour genetic profile but also on its origin and mode of growth. Moreover, the metabolic vulnerabilities we identified have the potential to inform the search of metabolic interventions that specifically inhibit tumours without affecting normal cells.

D157 Ecology of the gut microbiome determines fly health. *W. B. Ludington*, V. Zhang, Z. T. Guvener. UC Berkeley, Berkeley, CA.

Gut microbiome diversity is thought to be good for animal health. However, no solid body of evidence supports the generalities of that claim, and several recent papers have shown that germ free flies live longer than flies with their typical bacteria. Here we present evidence that intermediate levels of microbial diversity in the *Drosophila melanogaster* gut promote fly health even when a suitably nutritious diet is provided. We assembled a gnotobiotic fly colonized with all of the bacterial strains consistently found in our laboratory stock flies, of which there are two *Lactobacilli* and three *Acetobacter* for a total of five strains. We then measured fly development time with every possible combination of the defined bacterial strains for a total of 32 combinations. We find overall that flies colonized with any single bacterial strain are the slowest to develop, but that flies colonized by all strains are also slow to develop. On nutritionally poor diets, however, some bacteria speed up development while others are detrimental. In flies colonized with combinations of bacterial strains, benign strains can offset the effects of detrimental strains. In a limited set of gnotobiotic fly lifespan experiments, we see a similar overall trend whereby germ free flies and flies with intermediate levels of microbial diversity live the longest.

We used the gnotobiotic fly development time data to build a microbial epistatic interaction map (i.e. a food web) to determine how microbe-microbe interactions affect fly development, noting an abundance of weak interactions. Separate measurements of microbial interactions inside the fly gut indicate that detrimental bacterial strains can promote colonization by beneficial strains. These combinations are more beneficial to the fly, providing an ecological mechanism by which specific microbial interactions and not just absolute strain numbers account for the health phenotypes we observe. In vitro growth assays and bioinformatics suggest syntrophic exchange of nutrients, specifically lactic acid and B-vitamins, as the mechanisms for these microbial interactions inside the fly.

We propose that maintaining a diverse gut microbiome serves as a bet hedge against the more severe consequences of dominance by detrimental strains.

D158 Genome-wide spatial-temporal gene expression pattern prediction in Drosophila melanogaster embryonic development. *J. Zhou*[1], I. Schor[3], V. Yao[1], O. Troyanskaya[1,2], E. Furlong[3]. 1) Princeton University, Princeton, NJ; 2) Simons Foundation, New York, NY; 3) European Molecular Biology Laboratory, Heidelberg, Germany.

Spatial-temporal gene expression patterns are fundamental information for understanding embryonic developmental program and tissue-specific gene functions. High-throughput *in situ* experiments have provide abundant measurements for *Drosophila* embryogenesis, but a high proportion of protein coding genes (>5000) have not been measured and the primary output of these experiments are qualitative. A genome-wide data-integration approach will complement the current spatial-temporal *in situ* data by training machine learning models that integrate all public expression and chromatin profiling data to provide genome-wide and quantitative predictions for all genes. To systematically predict spatial-temporal expression patterns in 282 tissue-stages of *Drosophila melanogaster* embryonic development, we developed structured *in silico* nano-dissection, a computational approach that predicts tissue and developmental stage specific gene expression using cell lineage information and gene co-regulation patterns from a diverse compendium of 6,378 genome-wide expression and chromatin profiling samples. Our method employs a two-stage approach, the first stage trains a predictor for each tissue-stage category, and the second stage integrates all predictions with a global cell lineage based probabilistic graphical model. We systematically evaluated our performance on holdout genes, and validated new predictions by literature and performing new experiments. On genes without previously known spatial temporal patterns or literature evidence for tissue-specificity, we verified the predictions by new *in situ* hybridization. Among all 13 *in situ* experiments with detectable expression signal, all five predicted genes for brain primordium and eight predicted genes for embryonic muscle systems were verified. The co-expression patterns of these genes in other tissues such as gut and plasmatocytes, as well as temporal patterns across developmental stages were also correctly predicted. Furthermore, we show that our spatial-temporal expression predictions can be applied to detect the tissue specificity signals in *twi*, *mef2*, and *trh* mutant embryos, demonstrating its potential in analyzing tissue specificity signal from non-tissue-dissected experiments. Our resource together with exploratory tools are available athttp://find.princeton.edu.

D159 Measuring exercise in *Drosophila:* Characterization of the Rotating Exercise Quantification System (R.E.Q.S.). *L.Patrick. Watanabe*, Nicole Riddle. University of Alabama at Birmingham, Birmingham, AL.

Recent estimates show that more than 78.6 million adults are obese in the United States with the annual medical cost at $147 billion dollars in 2008. The most common treatments for obesity come in the form of a dietary change or an increase in physical activity (exercise). Despite the popularity of exercise as a treatment for obesity, very little is known about how an individual's genetic background impacts his/her response to exercise. This has become increasingly relevant as new studies have indicated that up to 20% of the population may be "exercise non-responders", individuals who are programmed – genetically or epigenetically – to have weak or absent metabolic responses to exercise. In order to elucidate the genetic mechanisms underlying exercise response, we have developed an innovative exercise system for *Drosophila melanogaster* named the Rotating Exercise Quantification System (R.E.Q.S.). This system allows for the real-time quantification of exercise in Drosophila. One interval and one continuous exercise regime were performed on three fly lines from the Drosophila Genetics Reference Panel 2 (DGRP2). Our results show that there is significant variability in exercise levels between these lines, enough so to perform Genome Wide

Association Studies (GWAS) using the entire panel of 200 lines to identify candidate genes. This study validates the use of rotation-based exercise and demonstrates the potential for Drosophila as a model in the field of exercise genetics.

D160 Features and Applications of FlyCircuit Database – From Fluorescent Images to the Drosophila Connectome. *C. T. Shih*[1,2], A. S. Chiang[3,4]. 1) Tunghai University, Taichung, TW; 2) National Center for High-Performance Computing, Hsinchu, TW; 3) National Tsing-Hua University, Hsinchu, TW; 4) Kavli Institute for Brain and Mind, CA.

FlyCircuit (www.flycircuit.tw) is a database containing more than 28,000 image entries of individual neurons in the Drosophila brain. The database was constructed for online data archiving, browsing, searching, analysis, and 3D visualization for the single neurons. Using the genetic mosaic analysis with a repressible cell marker (MARCM), the single neurons were labeled with green fluorescent protein (GFP). Also, the birth date during the brain development and putative neurotransmitter for each neuron could be predicted with this transgenic technique. To handle these terabytes of image data, a fully automatic image-processing workflow was introduced to reconstruct the single neurons from the fluorescent raw images to their three-dimensional digital reconstruction.

With the image data in FlyCircuit, the structural connectome of the fruit flies could be assembled at different scales. The mesoscopic connectome was composed of the brain regions called the local processing units (LPUs) and their directed and weighted links. It showed small-world characteristics, hierarchical modular structure, and rich-club organization. The network consisted of five functional modules – olfactory, auditory, left and right visual centers, and the pre-motor center. Major loops with length from three to five were identified. Many loops were found which could sustain the electrophysiological signals for a longer period.

Microscopic connectome composed of the neuron-to-neuron connections was also analyzed. The network also showed hierarchical modular structure. Interestingly, the modules were corresponding to the functional modules in the mesoscopic version, rather than the expected LPUs or neuropils in the brain anatomy. The numbers of loops with length up to six in the brain network were significantly larger than their counterparts in the random networks. Finally, rich club formed from a population of neurons with high global centrality scores was also detected.

D161 High-speed imaging of neural spiking and dendritic dynamics in awake flies with a fluorescent voltage sensor. *Cheng Huang*[1], Yiyang Gong[1,2,4], Mark Schnitzer[1,2,3]. 1) James H. Clark Center, Stanford University, Stanford, CA; 2) CNC Program, Stanford University, Stanford, CA; 3) Howard Hughes Medical Institute, Stanford, CA; 4) Duke University, Durham, NC.

Genetically encoded voltage indicators (GEVIs) are a promising technology for visualizing the millisecond-scale dynamics of specific neuron types and their fine arbors in live animals, which presently neither electrophysiological nor Ca^{2+} imaging techniques do well. For researchers studying *Drosophila*, optical voltage imaging with GEVIs offers newfound possibilities to investigate cellular and sub-cellular electrophysiological phenomena that remain largely unexplored due to the technical difficulty of *in vivo* intracellular electrical recording methods. Here, to showcase the capabilities for optical voltage imaging in the intact fly brain, we imaged neuronal voltage dynamics in several parts of the fly olfactory system using our recently developed GEVI, Ace2N-mNeonGreen (Gong *et al., Science* 2015), in which a fast voltage-sensing domain from a rhodopsin family protein is coupled to a bright protein fluorophore through resonance energy transfer. Ace2N-mNeonGreen is sufficiently bright and fast to report neural action potentials and membrane voltage dynamics in awake flies, and resolves fast spike trains with 0.2-millisecond timing precision at spike detection error rates orders of magnitude better than prior GEVIs. *In vivo* imaging revealed sensory-evoked responses, including somatic spiking, dendritic dynamics, intracellular voltage propagation, and inter-hemispheric differences in neuronal activity. These results empower *in vivo* optical studies of neuronal electrophysiology and motivate novel experimental designs that will allow researchers to relate high-speed neuronal dynamics and information processing to fruit fly behavior..

D162 Effective knockdown of *Drosophila* long noncoding RNAs by CRISPR interference. *J. L. Liu*, S. Ghosh, C. Tibbit. Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3PT, United Kingdom.

Long noncoding RNAs (lncRNAs) have emerged as regulators of gene expression across metazoa. Interestingly, some lncRNAs function independently of their transcripts - the transcription of the lncRNA locus itself affects target genes. However, current methods of loss-of-function analysis are insufficient to address the role of lncRNA transcription from the transcript which has impeded analysis of their function. Using the minimal CRISPR interference (CRISPRi) system, we show that co-expression of the catalytically inactive Cas9 (dCas9) and guide RNAs targeting the endogenous *roX* locus in the *Drosophila* cells results in a robust and specific knockdown of *roX1* and *roX2* RNAs, thus eliminating the need for recruiting chromatin modifying proteins for effective gene silencing. Additionally, we find that the human and *Drosophila* codon optimised dCas9 genes are functional and show similar transcription repressive activity. Finally, we demonstrate that the minimal CRISPRi system suppresses *roX* transcription efficiently *in vivo* resulting in loss-of-function phenotype, thus validating the method for the first time in a multicelluar organism. Our analysis expands the genetic toolkit available for interrogating lncRNA function *in situ* and is adaptable for targeting multiple genes across model organisms.

D163 Optimized synthetic lethal screening approaches for drug target discovery in *Drosophila*. *Benjamin E. Housden*[1], Alexander J. Valvezan[2], Colleen Kelley[1], Andrew P. Georgiadis[1], Yanhui Hu[1], Yuanli Wang[1], Stephanie Mohr[1], Brendan D. Manning[2], Norbert Perrimon[1,3]. 1) Harvard Medical School, Boston, MA; 2) Harvard School of Public Health, Boston, MA; 3) Howard Hughes Medical Institute, Boston, MA.

A major approach for the discovery of potential drug targets for tumorigenic disease is based around the identification of synthetic lethality. Synthetic lethality is defined as a genetic interaction where the combined disruption of two genes is lethal but either disruption alone has no

effect. Genes that have synthetic lethal interactions with tumor suppressors are attractive as drug targets for cancers because treatments will specifically kill tumor cells but will have little effect on the surrounding wildtype tissue. However, these interactions have proved difficult to identify using existing screening technologies and those that are identified are often not reproducible between screens.

We combined CRISPR and RNAi in a novel combinatorial screening method in *Drosophila* cells. Using this approach we identified almost 200 robust candidate drug targets for Tuberous sclerosis complex (TSC), a tumorigenic disease caused by mutation of either the TSC1 or TSC2 tumor suppressors. Based on this work, a promising drug is now in development for this disease. However, despite this success, overlap of hits between screens is limited, indicating that many interactions are still missed. We hypothesized that many synthetic lethal effects occur only with incomplete gene knockdown. These interactions are therefore missed in many cases because weak knockdown has no effect and strong knockdown is lethal to both mutant and wildtype genetic backgrounds. We therefore developed an approach called variable dose analysis (VDA), which uses cytometry-based analysis to profile viability effects over a range of knockdown efficiencies in a single population of cells treated with RNAi. Using VDA to compare viability profiles between wildtype and TSC mutant cells, we found that this method has reduced noise compared to previous screening approaches. We were able to detect 83% of known synthetic lethal interactions with TSC compared to only 22% using established screening technologies. This is therefore a robust method to detect synthetic lethality that will likely be applicable to the identification of drug targets for many tumorigenic diseases.

D164 CRISPR/Cas9-based tools for *in vivo* transcriptional activation and repression in *Drosophila*. *B. Ewen-Campen*[1], Lu-Ping Liu[1], Rong Tao[1], Donghui Yang-Zhou[1], Shauiliang Lin[1], Xingjie Ren[3], Sun Jin[3], Jian-Quan Ni[3], Shu Kondo[4], Norbert Perrimon[1,2]. 1) Harvard Medical School, Boston, MA; 2) Howard Hughes Medical Institute; 3) Medicine, Tsingua University, Beijing, China; 4) National Institute of Genetics, Mishima, Shizuoka, Japan.

CRISPR/Cas9-based transcriptional activation (CRISPRa) and repression (CRISPRi) provide a powerful new set of tools for studying gene function *in vivo*. A major advantage of these systems is that any gene-of-interest can be targeted using a ~20bp guide RNA, making this approach versatile and scalable. However, several different dCas9-fusions and sgRNA design rules have recently been published, and the most effective approaches have not been defined. In this study, we directly compare different dCas9-fusion proteins for both CRISPRa and CRISPRi *in vivo*, and identify highly effective approaches for each. We also present a growing collection of publically available *Drosophila* stocks for activating and repressing target genes in various tissues. Together, these studies lay a foundation for our ongoing effort to generate a genome-wide resource for CRISPRa and CRISPRi for the *Drosophila* community.

D165 A novel 96 well system for housing, manipulating and feeding flies. *M. D. Jaime*, B. Oliver. National Institutes of Health , Bethesda, MD.

The sophisticated genetic tools available in *Drosophila*, coupled with the evolutionary conservation of biological processes found in higher organisms, including humans, should encourage the use of whole organism in drug discovery, diet, and toxicology. For high-throughput work, this requires plate formats compatible with standard equipment, minimization of handling, and low volume feeding. To meet these design specifications, we have developed a 3D printed 96-well system called the Whole Animal Feeding Flat (WAFFL). The WAFFL is an apparatus that enables the housing and feeding of flies while reducing the number of manipulations. It has four main units for: 1) food 2) feeder (housing) 3) receiver and 4) transfer adapter. The liquid food (<10ul) is provided to the fly in a round bottom 96 well plate. The feeder plate is an array of 96 deep square shaped chambers where a single fly is housed that tightly fits into the food plate. The feeder plate includes inner ledges upon which a housed fly can perch while extending its proboscis through narrow capillary ports with liquid food. Cameras integrated into the system allow monitoring of behavior. The transfer adapter allows the interconnection of the chambers of the feeder plate to a receiver plate to harvest the flies by centrifugation to a standard deep well plate for homogenization and assay preparation. The WAFFL facilitates the transfer of flies from one condition to another in seconds by simply placing the feeder plate on a different microplate with the condition of interest without disturbing the flies. To ensure that compound doses are delivered at the same time, we used 6 hr treatments on starvation media, followed by 1 hr of feeding. A bolus of food (with dye) was observed and transit time through the gut is < 3 hrs. We have performed several small screens (600 - 800 flies) for exposure to dimethyl sulfoxide (DMSO), ethanol, and different diets. We will present time-course results (~600 flies) on the effect of DMSO on gene expression profiles following exposure to different concentrations of DMSO .The scalable WAFFL system allows for automated handling of individual flies exposed to different foods, drugs, and compounds, providing a unique high-throughput platform for drug screening in flies. The WAFFL and flies are a limitless discovery duo.

D166 Neuronal 3'UTR extension: ELAV links Pol II pausing to alternative polyadenylation. *V. Hilgers*[1], K. Oktaba[1], W. Zhang[1], D. Rio[1], M. Levine[2]. 1) University of California, Berkeley, Berkeley, CA; 2) Princeton University, Princeton, NJ.

Alternative polyadenylation (APA) has been implicated in a variety of developmental and disease processes. A particularly dramatic form of APA occurs in the developing nervous system of flies and mammals, whereby approximately 200 developmental genes undergo coordinate 3'UTR extension. The RNA-binding protein ELAV inhibits RNA processing at proximal polyadenylation sites, thereby fostering the formation of these exceptionally long 3'UTRs.

We found that paused Pol II promotes recruitment of ELAV to its target genes. Replacing promoters of extended genes with heterologous promoters blocks normal 3' extension in the nervous system, while extension-associated promoters can induce 3' extension in ectopic tissues expressing ELAV. Computational analyses show that promoter regions of extended genes tend to contain paused Pol II and associated cis-regulatory elements such as GAGA. ChIP-Seq assays identify ELAV in the promoter regions of extended genes. We provide the first evidence for a regulatory link between promoter-proximal pausing and APA.

Neuron-specific APA is conserved in humans. 3'UTR extensions are enriched in conserved regulatory motifs, indicating that extended mRNAs play a specific role in the regulatory networks that drive neural development and brain function. Our functional genetics studies reveal that deleting 3'UTR extensions in Drosophila results in diverse phenotypes such as neurodegeneration and life span lengthening.

D167 Mutant rescue by inhibition of nonsense mediated decay. *Mark M. Metzstein*. Univ Utah, Salt Lake City, UT.

The nonsense mediated mRNA decay (NMD) pathway targets and degrades mRNAs containing premature termination codons (PTCs). NMD often exacerbates the effects of nonsense mutation containing alleles by preventing the expression of partially-functional truncated proteins. As such, inhibition of NMD is considered to be a broadly applicable approach for treating numerous inherited diseases. In cases in which the truncated protein does not retain function, NMD inhibition may be synergistic with "readthrough" therapy: the administration of drugs that stimulate ectopic translation of PTCs, thus producing full length proteins.

However, before such an approach can be realized, the endogenous roles of the NMD pathway must be understood. In particular, loss of NMD is in itself completely lethal to most organisms, including *Drosophila*, probably because of a role of the NMD pathway in inhibiting the expression of a number of endogenous (non-mutated) transcripts. Recently, we have shown that this requirement of NMD for viability is due to degradation of only a single endogenous NMD target: the mRNA of the stress response gene *Gadd45* (Growth Arrest and DNA Damage)*. In particular, we have found deletion of *Gadd45* can rescue *Drosophila* carrying null mutations in core NMD genes to adulthood.

Based on these findings, we are now in the position to test whether administration of readthrough drugs does indeed work synergistically with loss of NMD in restoring functional protein expression of PTC-containing genes. To do this, we will combine known stop-codon containing alleles with mutations in NMD genes, while simultaneously suppressing NMD mutant lethality using the *Gadd45* deletion. This will be done both with and without drug-induced stop codon readthrough. Ultimately, our data should reveal the underlying relationship between RNA degradation and translation in regulating gene expression of mutated genes, in both an experimental and clinical setting.

*"Degradation of *Gadd45* mRNA by nonsense-mediated decay is essential for viability". Nelson *et al*. (**2016**) *eLife*, In press.

D168 The *Drosophila* hnRNP F/H homolog, Glorund, Uses Two Distinct RNA Binding Modes to Differentially Regulate its Targets. *E. R. Gavis*[1], J. V. Tamayo[1], T. T. Teramoto[2], T. M. T. Hall[2]. 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Laboratory of Structural Biology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC.

Post-transcriptional gene regulation by RNA-binding proteins has proved crucial for developmental events including establishment and patterning of body axes, asymmetric cell fate decisions, and morphogenesis. Among RNA-binding proteins, heterogeneous nuclear ribonucleoproteins (hnRNPs) are notable for their ability to interact with numerous target RNAs and function in multiple aspects of RNA metabolism. The basis for this functional diversity is poorly understood, however. We previously identified Glo as an ovarian repressor of *nanos* (*nos*) translation through its interaction with a double-stranded UA-rich motif in the *nanos* 3' untranslated region (3'UTR). In contrast, human hnRNP F/H proteins are best known for their role in the regulation of alternative splicing through their interaction with single-stranded G-tract sequences. Consistent with this, we have also uncovered a potential role for Glo as a splicing factor. To investigate the multifunctionality of Glo, we determined the crystal structures of Glo's three quasi-RNA recognition motifs (qRRMs) and performed a structure/function analysis. We found that Glo qRRMs recognize G-tracts similarly to hnRNP F but recognize the UA-rich motif in *nos* through a second, noncanonical RNA-binding interface. By assaying the effect of mutations that disrupt each recognition mode *in vitro* on Glo function *in vivo*, we demonstrated that regulation of a subset of Glo's targets *in vivo* is mediated solely using the G-tract binding mode whereas regulation of *nos* requires both modes of recognition. This latter result led to the identification a G-tract in the *nos* 3'UTR that mediates Glo binding and is indeed required for *nanos* regulation. Finally, we establish that Glo's requirement for both RNA binding modes to regulate *nos* translation reflects a requirement for both motifs in the *nos* 3'UTR.

D169 The TREX complex suppresses piRNA precursor splicing and promotes assembly of piRNA cluster heterochromatin. *G. ZHANG*, Shikui Tu, Zhiping Weng, William Theurkauf. UMass Medical School, Worcester, MA.

The PIWI interacting RNA pathway silences transposons and maintains genome integrity during germline development. In *Drosophila*, piRNA precursors are generated from discrete genomic loci, called piRNA clusters. The nuclear factors Rhino, Cutoff and Deadlock appear to be dedicated to processing germline cluster transcripts in *Drosophila* ovaries, and all three proteins localize to germline cluster chromatin. Germline cluster transcripts are not spliced, and mutations in *rhino, cuff* and *deadlock* that block piRNA production also lead to cluster transcript splicing. UAP56 is an ATP dependent RNA binding protein that functions in general mRNP biogenesis. We previously showed that UAP56 interacts with piRNA precursors and that a point mutation in UAP56 (*uap56-sz15*) predicted to weaken RNA binding specifically disrupts piRNA biogenesis and increases cluster transcript splicing. The THO complex and UAP56 forms the evolutionarily conserved TREX (transcription/export) complex. Using immunoaffinity purification and mass spectroscopy from fly ovaries lysate, we confirm the *Drosophila* UAP56 interacts with the THO complex. Significantly, we also show that this point mutation in *uap56-sz15* significantly reduces binding to the THO complex. We also show that THO complex components co-localize to nuclear foci with Rhino. Similar to UAP56, THO complex binds specifically to unsplicing cluster transcripts. Mutation in *thoc7,* one of the core components of the THO complex, disrupts localization of Rhino, Cuff and UAP56. It also blocks piRNA production and leads to global defects in transposons silencing. In addition, *thoc7* mutation increases cluster transcript splicing. We propose that stable cluster transcripts binding by the TREX complex is required to suppress piRNA precursor splicing, and promotes cluster chromatin assembly.

D170 From egg to adult: piRNA-mediated silencing throughout germline development in *Drosophila melanogaster*. *P. Marie*, S. Ronsseray, A. Boivin. IBPS, CNRS, Universite Pierre et Marie Curie, Paris, France.

In metazoan germ cells, transposable element activity is repressed by small noncoding PIWI-associated RNAs (piRNAs). Numerous investigations in *Drosophila* have enlightened the mechanism of this repression in the adult germline. However, very little is known about piRNA-mediated repression during germline development. Nevertheless, to maintain the integrity of the genome, repression should occur throughout lifespan of germ cells. Here, we show that piRNA-mediated repression is active in the female germline from late embryonic to pupal primordial germ cells, and that genes related to the adult piRNA pathway are required for repression during development. *rhino*-dependent piRNAs exhibiting the molecular signature of the piRNA pathway "ping-pong" amplification step are detected in larval gonads. Furthermore, as in adult ovaries, we can observe an incomplete, bimodal and stochastic repression resembling variegation at all developmental stages. We strongly suggest, using clonal analyses, that this variegation reflects a cellular memory of an early repression decision taken in embryonic germ cells. This study shows that piRNAs and their associated proteins are the epigenetic components of a continuous repression system throughout germ cell development.

D171 Nano-exons in Drosophila. *Stephen M. Mount*[1,2], Yifei Shi[1,2], Ashley E. Nazario-Toole[1,3], Louisa Wu[1,3]. 1) Dept. Cell Bio. & Mol. Genetics, University of Maryland, College Park, MD; 2) Center for Bioinformatics and Computational Biology, University of Maryland, College Park, MD; 3) Institute for Bioscience and Biotechnology Research, University of Maryland, MD.

Nano-exons are exons less than 4 nt. long. Because nano-exons are so small, they often go undetected, and the number of nano-exons remains unknown. We have examined a single high-quality RNA-seq data set in depth using a bioinformatics pipeline to discover and quantify nano-exons. These data are derived from Drosophila hemocytes, both wild-type and expressing an *A2bp1* RNAi transgene. *A2bp1* is a member of the highly conserved Fox-1 family of RNA-binding proteins. Human homologs *RBFOX1*, *RBFOX2* and *RBFOX3* have been linked to brain development, cardiac function, and autism spectrum disorders, and have been implicated in the regulation of microexons less than 51 nt. in humans. Our analysis has revealed new potential nano-exons in Drosophila, including eight previously undescribed nano-exons with strong support. We also found three likely cases of recursive splicing with deletion that can be considered to be exons of size -1, meaning that sequences within an intron, including the sequence AGT, result in the removal of one nucleotide due to recursive splicing. Each of these three cases (in *Rpl8*, *orb* and *Axn*) occurs within highly conserved 5' UTR sequences, but their regulatory significance remains unknown. Ongoing research explores the occurrence of nano-exons in other Drosophila data sets and in other species.

D172 Identifying genetic modifiers of FUS toxicity in a drosophila model of ALS. *Udai Pandey*[1], John Monaghan[1], Daniel Johnson[2], Lawrence Reiter[2], Ian Casci[1]. 1) University of Pittsburgh Medical Center, Pittsburgh, PA; 2) University of Tennessee Health Science Center.

Amyotrophic lateral sclerosis (ALS) is the most common form of Motor Neuron Disease, and is characterized by the loss of both upper and lower motor neurons. Recently, mutations in genes that code for RNA-binding proteins have been linked to ALS pathology, suggesting that perturbation of RNA metabolism may be the cause of disease onset. Mutations of the gene Fused in Sarcoma (*FUS*), which codes for the protein FUS, have been linked to both familial and sporadic forms of ALS. FUS is a DNA/RNA-binding protein that plays critical roles in RNA metabolism including RNA trafficking and alternative splicing. While investigating the molecular mechanisms of FUS in ALS, the following observations emerged that have formed the foundation of our ongoing research program. We found that RNA binding abilities of FUS are required for causing neurodegeneration, cytoplasmic mislocalization, and incorporation of mutant FUS into cytoplasmic RNA granules (also called stress granules). We observed that ALS-causing mutations perturb the ability RNA granules to rapidly disassemble as compared to control preventing the proteins and RNA constituents to dissociate in the cytoplasm.

Using a *Drosophila melanogaster* model for FUS-associated ALS that was developed by our laboratory, we performed an unbiased genetic screen to identify modifiers of ALS-associated phenotypes. One gene identified in this screen, muscleblind (*mbl*), is the *Drosophila* homolog of human muscleblind-like (*MBNL*). MBNL is also an RNA-binding protein involved in regulating alternative splicing. Muscleblind-like proteins have been linked to several neurodegenerative diseases, and understanding how MBNL can modulate FUS toxicity in ALS will help to elucidate its role in other diseases, including myotonic dystrophy, Huntington's disease and spinocerebellar ataxia. We found that RNAi-mediated knockdown of *Drosophila* mbl rescues neurodegenerative phenotypes caused by ALS-associated mutant FUS in our model. We performed RNA sequencing using *Drosophila* brains expressing WT or mutant FUS with or without *mbl* to understand molecular mechanisms of mbl mediated suppression. Our RNA sequencing approach identified several genes whose expression is altered when FUS is overexpressed, and subsequently returned to almost normal following knockdown of endogenous mbl. Quantitative, reverse transcription, polymerase chain reaction (Q-RT-PCR) confirmed expression changes of identified genes. Taken together, the results of these experiments not only provide new insights into the mechanisms by which mutant FUS is toxic in patients with ALS, but they will also have broader implications for other related neurodegenerative diseases.

D173 A high-throughput pipeline for the production of synthetic antibodies for analysis of ribonucleoprotein complexes. *J. D. Laver*[1], H. Na[1], J. Jeon[1], F. Singh[1], K. Ancevicius[1,2], Y. Fan[1], W. X. Cao[1], K. Nie[1], Z. Yang[1], H. Luo[1], M. Wang[1,3], O. Rissland[1,3], J. T. Westwood[2], P. M. Kim[1], S. S. Sidhu[1], C. A. Smibert[1], H. D. Lipshitz[1]. 1) University of Toronto, Toronto, Ontario, CA; 2) University of Toronto at Mississauga, Mississauga, Ontario, Canada; 3) Hospital for Sick Children, Toronto, Ontario, Canada.

Post-transcriptional regulation of mRNAs plays an essential role in the control of gene expression. mRNAs are regulated in ribonucleoprotein (RNP) complexes by RNA-binding proteins (RBPs) along with associated protein and noncoding RNA (ncRNA) cofactors. A global understanding of post-transcriptional control in any cell type requires identification of the components of all of its RNP complexes. We have previously shown

that these complexes can be purified by immunoprecipitation using anti-RBP synthetic antibodies produced by phage display. To develop the large number of synthetic antibodies required for a global analysis of RNP complex composition, we have established a pipeline that combines (i) a computationally aided strategy for design of antigens located outside of annotated domains, (ii) high-throughput antigen expression and purification in *Escherichia coli*, and (iii) high-throughput antibody selection and screening. Using this pipeline, we have produced 279 antibodies against 61 different protein components of *Drosophila melanogaster* RNPs. Together with those produced in our low-throughput efforts, we have a panel of 311 antibodies for 67 RNP complex proteins. Tests of a subset of our antibodies demonstrated that 89% immunoprecipitate their endogenous target from embryo lysate. We have successfully used synthetic antibodies in RNA co-immunoprecipitation experiments to identify the entire complement of mRNAs associated with each of three different RBPs – Staufen, Pumilio, and Brain Tumor – analysis of which provided significant insights into the functions of each of these proteins. This panel of antibodies will therefore serve as a valuable resource for global studies of RNP complexes in *Drosophila*. Furthermore, our high-throughput pipeline permits efficient production of synthetic antibodies against any large set of proteins.

D174 Intercellular Ca^{2+} transients integrate spatiotemporal morphogenetic patterning in the *Drosophila* wing imaginal disc. *Qinfeng Wu*, Pavel Brodskiy, Cody Narciso, Jeremiah Zartman. University of Notre Dame, Notre Dame, IN.

Tissue development requires instructive input from multiple morphogenetic signals. To guide cellular decision making, cells must correctly integrate these diverse signals as well as coordinate behavior with their neighbors. Calcium (Ca^{2+}) is a ubiquitous second messenger that plays important roles in many physiological activities such as fertilization, proliferation and apoptosis and is regarded as a central signal integrator. While extensive work has identified core components of Ca^{2+} signaling, the regulation between Ca^{2+} signaling and morphogen pathways is still poorly understood. Here we report a novel phenomenon of oscillating, intercellular Ca^{2+} transients (ICT) in *Drosophila* wing imaginal discs, and their relation to morphogen signaling. We found that the ICT exhibits a patterned behavior, both spatially and temporally. We have elucidated the Ca^{2+} pathway components that are responsible for ICT through a combination of genetic and chemical perturbations using an *ex vivo* imaging assay. We also demonstrated that Ca^{2+} dynamics are modulated by signaling pathways including Bone Morphogenetic Protein (BMP) and Hedgehog (Hh) signaling. These observations lead to a working model of Ca^{2+} signaling as a mediator of signal integration for morphogen pathways.

D175 Rewiring regulatory feedback in BMP morphogen signaling. *J. Gawlik*[1,2], A.-H. Springhorn[1,2], G. Pyrowolakis[2,3]. 1) Spemann Graduate School for Biology and Medicine (SGBM), Freiburg, Germany; 2) BIOSS-Centre for Biological Signalling Studies, Freiburg, Germany; 3) University of Freiburg- Institute of biology I, Freiburg, Germany.

Proper growth and patterning of tissues and organs require tight control of intercellular signaling which is assured by the existence of different feedback regulatory mechanisms. We are interested in identifying the impact of feedback regulators in BMP morphogen signaling and their influence on the establishment and maintenance of graded signaling. In Drosophila BMP morphogen signaling one regulatory feedback loop involves the BMP receptor Thickveins (Tkv). In the wing imaginal disc, BMP dependent transcriptional repression of tkv keeps the level of the receptor low near the source of the BMP ligand Decapentaplegic (Dpp). This allows efficient spreading of the ligand and the formation of a long range Dpp gradient. In contrast, in the haltere precursor, the evolutionary homologue of the wing, the Hox gene Ubx disables BMP from repressing tkv and high receptor levels trap the ligand and restraint its spreading. The resulting short-range Dpp gradient has been suggested to contribute to the smaller size of the haltere. We will report on the molecular circuit implementing differential tkv regulation. In particular, we will show how BMP target genes, previously assigned as effectors of the pathway, interact with Ubx and with cis–elements on tkv regulatory modules to differentially affect tkv transcription in the wing and haltere precursors. Based on our cis-analysis we use genome editing approaches to actively reshape tkv expression, the shape of the Dpp gradient and final organ size *in vivo.* Our results provide insights on the mechanisms by which evolution rewires regulatory feedback circuitry to produce signaling gradients of new shapes and, consequently, organs of different size and ultimately new function.

D176 Minibrain and Wings apart control organ growth and tissue patterning through downregulation of Capicua. *L. Yang*[1], S. Paul[1], K. Trieu[1], F. Froldi[2], K. Harvey[2], L. Cheng[2], G. Jimenez[3], S. Shvartsman[4], A. Veraksa[1]. 1) Umass Boston, Boston, MA; 2) Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia;; 3) Institut de Biologia Molecular de Barcelona-CSIC, and Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain; 4) Princeton Univ., Princeton, NJ, USA.

The transcriptional repressor Capicua (Cic) controls tissue patterning and restricts organ growth, and has been recently implicated in several cancers. Cic has emerged as a primary sensor of signaling downstream of the receptor tyrosine kinase (RTK)/extracellular signal-regulated kinase (ERK) pathway, but how Cic activity is regulated in different cellular contexts remains poorly understood. We found that the kinase Minibrain (Mnb, ortholog of mammalian DYRK1A), acting through an adaptor protein Wings apart (Wap), physically interacts with and phosphorylates the Cic protein. Mnb and Wap inhibit Cic function by limiting its transcriptional repressor activity. Downregulation of Cic by Mnb/Wap is necessary for promoting the growth of multiple organs, including the wings, eyes, and the brain, and for proper tissue patterning in the wing. We have thus uncovered a previously unknown mechanism of downregulation of Cic activity by Mnb and Wap. This mechanism operates in parallel to ERK-dependent control of Cic, indicating that Cic functions as an integrator of upstream signals that are essential for tissue patterning and organ growth. Finally, since DYRK1A and CIC exhibit, respectively, pro-oncogenic versus tumor suppressor activities in human oligodendroglioma, our results raise the possibility that DYRK1A may also downregulate CIC in human cells.

D177 Muscle derived TGF-β growth factor Myoglianin regulates size of imaginal wing discs. *Ambuj Upadhyay*, Aidan Peterson, Michael O'Connor. University of Minnesota, Minneapolis, MN.

How the size of animal appendages are coordinated to scale with body size has been a longstanding question in developmental biology. We are using the *Drosophila* wing imaginal disc as a model to identify novel genes and mechanisms which finely tune this process. In the wing imaginal disc, Decapentaplegic (Dpp), a member of the Transforming Growth Factor β (TGF- β) superfamily of ligands and homologous to vertebrate Bone Morphogenic Protein (BMP) 2/4, signaling plays a crucial role in regulating organ size and patterning. In contrast, little is known about the related branch TGF-β/Activin pathway in regulating organ size. Here we show that *myoglianin* (*myo*), a TGF-β/Activin ligand, is required for proper growth of wing, haltere, and leg imaginal discs. The smaller imaginal discs of *myo* mutants phenocopies *baboon*, the TGF-β/Activin Type-I receptor, mutants. Using tissue specific RNAi we show that only Baboon-a isoform, but not Baboon-b or Baboon-c, is required for growth. Surprisingly, we also found that only the muscle derived Myoglianin, but not the glial derived, is required for proper growth of the wing disc. In summary, our work has identified a novel function of Myoglianin in wing disc growth control. More importantly this study highlights how the size of appendages like the insect wing can be modulated by structural organs like the body wall muscle using long range TGF-β ligands. In our future studies we will explore the intracellular molecular mechanism of Myo and Baboon signaling to determine whether they feed into the same pathways as the Dpp branch. .

D178 The Drosophila tumor suppressor Tid/Alg3 controls TNFR/JNK signaling through glycosylation. *Geert de Vreede*[1,4], Holly Morrison[2], Ditte Andersen[3], Julien Colombani[3], Pierre Leopold[3], David Bilder[1]. 1) Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) Department of Pediatrics, University of California, San Francisco, San Francisco, CA; 3) University of Nice-Sophia Antipolis, CNRS, INSERM, Institute of Biology Valrose, Parc Valrose, Nice, France; 4) Developmental Biology, Utrecht University, Utrecht, The Netherlands.

Drosophila tumor suppressor genes have given great insight into the control of tissue size by cell signaling and organization. However, the molecular events required for appropriate organ growth remain poorly understood. Here we report that the Drosophila tumor suppressor *tumorous imaginal discs (tid)*, whose phenotypes were previously attributed to mutations in a DNAJ-like chaperone, are in fact driven by the loss of the N-linked glycosylation pathway component ALG3. *tid/alg3* imaginal discs display tissue architecture defects and moderate overgrowth, sharing characteristics of both 'neoplastic' and 'hyperplastic' mutants. Apicobasal polarity is largely intact, but growth-promoting Hippo signaling is upregulated, and suppressing Yki blocks tumorous overgrowth. Aberrant Yki activity results from excess JNK signaling through the recently described TNF receptor homolog Grindelwald (Grnd), and requires the Eiger/TNF ligand. Interestingly, Grnd contains an N-linked glycosylation site in its ligand binding domain, while loss of N-linked glycosylation alters Egr-Grnd signaling and leads to ectopic Grnd activation in *tid/alg3* mutant discs. Our results suggest that TNFR glycosylation modulates JNK signaling through modifying ligand-receptor interactions, opening up a potential model for general TNFR signaling regulation.

D179 A kinome-wide RNAi screen in *Drosophila* glia and human GBM models reveals Stk17A drives neoplastic glial proliferation. *Joanna Wardwell-Ozgo*[1], Colleen Mosley[1], Harley Kormblum[2], Renee Read[1]. 1) Emory University School of Medicine, Atlanta, GA; 2) Intellectual and Developmental Disabilities Research Center, The Jonsson Comprehensive Cancer Center and Departments of Psychiatry, Molecular and Medical Pharmacology, and Pediatrics, David Geffen School of Medicine, University of California Los Angeles,.

Glioblastoma multiforme (GBM), the most common primary malignant brain tumor, is highly proliferative, diffusely invasive, and incurable by current therapies. Genetic and molecular analyses reveal that GBMs frequently harbor activating mutations in the EGFR receptor tyrosine kinase (EGFR) and Phosphoinositide 3-kinase (PI3K) signaling pathways. While the ability of these mutations to drive gliomagenesis has been verified in mouse models, current data also reveal that EGFR and PI3K signaling cooperates with as yet unknown factors to drive tumor pathology in GBM. To uncover novel genes that augment EGFR- and PI3K- dependent neoplasia, we leveraged use of our novel *in vivo Drosophila* glioma model and performed a cross-species, multidisciplinary genetic modifier screen in both *Drosophila melanogaster* and mammalian GBM model systems that identified multiple suppressors of neoplastic growth including, drak, a cytoplasmic serine-threonine kinase.

Our preliminary results in both *Drosophila* and mammalian GBM model systems indicate that drak and its human ortholog, Stk17A, is necessary for GBM cell proliferation. Our data also illustrate that overexpression of drak in combination with oncogenic EGFR mutations is sufficient to promote increased glial cell proliferation. Furthermore, in our *in vivo Drosophila* model, we find that drak overexpression stimulates increased phosphorylation and activity of spaghetti squash (sqh), non-muscle myosin II regulatory light chain, a known drak substrate and key regulator of the cell cycle and cellular motility, which itself is required for neoplastic glial transformation. Finally, oncogenomic analysis of GBMs and other gliomas reveal that Stk17A becomes overexpressed in association with EGFR mutations, and Stk17A copy-gain and overexpression are significantly associated with poor prognosis in patients.

Together, our data suggest that drak potentiates the oncogenenic effects of EGFR through the upregulation of sqh activity and suggests that Stk17A may serve as a novel therapeutic target for EGFR-PI3K dependent GBMs and high-grade gliomas. Current work is aimed at furthering our understanding of drak/Stk17A's contribution to tumor pathology. We are specifically exploring the molecular function of drak/Stk17A and the signaling pathways in which drak/Stk17A enacts oncogenic transformation.

D180 Wnt proteins serve as directional cues for the Par-complex polarity and the *Drosophila* nervous tissue growth. *S. Yoshiura*, F. Matsuzaki. RIKEN CDB, Kobe, Hyogo, JP.

Correct orientation of cell polarity and division is important for the directional tissue growth and morphogenesis, but the mechanism underlying this process is remained unclear. Here, we identified multiple Wnt proteins act as directional cues to regulate the orientation of the

cell polarity and division of *Drosophila* neural stem cells, leading to the correct orientation of the nervous tissue growth. Wnt proteins play essential roles in the efficient formation and orientation of the Par-complex polarity in the neural stem cells through Frizzled/beta-Catenin signaling, which in turn dictate the central-to-peripheral polarity of the nervous tissue. Our results unveil the novel function of the Wnt signaling in the regulation of the tissue morphogenesis.

D181 Motile stem cells exhibit tissue-level spatial order during homeostasis but not growth of the adult *Drosophila* midgut. *X. Du*, J. Martin (co-first), S. Balachandra, I. Riedel-Kruse, L. O'Brien. Stanford University, Stanford, CA.

Many self-renewing, solid organs contain stem cell populations that are dispersed throughout the expanse of the tissue. Spatial dispersal ensures that each tissue field contains stem cells. How dispersed stem cells maintain proper spacing during organ renewal is unknown. Here we combine quantitative spatial analyses with long-term live imaging to investigate the underlying basis of stem cell spacing. Comprehensive statistical maps of stem cell-stem cell distances reveal that stem cells are spatially ordered during steady-state organ renewal, but not during adaptive organ growth. Overnight imaging of midguts within living animals, together with tissue-wide cell tracking and spatio-temporal analysis of individual cell displacements, shows that stem cells are autonomously motile. Individual cells, propelled by actin-rich protrusions, flatten and slither beneath the basal epithelium. At the population level, stem cell motility appears stochastic in frequency and direction. Progenitor-specific depletion of motility factors disrupts the spatial ordering of stem cells, implying that motility is needed for tissue-level dispersal. Our findings show that spatial order is a hallmark of steady-state but not expanding stem cell populations and suggest that autonomous motility is involved in stem cell distribution during tissue homeostasis.

D182 Identification of Alzheimer's disease as a neurodegenerative laminopathy. *Bess Frost*[1], Farah Bardai[2], Mel Feany[2]. 1) University of Texas Health Science Center, San Antonio, TX; 2) Brigham and Women's Hospital, Boston, MA.

Hutchinson-Gilford progeria syndrome (HGPS) is a fatal disorder involving the appearance of accelerated aging in young children. HGPS is caused by a mutation in the lamin A gene, *LMNA*, the study of which has provided fundamental insights into basic cellular biology and the biology of normal aging. Cellular consequences of lamin dysfunction in progeria and other "laminopathies" resulting from mutations in *LMNA* include invaginations and herniations of the nuclear envelope and relaxation of heterochromatic DNA. The segmental nature of progeria has been a longstanding mystery in aging research: certain aspects of aging appear to be recapitulated in the syndrome, while others, particularly neurodegeneration, are not. We have found that acquired lamin misregulation though aberrant cytoskeletal-nucleoskeletal coupling mediates neuronal death in a *Drosophila* model of Alzheimer's disease and related tauopathies. In addition, we observed robust alterations in the nuclear lamina in neurons from patients with Alzheimer's disease. To demonstrate definitively that lamin dysfunction can promote brain aging, we depleted lamin function genetically in the *Drosophila* nervous system and observed markedly decreased lifespan accompanied by age-dependent neurodegeneration. Our findings challenge the paradigm that the brain is resistant to lamin dysfunction, and identify new avenues for the therapeutic treatment of Alzheimer's disease and related tauopathies.

D183 Defects in synaptic vesicle endocytosis are caused by TDP-43 dependent translation inhibition in a Drosophila model of ALS. *A. Coyne*, J. Johannesmeyer, D. Zarnescu. University of Arizona, Tucson, AZ.

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease affecting upper and lower motor neurons. TDP-43, an RNA binding protein linked to the majority of ALS cases, is involved in several aspects of RNA metabolism. Using a *Drosophila* model of ALS based on TDP-43 we have previously identified a role for TDP-43 in the translation regulation of specific mRNA targets. Here we use a combination of genetic, molecular, and imaging approaches to show that TDP-43^{G298S} but not TDP-43^{WT} regulates the translation of *hsc70-4* mRNA. Hsc70 is a molecular chaperone that functions at multiple steps in the synaptic vesicle cycle. FM1-43 dye uptake experiments reveal defects in endocytosis and a reduction in the size of the readily releasable and recycling vesicle pools in both TDP-43^{WT} and TDP-43^{G298S} variants. However, upon overexpression of Hsc70, endocytosis is restored specifically only in the disease associated mutant. Genetic interaction approaches reveal the ability of synaptic proteins namely cysteine string protein (CSP), dynamin, clathrin, lap, and auxilin to modulate TDP-43 mediated locomotor dysfunction. The results of these experiments suggest that synaptic vesicle cycling defects resulting from TDP-43 expression occur during early endocytic events at the presynaptic membrane. In addition, overexpression of Hsc70 in the context of TDP-43 mitigates multiple aspects of TDP-43 toxicity including locomotor dysfunction and reduced lifespan. Notably, this rescue is dependent on both the chaperone and membrane bending activities of Hsc70 as evidenced by genetic interactions with Hsc70 ATPase or membrane binding mutants, respectively. Thus, both chaperone and membrane bending activities, both of which contribute to the maintenance of synaptic protein pools, are affected upon TDP-43 overexpression and contribute to ALS phenotypes and toxicity. Our results provide the first evidence for TDP-43 regulating the synaptic vesicle cycle through the translational regulation of its synaptic mRNA targets. Thus, altered ribostasis, a key event in the progression of ALS, contributes to synaptic failure preceding neurodegeneration in ALS pathogenesis.

D184 Glial expression of spen confers a Notch-dependent resistance to paraquat. *Nathalie Davoust*[1], Valérie Goubard[1], Matthieu Quérenet[1], Laurent Seugnet[2], Victor Girard[1], Laurent Pays[3], Serge Nataf[3], Bertrand Mollereau[1]. 1) LBMC, ENS de Lyon, Lyon, FR; 2) CRNL, Waking team, Lyon, FR; 3) CARMEN, HCL, University of Lyon, Lyon, FR.

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease after Alzheimer disease. It is characterized by the selective loss of dopaminergic neurons in the substantia *nigra pars compacta*. Dopaminergic signalling deregulation leads to resting tremor and postural instability. Genetic factors as well as environmental stress such as the use of the pesticide paraquat, have been shown to trigger PD. Although several key molecular players have been identified and characterized in PD models, little is known about the cellular glial mechanisms that may

regulate the survival versus death of dopaminergic neurons.

To identify glial genes involved in dopaminergic neuronal cell death, we combined a candidate gene approach in Drosophila melanogaster and a transcriptomic analysis on the *substantia nigra* from PD patients. Among the *Drosophila* candidate genes, 20 were found to have homologous genes in human that were differentially expressed in PD. And among these 20 genes, we identified *split-ends* (*spen*) has being protector in a chemical model of Parkinson induced by paraquat treatment in *Drosophila*. Spen belongs with spenito to the evolutionary conserved SPEN proteins family. Spen is implicated in multiple cellular processes such as neuronal and glial cell fate or axon guidance during nervous system development and we have recently shown that spen was necessary for interomatidial cells (IOCs) survival during retina development.

We found that brain expression of spen is modulated by paraquat treatment and that flies heterozygous for a loss of function mutation in *spen* exhibit an enhanced vulnerability to paraquat, which indicates a prosurvival function of spen. Importantly, specific depletion of spen in glial cells also renders flies more sensitive to paraquat, while, reversely, over-expression of spen in glial cells has a protective effect. In support of previous reports, a bio-informatics large scale analysis of transcriptomic data indicates that spen and the Notch pathway are tightly interconnected. Using a Notch reporter construct, we indeed demonstrate that spen is required for Notch activity in glial cells. Furthermore, modulation of Notch in glial cells is associated with higher sensitivity to paraquat treatment.

Together, these results suggest that in pharmacological models of PD, spen expression in glial cells is required for a Notch-dependent mechanism of neuronal resistance to oxidative stress. . This finding further supports the importance of glia-mediated neuroprotective pathways under neurodegenerative conditions.

D185 The ecdysone and JAK/STAT pathways regulate proper morphogenetic movement of squamous cells by suppressing Notch-induced Broad. *Dongyu Jia*[1], Allison Jevitt[1], Yoichiro Tamori[2], Yi-Chun Huang[1], Gabriel Calvin[1], Wu-Min Deng[1]. 1) Florida State University, Tallahassee, FL, USA; 2) National Institute of Genetics, Mishima, Japan.

During development, proper formation of epithelial tissues is critical for normal cellular functions, and improper morphological changes could potentially contribute to tumor malignancy. To study epithelial morphogenesis, many animal models have been established, and the *Drosophila* egg chamber is one important system to understand morphogenetic movements. Epithelial cells have three major shapes: cuboidal, columnar and squamous. Here, we focus on squamous cells (SCs, also known as stretched cells), which are restricted at the surface of anterior egg chambers in cuboidal shape at the beginning, then rapidly become flattened in twelve hours to spread over half of the surface. The regulation of this drastic morphological change is still unclear. Our report indicates Broad (Br) interacts with the Notch, ecdysone and JAK/STAT pathways, serving as an important spatiotemporal cue for proper cell differentiation, elongation and movement. The early uniform pattern of Br in the follicular epithelium is directly established by Notch signaling at stages 5/6 during *Drosophila* oogenesis. Ecdysone and JAK/STAT signaling synergize to suppress Br in SCs to de-repress themselves from stage 8 to 10a, which contributes to proper SC stretching. During the process, ecdysone signaling is essential for initiation of SC stretching, while JAK/STAT regulates SCs distribution and cell fate determination. Our findings also shed new light on understanding of squamous cell carcinomas (SCCs), as the SC stretching process is accompanied by loss of epithelial cell marker, and our meta-analysis results implicate that human homologs of ecdysone and JAK/STAT are associated with patient survival outcomes in SCCs.

D186 Selective removal of deletion-bearing mitochondrial DNA in heteroplasmic muscle. *Nikolay Kandul*[1], Ming Guo[2], Bruce Hay[1]. 1) Division of Biology and Biological Engineering, Mail Code 156-29, California Institute of Technology, Pasadena, CA 91125, USA; 2) Departments of Neurology and Pharmacology, Brain Research Institute, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA.

Mitochondrial DNA (mtDNA) often exists in a state of heteroplasmy, in which deleterious mutant mtDNA co-exists in cells along with wildtype mtDNA. Heteroplasmy for pathogenic mutations is common, and high frequencies of mutant mtDNA result in severe maternally inherited syndromes. Inherited and somatically acquired mutations also accumulate over time and contribute to a number of diseases of aging. Reducing heteroplasmy is therefore an important therapeutic goal. Intergenerational selection against mtDNA mutations occurs in germ cells, but whether it also acts in somatic non-diving cells is unclear. To address this question, and provide a system in which genes and drugs that increase mtDNA quality control can be identified, we created a transgene-based model of a lethal heteroplasmic mtDNA deletion ($mtDNA^{\Delta}$) in a non-dividing somatic tissue, the *Drosophila* indirect flight muscle (IFM). A pulse of transcription driven by the *Flightin* promoter drives the expression of two transgenes, which together create $mtDNA^{\Delta}$ directly in IFMs. Quantitative PCR (qPCR) and *in situ* target primed rolling circle amplification show that up to 75% of the total mtDNA in the IFM carries the deletion. High amounts of $mtDNA^{\Delta}$ activate autophagy in the IFM. To determine if mtDNA quality control can be manipulated we overexpressed and knocked down genes in the $mtDNA^{\Delta}$ background, and monitored changes in numbers of $mtDNA^{\Delta}$ molecules. We found that increasing expression of genes involved in autophagy and *PINK1/parkin* pathway, and knocking down *Mitofusin* (*Mfn*) result in a decrease in $mtDNA^{\Delta}$, but not wildtype mtDNA. Conversely, suppressing the autophagy pathway causes a further increase of $mtDNA^{\Delta}$. The selective removal of $mtDNA^{\Delta}$ by *parkin* overexpression is prevented in an autophagy mutant background. In summary, we have developed a system in which the fate of an mtDNA deletion can be monitored *in vivo*, in a tissue important for aging. We also provide evidence that levels of mutant mtDNA can be decreased through manipulation of autophagy/mitophagy, suggesting pharmacological approaches to inhibiting a major cause of age-related decline in cell function.

D187 A Drosophila model for XX Gonadal Dysgenesis. *O. Gerlitz*[1], A. Weinberg-Shukron[2,3], R. Kalifa[1], D. Rekler[1], A. Dreifuss[1], N. Fardian[1], T. Shore[1], P. Renbaum[2,3], E. Levy-Lahad[2,3], D. Zangen[4]. 1) Department of Developmental Biology and Cancer Research, IMRIC, Faculty of Medicine - Hebrew University, Jerusalem, Israel; 2) Medical Genetics Institute, Shaare Zedek Medical Center, Jerusalem, Israel; 3) Hebrew University

Medical School, Jerusalem, Israel; 4) Division of Pediatric Endocrinology, Hadassah Hebrew University Medical Center, Jerusalem, Israel.

Ovarian development and maintenance are poorly understood; however, diseases that affect these processes can offer insights into their underlying mechanisms. XX Gonadal Dysgenesis (XX-GD) is a rare heterogeneous genetic disorder characterized by underdeveloped, dysfunctional ovaries, with a subsequent lack of spontaneous pubertal development, primary amenorrhea, uterine hypoplasia, and hypergonadotropic hypogonadism. Using homozygosity mapping and whole exome sequencing, we identified a missense recessive mutation in Nucleoporin107 (Nup107) in a consanguineous family with multiple females affected by XX-GD (with no other apparent developmental deficits). Nup107 is an essential component of the nuclear pore complex. We use *Drosophila* as a model to study the potential function of Nup107 in human female gonadal dysgenesis. A deletion allele of Nup107 is embryonically lethal in the fly, but can be rescued by exogenously expressing WT Nup107. Transgenic expression of the functionally equivalent *Drosophila* Nup107 missense mutation in flies, while rescuing viability, results in abnormal ovarian development accompanied by an almost complete sterility with a marked reduction in progeny, morphologically aberrant eggshells, disintegrating egg chambers and increased apoptosis, indicating defective oogenesis. RNAi knockdown of Nup107 in somatic gonadal cells causes complete female sterility, whereas males remain fully fertile. Interestingly, this specific RNAi knockdown in follicle cells causes defects in nurse cells and oocytes, both of which are germline cells. In contrast, specific knockdown of Nup107 in the germ line has no apparent effect in either sex. In summary, these results indicate Nup107 as a novel genetic source of ovarian dysgenesis and suggest that nucleoporin defects may play a role in milder and more common conditions such as premature ovarian failure. Future research using this XX-GD *Drosophila* model system will elucidate signaling pathways and new genes involved in both ovarian development and dysgenesis mechanisms.

D188 Mechanism of Ethanol Tolerance: ChIP-seq to identify the signature of ethanol tolerance genes. *Nigel Atkinson*, Alfredo Ghezzi. The University of Texas at Austin, Austin, TX.

Genomic studies often report that a significant fraction of the genome (up to ~30%) changes expression following exposure to ethanol. Unfortunately, responses important for producing alcohol behaviors can be obscured by this surfeit of changes. Functional alcohol tolerance is alcohol-induced alcohol resistance that is not mediated by a change in alcohol metabolism. To help identify changes important for producing functional behavioral tolerance to alcohol, we exploited the fact that two chemically distinct alcohols produce mutual cross tolerance through a related mechanism. This was demonstrated in detail using the Drosophila *slo* gene as a test case. In flies, the capacity to acquire tolerance to either ethanol or benzyl alcohol has been linked to the *slo* gene, which encodes BK-type Ca^{2+}-activated K^{+} channels. Mutations in *slo* block the acquisition of tolerance, sedation with either drug induces *slo* expression, and *slo* induction has been shown to phenocopy tolerance. A survey of the alcohol-induced changes in histone modification and transcription factor binding across the *slo* transcriptional control region proved to be an effective way to identify DNA regulatory elements that mediated the alcohol response. Based on the success of this approach, we performed a genomic survey to identify all genes and regions that respond similarly to both benzyl alcohol and ethanol. This technique identified a gene network, shown to play important roles in this alcohol response (tested by mutation, RNAi, or transgenic mis-expression). We also developed profiles for alcohol-induced histone H4 acetylation patterns and CREB-binding patterns across the *slo* promoter region. These profiles were then used in a ChIP-Seq genomic survey to find other genes with similar patterns. By doing so we have developed a refined profile of one class of alcohol-regulated gene.

D189 A Drosophila Model of Essential Tremor. *Lorraine N. Clark*[1,2], Ronald Arias[2], Philip Smith[3], Krasimira Tsaneva-Atanasova[4], James Hodge[3], Brian McCabe[5], Elan Louis[6]. 1) Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, New York, NY, USA; 2) Department of Pathology and Cell Biology, Columbia University, New York, NY, USA; 3) School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK; 4) Department of Mathematics, University of Exeter, Exeter, UK; 5) Brain Mind Institute, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; 6) Departments of Neurology and Chronic Disease Epidemiology, Yale School of Medicine and Yale School of Public Health, Yale University, New Haven, CT, USA.

Objective: To generate and characterize a *Drosophila* Model of *KCNS2* associated Essential Tremor (ET).

Background: ET is one of the most common neurological diseases, with an estimated 7 million affected individuals in the US. The most characteristic clinical feature of ET is a kinetic tremor in the hands or arms. As the disease progresses, tremor becomes more severe and more anatomically widespread (e.g., head, trunk). Recently, we identified a mutation (p.Asp379Glu) located in the pore domain of a potassium channel alpha subunit, *KCNS2*, in an early onset family with autosomal dominant ET. KCNS2 is highly and selectively expressed in the brain and modulates the activity of the *KV2.1* (*Shab*) and *KV2.2* (*Shab related*) channels.

Methods: The pBID-UAS *Drosophila* vector (McCabe lab) was used to express human normal *KCNS2* and mutant *KCNS2* in *Drosophila*. Transgenic flies were crossed to a number of well-characterized lines that express and drive expression in tissue- and cell-type-specific patterns (e.g. panneuronally or motor neurons). To determine the effects of human wildtype and mutant *KCNS2* on adult clock neuron activity, electrophysiology recordings were performed by using whole cell voltage-clamp. Using a Shab specific toxin we were able to isolate the Shab current which is a voltage-senstive non-inactivating potassium current. Expression of human wildtype *KCNS2* caused the Shab current to become an inactivating current activating at more hyperpolarised currents. Expression of human mutant *KCNS2* caused both phenotypes but to a greater extent. In addition to neuronal degeneration we also investigated the behavioral manifestations of nervous system dysfunction in the transgenic flies.

Results: Here we express human normal and mutant (p.Asp379Glu) forms of *KCNS2* in *Drosophila* (panneuronally and restricted to subsets of neurons) and produce adult onset leg shaking, abdomen pulsations and shuddering under ether anesthetization in addition to bilateral wing elevation indicative of loss of neurons during aging. Electrophysiological recordings show expression of human *KCNS2* (particularly the mutant form) switched Shab into an inactivating current which would be expected to contribute to neuronal hyperexcitability.

Conclusion: Our *Drosophila* model recapitulates some of the features of ET, and can now be used to advance our understanding of disease pathogenesis and to identify pathways that can be targeted for pharmaco-therapeutics for ET.

D190 Modulation of bursting kinetics generates specific gene expression rates in the early embryo. *Shawn Little*, Eric Wieschaus, Thomas Gregor. Princeton University, Princeton, NJ.

Precise control of gene expression is a hallmark of early development. To understand how random molecular interactions generate reproducible gene expression patterns, we have developed methods for absolute quantification of transcription rates. We examine gene expression dynamics at the level of individual gene loci using a combination of single molecule counting, live transcription imaging, and computer simulations. Several genes found in broad domains of the blastoderm stage embryo are expressed at equivalent rates within their respective domains of fastest accumulation. However, this fastest, or maximum, expression rate does not result from constitutive, fully active expression. Instead, transcription switches between expressing and non-expressing states during interphase, a phenomenon referred to as "bursting." Our analysis suggests that a single rate-limiting molecular interaction, occurring at random, determines bursting kinetics. We find that the on- and off-rates that determine bursting kinetics are finely tuned across genes in order to generate equivalent expression rates within domains of highest activity. We also find that a minimal promoter/enhancer from the gene *hunchback* is sufficient to confer bursting kinetics that are identical to those observed for the endogenous *hunchback* gene in the domain of highest activity. This suggests that multiple enhancers are not required to determine correct transcription dynamics.

D191 A fully synthetic transcriptional enhancer platform for study of regulatory protein function in a multicellular eukaryote. *J. Crocker*, D. Stern. HHMI Janelia, Ashburn, VA.

The complex spatial and temporal patterns of gene expression during development of multicellular organisms are determined by the binding of transcription factor proteins to regions of genomic DNA called enhancers. Traditionally, tests of the *in vivo* functions of transcription factors have been performed using modified native enhancers. These native enhancers have provided great insight, but can also yield unpredictable results, presumably because they also encode sites of unknown function. A synthetic, transcriptionally silent enhancer platform may allow cleaner tests of regulatory protein function, but, to date, attempts to build a synthetic enhancer in a multicellular eukaryote—by combining binding sites for transcriptional activators and repressors—have largely failed to produce predictable patterns of gene expression. Here we report construction of a fully synthetic transcriptional platform in *Drosophila* consisting of an engineered transcriptional activator and an artificial enhancer platform. We found that binding sites for a transcriptional activator are not sufficient to drive transcription. Instead, binding sites for a transcription factor that makes DNA accessible are required together with binding sites for transcriptional activators to produce a functional enhancer. Only in this context can changes in the number of activator binding sites mediate quantitative control of transcription. Our synthetic system provides a platform for testing models of transcriptional regulation and will provide an improved understanding of how natural networks function.

D192 Developmental Regulomes – Resolving Enhancer-Protein Interactions with Temporal and Tissue-Specificity. *Robert P. Zinzen*[1], Sabrina Krueger[1], Djordje Vasiljevic[2], Matthias Selbach[2], Robert P. Zinzen[1]. 1) Berlin Institute of Medical Systems Biology (BIMSB-MDC), Berlin, Germany; 2) Max Delbruck Centre for Molecular Medicine (MDC), Berlin, Germany.

Tightly coordinated control of gene expression is essential, especially in development. *Cis*-regulatory elements (CRMs) like enhancers act as information integration hubs to drive and regulate spatio-temporal gene expression by interpreting the specific cellular environment (transcription factor (TF) availability, chromatin state, etc.) to translate the available regulatory information into transcriptional responses.

While it is possible to identify CRMs globally, we are largely unable to explain the exact spatio-temporal activities that individual identified enhancers actually drive when tested *in vivo over the course of development*. Our knowledge of the suit of regulatory interactions that converge on individual enhancers is all too often incomplete. In order to accurately predict and model enhancer activity *in vivo*, identification of these inputs remains a crucial challenge.

We have developed a method that allows for the biochemical isolation of defined regulatory elements from whole animals to high purities. We show that protein:DNA interactions remain stable during purification and that mass spectrometry can identify specific CRM:protein interactions, including TFs, as well as chromatin modulators and local nucleosome components. The major advantage of our approach is that using the genetic accessibility of *Drosophila,* we are not limited to querying such interactions at developmental stages, but we can do so in a tissue- and cell type-specific manner. For the first time, we are able to extract specific DNA elements from embryos and identify their tissue-specific regulatory interactions (i.e. their '*regulomes')* to ask what renders an enhancer active in one tissue, but inactive in another, how these interactions change over the course of development, and how they depend on activity state?

Deploying this method to query enhancer:protein interactions of neurectodermal enhancers has revealed dozens of potential regulators, known and novel, which we are now being tested for their global roles in early nervous system patterning.

The method presented here is broadly applicable to the identification of specific DNA:protein interactions *in vivo* with temporal and tissue-specificity and should be expandable for the identification of other potential regulators including lncRNAs and local genome architecture.

D193 Application of ChIP-nexus to map transcription factors during development. *Robin Fropf*[1], Qiye He[2], Jeff Johnston[1], Julia Zeitlinger[1,3]. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Zhejiang University, Hangzhou, China; 3) University of Kansas Medical Center, Kansas City, MO.

A mechanistic understanding of how cis-regulatory sequences are decoded relies on high-resolution transcription factor binding footprints.

Since the function of enhancers can only be assayed in the cell type in which they are active, this task ultimately requires the analysis of a wide variety of different cell types, many of which are hard to isolate in large numbers. To map transcription factor binding in specific cell types *in vivo*, it is therefore vital to improve the efficiency of current ChIP-seq protocols, ideally also with increased resolution. Nucleotide resolution can be achieved through a ChIP-exo protocol, which uses a lambda exonuclease digestion step to precisely locate the boundary at which the cross-linked protein is bound to DNA. To apply ChIP-exo to *Drosophila* embryos, we recently developed a new technique termed ChIP-nexus (ChIP with nucleotide resolution through exonuclease, unique barcode and single ligation). ChIP-nexus introduces a novel library preparation protocol that relies on self-circularization and thus improves the efficiency by which DNA fragments are incorporated into the library. ChIP-nexus outperforms existing ChIP protocols in resolution and specificity and pinpoints relevant binding sites within enhancers containing multiple binding motifs. We are currently working on additional modifications to the protocol to further increase the efficiency, allowing lower amounts of starting material. This will enable the precise mapping of transcription factor binding in cell types of low abundance, facilitating the application of ChIP-seq to samples that were previously not accessible to such an analysis..

D194 Natural variation in binding site affinity controls stochastic gene expression in the fly eye. *C. Anderson*[1], C. Zhou[1], I. Reiss[1], A. Cho[1], H. Siddiqi[1], B. Mormann[2], C. Avelis[1], E. Roberts[1], J. Taylor[1], D. Vasiliauskas[3], R. Johnston[1]. 1) Johns Hopkins University, Baltimore, MD; 2) New York University, New York, NY; 3) Université Paris Diderot, Paris, France.

Stochastic mechanisms are critical to diversify cell fates during development. How the proportions of randomly determined cell fates is controlled is poorly understood. The stochastic on/off expression of the transcription factor Spineless (Ss) in R7 photoreceptors determines the patterning of two subtypes in the fly eye. Our studies of natural variation revealed a single nucleotide polymorphism (SNP) in a non-coding DNA element in the *ss* gene locus that lowers the percentage of Ss on cells ("*low ss SNP*"). This SNP lies within a binding site for the zinc finger transcriptional repressor Klumpfuss (Klu) and increases Klu binding affinity. Klu acts in R7s to lower the ratio of Ss on to off cells: loss of *klu* function causes more R7s to express Ss whereas increasing Klu levels causes a decrease in the number of Ss-expressing R7s. Thus, the levels of Klu protein are converted into a ratio of Ss on/off cells. Our data suggest that stochastic on/off gene expression is controlled by threshold levels of *trans* factors bound to low affinity sites.

D195 Towards a 4D understanding of chromatin architecture and transcriptional regulation. *H. Chen*[1], M. Fujioka[2], T. Gregor[1]. 1) Princeton University, Princeton, NJ; 2) Thomas Jefferson University, Philadelphia, PA.

We use Drosophila embryo as a model to study the dynamic properties of 3D genome organization and the functional significance of chromatin architecture on the regulation of gene transcription. Using a combination of genetics, genome editing and live imaging, we are able to mark specific genes with fluorescent markers and trace the movement of individual loci. Particularly, by simultaneously labeling promoter activity and the position of enhancers, we are able to measure kinetic parameters (e.g. looping probability, dwell time and dissociation constants) for long-range promoter-enhancer interactions. Furthermore, by synthetically tuning the promoter-enhancer distance and the insulator elements that mediate the interactions, we are able to investigate the mechanistic details of chromatin looping and enhancer searching through physical polymer modeling.

D196 Zelda pioneers early enhancers during genome activation. *C. A. Rushlow*[1], Y. Sun[1], C. Y. Nien[1], K. Chen[2], J. Chen[1], H. Crimmins[1], J. Johnston[2], N. Kirov[1], J. Zeitlinger[2]. 1) New York Univ, New York, NY; 2) Stowers Institute for Medical Research.

The Zn finger transcription factor Zelda serves as a major hub in the early gene network. Zelda binds to CAGGTAG motifs in enhancers of early-expressed genes, which can be categorized into two types. Class I genes are uniformly expressed like Zelda, and absent in *zelda*$^-$ mutants, suggesting direct activation by Zelda. Class II genes are spatially-restricted patterning genes, which are delayed and sporadic in *zelda*$^-$ but nevertheless expressed, as they are primarily regulated by patterning factors such as Dorsal and Bicoid, suggesting a timing and potentiating role for Zelda. What determines whether Zelda will be a direct activator or a potentiator? We will discuss differences in RNA Polymerase II pausing, promoter motifs, and transcription factor binding that distinguish Zelda's two different roles. In addition, we will present evidence that Zelda's potentiating role is based on its ability to act like a pioneer factor and bind its motifs in nucleosomes. We discuss our model of how Zelda "scouts out" regions of high nucleosome occupancy, then upon binding to CAGGTAG motifs, displaces nucleosomes just enough for other factors to access the genome. In this way Zelda imparts "enhancer competence" globally during genome activation.

D197 Highly accurate prediction of early anterior-posterior enhancer sequences from ChIP-seq data. *H. Arbel*[1], P. Bickel[1], S. Celniker[2], M. Biggin[2], J. B. Brown[1,2]. 1) University of California, Berkeley, Berkeley, CA; 2) Lawrence Berkeley National Laboratory, Berkeley, CA.

In animals, definitive epigenetic signatures of enhancer elements have been challenging to identify– the best prediction tools offer weak positive predictive power at genome-scales and are not accurate enough to conduct in silico enhancer annotation. In the early Drosophila embryo, a small cohort of ~40 transcription factors drive body patterning. Hence, fly development offers a simplified model system in which to study the relationship between transcription factor binding and tissue-specific enhancer activity. We studied the DNA binding patterns for 22 of these factors, as well as chromatin marks during embryonic stages 4 and 5. We applied supervised machine learning to identify enhancer sequences active during embryogenesis using a test set of over 7000 whole-embryo in situ imaging experiments. We find that we have nearly perfect predictive accuracy for early anterior-posterior (AP) enhancer sequences (>97% predictive accuracy, suitable for whole-genome scans), while dorsal-ventral (DV) and other classes of enhancers are far more challenging to predict. Further examination of "false positives" identified by our methods reveals manual annotation errors in the labeling of in situ experiments – our actual correct classification rate is likely higher than reported here. Well-predicted enhancers admit a unique epigenetic signature involving interactions between both AP and DV

transcription factors. Our model identifies nonlinear interactions between cohorts of transcription factors, suggesting the presence of combinatorial activation rules. Using a whole genome scan, we predict that around 1.6% of 1kb windows in the genome are likely AP enhancers (recovering 98% of known enhancers from our training and test data). For the first time, we demonstrate a sufficiently accurate enhancer prediction algorithm to enable the near-comprehensive discovery of a subclass of enhancer sequences.

D198 ESCRTs and intraluminal vesicles play novel roles in controlling exosome heterogeneity, late endosomal acidification and regulated secretion. *Benjamin Kroeger*, Shih-Jung Fan, Felix Castellanos, Sumeth Perera, Irina Stefana, Siamak Redhai, Mark Wainwright, Carina Gandy, Deborah Goberdhan, Clive Wilson. Dept. of Physiology, Anatomy & Genetics, University of Oxford, Oxford, UK.

The appropriate functioning of the endosomal system is an essential aspect of cell biology, intimately linked to cell-cell signalling and growth regulation. One component of this system, the late endosomal multivesicular body (MVB), is characterised by its many intraluminal vesicles (ILVs), whose formation is dependent on the evolutionarily-conserved Endosomal Sorting Complexes Required for Transport (ESCRT) 0-III. Although some functions of ILVs are yet to be fully elucidated, MVBs can fuse to the plasma membrane, releasing their ILVs as exosomes (nano-sized extracellular vesicles that transmit multiple signals between cells). ILVs are also thought to prevent excessive receptor-ligand mediated signalling and can be degraded following MVB fusion to lysosomes. We are studying the roles of ILVs *in vivo* in secondary cells of the *Drosophila* male accessory gland and aim to translate these findings into human systems. These cells have extraordinarily large (5-10 µm diameter) endosomes, allowing us to readily visualise their diversity, abundance and intraluminal contents. We utilise a combination of molecular genetic approaches and advanced microscopy techniques, including super-resolution 3D structured illumination microscopy (SIM), electron and time-lapse wide-field microscopy. Using 3D-SIM we visualise and resolve ILV populations in living cells for the first time. We observe that ILVs carrying exosome markers are formed within the late endosomal MVBs, as expected, but also within recycling endosomes. These two types of ILV are loaded with different cargos, and secreted via different signalling-dependent mechanisms. We provide evidence that ESCRT complexes localise to and are required for the biogenesis of ILVs in both late and recycling endosomes. We show that human cancer cells also form at least two exosome subtypes in the equivalent compartments, suggesting these cell biological processes are evolutionarily conserved. Functional characterisation reveals at least two previously unsuspected roles for the different ILV populations. We show that late endosomal ILVs carry the v-ATPase proton pump, which sets up acidic microdomains around their exterior, which appears to be required for acidification of this compartment. In addition, ILVs in recycling endosomes play an essential role in the maturation of the dense-core compartments involved in regulated secretion. Our studies therefore reveal multiple new sub-compartmental functions for ILVs and ESCRTs, and provide key evidence to help explain the diversity of exosomes and their biological functions.

D199 Mitophagy is dispensable for axonal maintenance during normal aging in *Drosophila*. *Y. Fang*[1], X. Cao[1], H. Wang[1], Q. Wang[1], N. M. Bonini[2], Y. Fang[1]. 1) IRCBC, SIOC, Chinese Academy of Sciences, Shanghai, China; 2) Dept of Biology, UPenn, Philadelphia.

Healthy mitochondria are required for maintaining neuronal integrity and function. Mitochondria undergo dynamic fission-fusion and damaged mitochondria are cleared by autophagy (termed as mitophagy). Recently, there have been debates on whether mitophagy occurs locally in axons or whether damaged mitochondria need to be transported to the neuronal soma for lysosome-mediated turnover (Cai et al., 2012; Ashrafi et al., 2014; Maday and Holzbaur, 2014). To address this question, we have developed a *Drosophila* wing nerve model, which allows us to label axons and mitochondria by different fluorescent proteins to study their changes during aging *in vivo*.

We found mitochondria in axons were significantly shorter in aged flies, suggesting that mitochondria become fragmented during aging. Alternatively, it may be due to reduced mitophagy in aged axons. We hence studied the consequence of manipulating key mitophagy genes *Pink1* and *Parkin* in axons. To our great surprise, unlike in muscles where the $Pink1^{B9}$ null mutation caused severe mitochondria abnormality and muscle degeneration (Clark et al., 2006; Park et al., 2006), $Pink1^{B9}$ flies showed normal mitochondria morphology, size and density in neurons, and did not cause spontaneous axon degeneration. This result suggests that local axonal mitophagy might not be an essential mechanism in maintaining neural integrity during aging. Consistently, we found that co-localization of mitochondria and autophagosomes almost exclusively occurred in the neuronal soma and was essentially not observed in axons, even in aged flies. The co-localization occurrence in soma did not decrease with age, suggesting a constant mitophagy level in neurons during aging. Co-localization of axonal mitochondria and autophagosomes *in vivo* were observed only after axotomy in the injured wing nerve. This is consistent with previous *in vitro* studies where extreme insults such as H_2O_2 or CCCP were used to induce mitophagy in cultured neurites.

Next, we overexpressed *Pink1* or *Parkin* to see if promoting mitophagy mitigated age-associated accumulation of damaged mitochondria in neurons. Unexpectedly, however, both flies showed increase in fragmented mitochondria and severe spontaneous axon degeneration. Upregulation of mitochondria fusion genes could fully rescue the axonal phenotypes of *Parkin* OE. Together, our results indicate that mitophagy is dispensable for axonal integrity and we have additional evidence suggesting that fission-fusion plays a major role in keeping healthy and functional mitochondria in axons during normal aging.

D200 Endosomal Microautophagy: a genetic model in *Drosophila*. *A. Mukherjee*, B. Patel, H. Koga, A. M. Cuervo, A. Jenny. Albert Einstein College of Medicine, Bronx, NY.

Autophagy is an essential pathway that maintains the energy balance of cells by lysosomal degradation and recycling of dysfunctional proteins and organellar components. Importantly, autophagy also provides nutrients including amino acids and lipids to cells under stress conditions and is thus essential for energy balance. As such, autophagy counteracts various human diseases (related to liver, kidney and the nervous system), and its reduction leads to aging like phenotypes. Macroautophagy (MA), the best studied form of autophagy to this date, selectively degrades organelles or aggregated proteins, but selective degradation of single proteins has only been described for Chaperone-

mediated autophagy (CMA) and endosomal Microautophagy (eMI). These two autophagic pathways, described to date only in mammals, are specific for cytosolic proteins containing KFERQ-related targeting motifs. However, CMA and eMI have only been described in mammals and thus lack a model in a genetically easily tractable organism.

Using transgenic flies expressing a KFERQ-tagged fluorescent biosensor, we have identified and functionally characterized an eMI-like pathway in *Drosophila melanogaster*. Our functional *in vivo* studies in fly fat body (functionally equivalent to the mammalian liver) reveal that the biosensor forms puncta in response to prolonged starvation and co-localizes with lysosomes/late endosomes in a KFERQ- and Hsc70-dependent manner. Furthermore, fly eMI requires endosomal multivesicular body formation mediated by ESCRT complex components. Importantly, induction of *Drosophila* eMI requires longer starvation than the induction of MA and is independent of the critical MA genes *atg5*, *atg7*, and *atg12*.

Tor signaling represses MA by inactivating the Atg1/Atg13 complex. Interestingly, inhibition of Tor signaling is also sufficient to induce eMI in flies under nutrient rich conditions, and, as eMI in *Drosophila* also requires *atg1* and *atg13*, our data suggest that these genes may have a novel, additional role in regulating eMI in flies.

Overall, our data provide the evidence for a novel, starvation inducible catabolic process resembling endosomal Microautophagy in a non-mammalian species *in vivo*. It is tempting to speculate that *Drosophila* eMI is an older form of selective autophagy that fulfills functions that in mammals are shared between eMI (likely the constitutive form) and CMA (the starvation-induced variant).

D201 Mechanisms of rapid, membrane-dependent furrow formation in the early Drosophila embryo. *J. Todd Blankenship*, Lauren Mavor, Yi Xie, Ryan Holly, Hui Miao. University of Denver, Denver, CO.

One of the primary events that must occur repeatedly throughout a complex animal's lifetime is the ingression of a plasma membrane furrow. Furrow formation and ingression are obligate parts of cell division, and drive the physical separation of one cell into two cells. Furrow formation in early syncytial Drosophila embryos is exceptionally rapid, with hundreds of furrows forming synchronously in as little as 4 minutes. Here, we use 4D imaging to identify furrow formation, stabilization, and regression periods, and identify a rapid, membrane-dependent pathway that is essential for plasma membrane furrow formation *in vivo*. While Myosin II function is thought to provide the ingression force for cytokinetic furrows, the role of membrane trafficking pathways in guiding furrow formation is less clear. We demonstrate that a membrane trafficking pathway centered on RalA, Rab8, Rab11, and the exocyst complex is required for fast furrow ingression in the early fly embryo. This trafficking pathway is absolutely required for furrow formation and initiation, and genomic instability occurs when its function is disrupted. We show that Rab8 vesicular intermediates direct the delivery of internal cytoplasmic Rab11 membrane pools to the cell surface through a RalA/exocyst targeting function. In contrast, Myosin II activity is not required for furrow ingression, but is needed to enforce furrow uniformity. These studies identify a pathway, which stretches from Rab11 to Rab8 to RalA and the exocyst complex that mediates rapid furrow formation in early Drosophila embryos.

D202 The Voltage Gated Chloride Channels CLC-b and CLC-c play critical roles in lifespan and cell viability respectively. *R. E. Burke*, S. Judd-Mole. Monash University, Melbourne, Victoria, AU.

Mammalian members of the Voltage Gated Chloride Channel family of Cl^-/H^+ exchanger proteins (CLC-3 to 7) are thought to play a range of cellular roles in transepithelial transport, endocytosis, cell volume regulation and the acidification of intracellular organelles. Mutations of these proteins in mice or humans have been shown to cause disease symptoms such as neuronal ceroid lipofuscinosis (CLC-6 and 7), osteopetrosis (CLC-7), Dents disease (CLC-5) and lysosomal storage disorders (CLC-3).

Drosophila has fewer CLC proteins, with CLC-c homologous to mammalian CLC-3, 4 and 5 and CLC-b homologous to CLC-6 and 7. As such it represents an excellent system in which to study the precise cellular role of each class of chloride channel. We have characterised the function of CLC-b and CLC-c.

A CLC-b:GFP fusion protein was found to localize to lysosomes. *ClC-b* null homozygotes are viable but exhibit a dramatic reduction in both lifespan and locomoter activity, plus a mild pigmentation defect. These phenotypes are reminsicent of the symptoms of neuronal ceroid lipofuscinoses caused by disruption of CLC-6 or 7 and may represent a defect in lysosomal acidification leading to lysosomal storage disorder-like pathologies. The *ClC-b* mutant flies are therefore a promising model for studying the molecular mechanism underlying such disorders.

A CLC-c:GFP fusion protein was shown to localize both to the apical plasma membrane and to endolysosomal vesicles. *ClC-c* null mutant homozygotes die in early larval development indicating that, unlike any of its three mammalian homologues, *ClC-c* is essential for viability. Mosaic analysis demonstrated that *ClC-c* homozygous mutant cells have a survival defect and are out-competed by surrounding wild type cells in the larval wing imaginal disc. Prior to undergoing apoptosis, these *ClC-c* mutant cells exhibit an increase in LysoTracker staining that is independent of autophagy. Therefore unlike proposed roles for its mammalian homologues in lysosomal acidification, CLC-c is more likely to be required either in the recycling of endosomes back to the plasma membrane, or the transition of late endosomes to lysosomes. A block in either function may result in an increase in acidified late endosomes, leading to the elevated LysosTracker signal observed.

Our initial charaterization of CLC-b and CLC-c function paves the way to an in-depth investigation of the molecular pathology underlying disorders such as Dent's disease and neuronal ceroid lipofucinoses, which could then be used as a platform for the identification of drugs able to restore function to defective chloride channel proteins.

D203 A screen for systemic growth regulators reveals *hobbit*, a novel and conserved regulator of insulin secretion. *Sarah Neuman*, Jessica Smoko, Arash Bashirullah. University of Wisconsin-Madison, Madison, WI.

Insulin signaling plays a central role in the regulation of systemic growth in *Drosophila*, and therefore is critical in the determination of final

body size. However, the mechanisms regulating insulin secretion remain poorly understood. In a forward genetic screen for mutants with reduced body size, we identified 5 alleles of a novel gene, which we named *hobbit*. *Hobbit* is highly conserved from yeast to humans, but no function has been assigned to the protein in any organism. Our results demonstrate that *hobbit* has a non-cell autonomous effect on growth, indicating that the small body size of the mutants is due to a systemic growth defect. We found that *hobbit* mutants exhibit dramatic defects in insulin secretion, and this defect is directly responsible for the small body size of the mutant animals. Importantly, these insulin secretion defects can be rescued by expressing Hobbit specifically in tissues required for insulin secretion. Furthermore, we demonstrate that expression of the human ortholog of *hobbit* is sufficient to rescue the fly mutant, suggesting that the function of *hobbit* in insulin secretion is conserved. We are currently focused on analyzing the mechanism by which *hobbit* regulates secretion of insulin, and our results indicate that *hobbit* is required for a novel step in the regulated exocytosis pathway. Overall, we have identified a novel regulator of exocytosis that is required for insulin secretion and the control of systemic growth during development.

D204 Spastic paraplegia proteins help model the axonal endoplasmic reticulum network in *Drosophila*. *Cahir J. O'Kane*[1], Belgin Yalçin[1], Martin Stofanko[1], Niamh C. O'Sullivan[1], Lu Zhao[1], Annika Roost[1], Zi Han Kang[1], Matthew R. Thomas[1], Sophie Zaessinger[1], Olivier Blard[1], Valentina Baena[2], Mark Terasaki[2]. 1) University of Cambridge, Cambridge, GB; 2) University of Connecticut Health Centre, Farmington, CT.

The length of motor axons entails significant engineering for their formation and maintenance. Failures of this are seen in the Hereditary Spastic Paraplegias (HSPs), with lower limb weakness, and degeneration of longer upper motor axons. To date over 60 causative Spastic Paraplegia Genes (SPGs) are known. Several encode endoplasmic reticulum membrane proteins, with intramembrane hairpins that curve and model ER membrane, suggesting the axonal ER network as an important component to maintain function and integrity of long axons.

We have explored this model in Drosophila by testing for abnormalities of the axonal ER network in Drosophila that lack SPG-encoded intramembrane hairpin proteins. We have focused on two of these protein families, reticulons and REEPs, which are together required for formation of most tubular ER in yeast. We have developed axonal ER markers in Drosophila, and mutants that lack one or more members of these protein families. We see widespread if not ubiquitous occurrence of tubular ER in axons, and a range of loss-of-function phenotypes, and partial redundancy of gene function. Loss of either reticulon, or one REEP gene, leads to partial loss of ER from distal but not proximal axons, recapitulating the susceptibility of longer distal axons in the human diseases. Loss of multiple genes can lead to phenotypes consistent with loss of ER membrane curvature, including some loss of continuity of the ER network in axons.

We therefore propose a role for SPG genes in establishing or maintaining the axonal ER network, and a role for ER continuity in axons, which could account for the preferential susceptibility of longer axons to HSP.

D205 Asymmetric Endoplasmic Reticulum partitioning is dependent on Jagunal in the early Drosophila embryo. *B. Riggs*, A. Eritano, A. Altamirano, S. Beyeler. San Francisco State University, San Francisco, CA.

Cell division is classically described as the division of genetic material and the corresponding reorganization of the cytoskeleton. However, the mechanisms by which organelles are segregated into daughter cells remain poorly understood. In investigating the molecular mechanisms underlying partitioning of the Endoplasmic Reticulum (ER), we sought to quantify ER segregation during cell division in the early gastrulating *Drosophila* embryo. Interestingly, we found, that at the start of gastrulation (14^{th} mitotic division), the ER divides asymmetrically in a population of asynchronously dividing epithelial cells at the anterior end of the embryo. Asymmetric division of the ER is restricted to this anterior population of cells, as other cellular domains during the 14^{th} mitosis display a symmetrical division. Furthermore, we found this asymmetric division of the ER dependent on the highly conserved membrane protein Jagunal. RNA inhibition of Jagunal, just prior to the start of gastrulation, disrupts this asymmetric division of the ER. This anterior cell population will eventually give rise to the asymmetrically dividing procephalic neuroectoderm, suggesting a link between ER distribution and the establishment of cell fate.

D206 Inhibiting lipid transfer between neurons and glia by modulating lactate levels delays neurodegeneration. *Lucy Liu*[1], Hugo Bellen[1,2]. 1) Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Houston, TX.

High levels of Reactive oxygen species (ROS) and the presence of mitochondrial defects in neurons are implicated in neurodegeneration. Previously, we have shown that lipid droplets (LD) accumulate in glia prior to neurodegeneration due to high levels of ROS activating neuronal C-Jun N-terminal Kinase (JNK) and Sterol Regulatory Element Binding Protein (SREBP). Activating lipogenesis in neurons alone will cause glial LD accumulation, suggesting that lipids are transferred from neurons to glia. However, the mechanism by which these lipids transfer is unexplored. Through a candidate gene screen designed to isolate genes involved in lipid transfer, we isolated monocarboxylate transporters (MCTs) along with fatty acid transporters and apolipoproteins, all of which are critical for eventual lipid transfer and glial LD accumulation. Furthermore, we have established a potential mechanism by which the lipids are transferred from the neuron to the glia. The pathway starts with the observation that glial cells secrete lactate via MCTS. This lactate is taken up by the neuronal MCTs. The neuron converts lactate to pyruvate to be used as an energy source in the TCA cycle. However, in the disease state, excess energy is diverted into the lipid synthesis pathway and lipogenesis is upregulated due to elevated SREBP and Acetyl coA carboxylase (ACC). The lipids are then transfer from the neuron via fatty acid transport protein (FATP) and carried by extracellular apolipoproteins to the glia. Importantly, blocking lactate transfer either genetically or pharmacologically will inhibit glial LD accumulation and delays cell death in mutant flies and in a murine neuronal-glial co-culture model. Similarly, removing FATP or the apolipoproteins also reduces LD accumulation. In brief, we are unraveling a mechanism that leads to lipid production, lipid transfer and lipid accumulation in the nervous system that has not yet been documented and that promotes neurodegeneration.

D207 Mitochondrial-nuclear incompatibility during oogenesis causes embryonic lethality. *C. Zhang*[1], K. Montooth[2], B. Calvi[1]. 1) Indiana University, Bloomington, IN; 2) University of Nebraska-Lincoln,Lincoln, NE.

Female reproduction is strongly affected by age, nutrition and metabolic homeostasis. There is increasing evidence that age-related female infertility is associated with reduced mitochondrial function. Although mitochondria have their own genomes, ~1500 nuclear-encoded gene products are transported into mitochondria and are required for mitochondrial function. Mutations in either these nuclear or mitochondria-encoded genes are known causes of a number of human mitochondrial diseases. It is less clear, however, whether mitochondrial diseases can result from specific incompatibilities between the nuclear and mitochondrial genomes.

Here, we use a previously established mito-nuclear incompatible Drosophila model to investigate its impact on female fertility. The (simw501); OreR mito-nuclear genotype carries mitochondria from D. simulans in a D. melanogaster OreR nuclear background. It was previously shown that a mito-nuclear incompatibility in this strain results in developmental delay, defective muscle function, and reduced oxidative phosphorylation. The basis for this mito-nuclear incompatibility are polymorphisms in the mitochondria-encoded tyrosyl-tRNA and the nuclear-encoded mitochondrial tyrosyl-tRNA synthetase (Aats-tyr-m or Aatm) genes. We now show that this incompatible (simw501); OreR strain has severe oogenesis defects, including death of germline stem cells and cystocytes, and egg chamber degeneration, which together result in reduced egg production. In addition, these strains have a strict temperature-sensitive maternal-effect embryonic lethality that is completely dependent on the developmental temperature of the mother. The temperature sensitive period in the mother is during late 3rd instar / pupation, and is not reversible by shifting the temperature of adult females. These last observations suggest that continued embryonic lethality results from some type of maternal metabolic memory. Our findings show that maternal mito-nuclear incompatibility has severe consequences for oogenesis and embryonic survival. More broadly, our results raise the possibility that mito-nuclear incompatibility could cause female infertility in humans.

D208 *dSod1* knock-in mutations cause ALS-like phenotypes in *Drosophila*. *A. H. Held*, A. Sahin, P. Major, D. Lipscombe, R. A. Reenan, K. A. Wharton. Brown University, Providence, RI.

Amyotrophic Lateral Sclerosis (ALS) is the most common adult onset motor neuron disease. ALS patients experience a rapid loss of motor function over the course of 2-5 years due to motor neuron death and neuromuscular junction (NMJ) deterioration. Approximately 20% of familial ALS cases are caused by mutations in superoxide dismutase-1 (hSOD1), and overexpression of mutant hSOD1 alleles has been used to model ALS in *Drosophila, C. elegans,* zebrafish, and mice. Overexpression of mutant hSOD1 in mice causes early lethality, progressive loss of motor function, NMJ deterioration, and motor neuron death, but overexpression of WT hSOD1 mimics several of these ALS-like phenotypes as well. The fact that WT hSOD1 overexpression can cause motor neuron death and impair motor function raises the possibility that high levels of SOD1 via overexpression may not accurately recapitulate the pathogenesis of ALS. To model ALS, the Reenan lab used homologous recombination to place synonymous disease causing dSod1 alleles under the control of the endogenous dSod1 promoter. Our characterization demonstrates that two mutations, $dSod1^{G85R}$ and $dSod1^{H71Y}$, cause 1) early lethality, 2) a loss of neuromuscular junction integrity, 3) electrophysiological defects and 4) a progressive loss of motor function. 1) Both alleles show profound lethality at eclosion with 100% of $dSod1^{G85R}$ and 75% of $dSod1^{H71Y}$ failing to fully eclose. The remaining 25% of $dSod1^{H71Y}$ flies that eclose show a reduced lifespan with a median survival of 14 days and a reduction in climbing ability as they age. 2) To identify why these dSod1 mutants fail to eclose, we examined nerve integrity in legs and the abdominal NMJ that both play an important function in successful eclosion. We found that $dSod1^{G85R}$ and $dSod1^{H71Y}$ mutants have disrupted leg nerve integrity and NMJ structure. 3) Furthermore, electrophysiological recordings from the adult abdominal NMJ show a decrease in mEPSP frequency, consistent with the observed decline in NMJ integrity. 4) To investigate whether these defects were progressive, we studied the motor function of larvae and their NMJs. We found that late 3rd instar larvae, but not mid 3rd instar larvae, have a profound crawling defect. Interestingly, the neuromuscular junctions of late 3rd instar larvae are largely normal in appearance and electrophysiological function, suggesting that other changes in the motor circuit account for the decline in motor function. Overall, we observed defects in 1) survival, 2) NMJ integrity, 3) electrophysiology, and 4) motor function. Given the similarity between the *dSod1* knock-in phenotypes and ALS patient symptoms, we anticipate that this model will be a valuable tool for ALS research.

D209 JmjC demethylases regulate resistance and tolerance to alcohol in *Drosophila*. *J. H. Pinzon*, N. A. Shalaby, M. Buszczak, A. Rothenfluh. UT Southwestern Medical Center Dallas, Dallas, TX.

Alcohol exposure affects gene expression in the brain and can lead to addiction in humans and other organisms. Epigenetic changes act on DNA or Histones to alter the transcription, or expression, of genes. Histone modifications include both acetylation and methylation; the latter was considered stable until the discovery of histone demethylases (KDM). Histone methylation/demethylation can promote or silence transcription. The majority of KDMs belong to a group of enzymes that contain a jumonji C (JmjC) domain; these JmjC demethylases regulate several physiological processes. The fact that alcohol and KDMs produce long lasting transcriptional changes suggests JmjC demethylases have a role in alcohol consumption and development of addition. In this study, we assess behavioral changes related to JmjC mutations in *Drosophila melanogaster*. We measured resistance to sedation (ST50 on an initial exposure) and tolerance (percent change in ST50 in a second exposure) towards alcohol in mutants of all 13 *Drosophila* JmjC demethylases genes. Our data shows that mutations in *NO66*, *lid* and *KDM3* (*JHMD2*) alter alcohol resistance and tolerance of flies. Mutations in these three genes cause a reduction in ST50 during an initial exposure to vaporized ethanol. During the second exposure (4hrs after the first), *NO66* and *lid*, showed increased tolerance to alcohol, while *KDM3* displays the opposite with a reduction in tolerance. An analysis of interactive networks revealed *NO66*, *lid*, and *KDM3*, interact with *snr1*, a transcription co-activator involved in dendrite morphogenesis and guidance, that interacts with *rho* and *S* both known to regulate behavioral responses to ethanol. These results suggest JmjC demethylases play a significant role modifying alcohol-related behaviors and further studies are needed to determine its effects on addiction.

D210 Loss of Nardilysin, a chaperone for α-Ketoglutarate Dehydrogenase, causes neurodegeneration in flies and humans and promotes mTORC1 activation. *Wan Hee Yoon*[1,2], Hector Sandoval[2], Sonal Nagarkar-Jaiswal[2], Manish Jaiswal[2], Shinya Yamamoto[2,3], Nele A. Haelterman[2], Nagireddy Putluri[2], Vasanta Putluri[2], Taraka Donti[2], Brett H. Graham[2], Mikiko Ohno[4], Eiichiro Nishi[4], Jill Hunter[5], Donna M. Muzny[2,5,6], Valerie A. Arboleda[7], Stanley F. Nelson[7], Michael F. Wangler[2,3], Ender Karaca[2], James R. Lupski[2,5,6], Hugo J. Bellen[1,2,3]. 1) Howard Hughes Medical Institute, Houston, TX; 2) Baylor College of Medicine, Houston, TX; 3) Jan and Duncan Neurological Research Institute at Texas Children's Hospital, BCM, Houston, TX; 4) Graduate School of Medicine, Kyoto University, Japan; 5) Texas Children's Hospital, BCM, Houston, TX; 6) Human Genome Sequencing Center, BCM, Houston, TX; 7) David Geffen School of Medicine, University of California, Los Angeles, CA.

Here, we isolated *Drosophila Nardilysin (NRD1) (dNrd1)* from a forward genetic screen designed for identification of genes whose loss causes neurodevelopmental or neurodegenerative phenotypes. We show that NRD1 is localized to the mitochondria whereas previous reports have documented a cytosolic, nuclear and plasma membrane localization. Unlike other mitochondrial mutants isolated from the screen, loss of *dNrd1* mutants did not affect reactive oxygen species, and ATP production, or mitochondrial contents. Rather, metabolomic studies and enzymatic assays revealed that *dNrd1* mutants exhibit a striking defect in α-Ketoglutarate Dehydrogenase (OGDH), a rate-limiting enzyme in Krebs cycle. To define the function of NRD1, we performed IP-mass spec and identified OGDH as well as numerous mitochondrial chaperones as NRD1-binding partners. Based on biochemical assays, we showed that NRD1 acts in a mitochondrial chaperoning complex to fold OGDH. Moreover, we show that loss of OGDH phenocopied the loss of NRD1, suggesting that OGDH is a major target of NRD1. A decreased OGDH activity in *NRD1* mutants or *OGDH* knockdown animas caused an increase in α-ketoglutarate, and glutamine levels, which in turn leads to mTORC1 activation, and inhibition of autophagy, resulting in a progressive neurodegeneration. Inhibition of mTOR activity by rapamycin delays the neurodegeneration phenotype in *dNrd1* mutant or OGDH knockdown flies. Furthermore, we describe the identification of two patients with rare variants in *NRD1* or *OGDH-like (OGDHL)* who exhibit a progressive neurodegeneration with an acquired microcephaly and ataxia. Using state of the art technology in *Drosophila*, we demonstrated that the variant in OGDHL in the patient is pathogenic. The cross-species functional studies described here reveal a novel role for NRD1 as a mitochondrial chaperone for OGDH, foster the discovery of two novel human diseases, and provide a potential therapeutic strategy.

D211 A multi-omics strategy for fly models of human disease in exposure biology. *James B. Brown*[1], Sasha Langley[1], Ken Wan[1], Sarah Morris[1], Soo Park[1], Charles Yu[1], Jennifer Kirwan[2], Mark Viant[2], Susan E. Celniker[1]. 1) Lawrence Berkeley National Laboratory, Berkeley, CA; 2) University of Birmingham, UK.

Several of the most prevalent forms of cancer and neurodegenerative disease are linked to chronic low dose pesticide exposure. Low-dose pesticide exposure occurs daily for over 100M Americans. Children exposed to pesticides are twice as likely to develop brain cancer as those unexposed, and mothers exposed to organochlorine pesticides have children with six-fold increased risk for autism spectrum disorders. We model pesticide-induced human disease using chronic exposures in *Drosophila*. Pathways targeted by pesticides exist in off-target organisms, including gut microbes, and low dose exposures may result in microbiome-mediated adverse health outcomes. Ingested chemicals are metabolized, and exposure involves all secondary and tertiary host and microbial metabolites, which may exhibit emergent or compound toxicity. The commensal microbiome of the *Drosophila* gut constitutes an exquisitely simplified model system in which to study the molecular basis of low-dose pesticide susceptibility and resiliency. We used a multi-omics approach (RNA-seq, metabolomics, 16S and metagenomics, phenomics) to study the modes of actions of three widely used herbicides, atrazine, glyphosate and paraquat on fly health. As controls, we studied cadmium chloride (positive control), WY14643 (vertebrate-specific drug, negative control), and alcohol (negative control). All three herbicides radically reshape the gut microbiome community compositions at environmentally relevant doses, and induce concomitant alterations in the fecal metabolome. Cadmium chloride, in contrast, had toxic effects for the host, but no statistically significant action on the gut microbiome. Whole genome PacBio sequencing of gut microbes identifies thousands of new microbial genes, and provides insights into the molecular basis of microbiome remodeling in response to pesticide exposures. Integration with host transcriptomics reveals adaptive responses to atrazine and glyphosate, which are both fully metabolized and undetectable in the feces, and more pronounced stress response to paraquat. Analysis of differentially expressed genes indicates that glyphosate acts as an endocrine disruptor in flies, while atrazine may modify lipid and cholesterol transport and metabolism. Paraquat eliminates Acetobacter in the gut, while atrazine and glyphosate both serve as prebiotics for several microbial clades – increasing population frequencies of basally rare families. The genes modulated by paraquat and atrazine are deeply conserved across the metazoan phylogeny and human. Machine learning on behavioral imaging data identifies phenotypes at all tested dosages, interpretable as lack of coordination – consistent with neurological disease. We predict that epidemiological studies of human populations exposed to Atrazine (33M Americans) will show increased incidence of neuromuscular disease.

D212 Using the DGRP to identify gene networks associated with autism-like behaviors. *L. T. Reiter*[1], D. Flatten[1], D. Johnson[2], K. Hope[1]. 1) Department of Neurology, UTHSC, Memphis, TN; 2) Molecular Bioinformatics, UTHSC, Memphis, TN.

Appropriate social behaviors are essential to humans and animals for reproduction and survival. Defects in social interaction and communication in humans can result in autism spectrum disorder (ASD). Little is understood about the basic neurogenetics controlling complex ASD-like behaviors in humans. Here we use an unbiased quantitative trait locus (QTL) approach in *Drosophila melanogaster* to identify genes and gene interactions that influence 1) social communication during mating (receptive and expressive) and 2) social space interactions in flies.

The Drosophila Genetic Reference Panel (DGRP) has been used to analyze a range of naturally occurring behaviors including sleep, appetite, olfaction, and aggression. The DGRP represent >4,800,00 single nucleotide polymorphisms (SNPs) and >2,900,00 non-SNP variants mirroring the variation for each of 205 individuals in a wild type population. Genetic variation within each line is fixed, while variation across lines represents the naturally occurring variation in the original population. The DGRP is thus the most powerful system available for behavioral genetics among model organisms. Using online genome wide association tools for the DGRP (http://dgrp.gnets.ncsu.edu), we identified genes

associated with ASD-like behaviors across the DGRP lines.

Social communication during mating was used as a proxy for expressive and receptive social communication and social space measured using a social space assay triangle to measure average resting distance among flies. The measures used for genome wide association were time to latency (expressive/receptive communication) and average distance between flies (social space).

We have now identified at least one gene, *sfl* (*sulfateless*), associated with both social communication and social spacing behavior. *sfl* encodes a heparan sulfate N-deacetylase known to be essential for synaptic vesicle endocytosis. We have also identified QTLs in two known ASD gene homologs affecting receptive (neuroligin2) and expressive (Nrx-IV) communication. We have identified QTLs in 130 genes affecting ASD-like behaviors but have only tested ~20% of the fully sequenced DGRP lines. Optimizing resolution and statistical power of these associations will require assessment of all 205 lines for all ASD-like behaviors. Our end goal is to identify new gene-gene relationships or even variants in known ASD genes that are associated with these behaviors in humans.

D213 Defects in phagocytosis by glia and immune cells in a *Drosophila* model of Fragile X syndrome. *M. Shirasu-Hiza*, R. M. O'Connor, E. F. Stone, C. R. Wayne, E. V. Marcinkevicius, J. S. Ziegenfuss, W. B. Grueber, J. C. Canman. Columbia University Medical Center, New York, NY.

We have identified defects in phagocytosis by immune cells in both the brains and bodies of *Fmr1* mutants, a *Drosophila* model of Fragile X Syndrome. Fragile X syndrome is the most common known monogenic cause of intellectual disability and autism, resulting from loss of an evolutionarily conserved translational inhibitor, *Fmr1*. Glia-mediated phagocytosis, the engulfment of extracellular material, is known to play an important role in neuronal structure and function but has not previously been implicated in neurodevelopmental diseases such as Fragile X syndrome. Here we investigate phagocytic immune cell function in *Drosophila Fmr1* mutants. We show that *Fmr1* mutants exhibit delays in phagocytosis by glia—both in the adult, after neuronal injury, and during neurodevelopment of the mushroom body, a brain structure required for learning and memory. In adults, the delay in phagocytosis is associated with a reduction in glial activation--specifically, expression of a crucial glial phagocytic receptor protein, Draper. We further demonstrate that these defects in phagocytosis are common to a second, developmentally independent lineage of blood immune cells in the body, or hemocytes. These results are the first demonstration of a defect in immune cell-mediated phagocytosis in any model of Fragile X Syndrome or autism and suggest the possibility that these defects contribute to the molecular mechanisms underlying neuronal dysfunction in these human diseases. .

D214 Epigenetic control of ribosome biogenesis homeostasis. *Jérôme Deraze*[1], Hélène Thomassin-Bourrel[1], Immane R'kiki[1], Sébastien Bloyer[2], Frédérique Peronnet[1]. 1) Sorbonne Universités UPMC CNRS IBPS UMR 7622, Developmental Biology, 75005, Paris, France; 2) I2BC U Paris Sud CEA CNRS,UMR 9198, 91198 Gif-sur-Yvette, France.

Ribosome biogenesis is an essential yet highly energy-demanding process. Both up- and down-regulation of ribosome biogenesis displays risks of cancer transformation, underlining the necessity for proper maintenance of ribosome metabolism. Proper coordination of its hundreds of factors requires monitoring mechanisms to assess the rate and quality of ribosome synthesis. An increasing body of evidence shows that ribosomal proteins possess regulatory functions outside the ribosome. Those extra-ribosomal functions have been linked in many occasions to the regulation of proliferative and stress pathways in response to perturbation of ribosome biogenesis. As such, they may act both as sensors and regulators of ribosome homeostasis.

We discovered that *Drosophila* Ribosomal Protein L12 (RpL12/uL11) may possess such an extra-ribosomal function. Indeed, we demonstrated that RpL12 can be trimethylated on lysine 3, and that this modification is specifically recognized by the chromodomain of the epigenetic cofactor Corto. As a member of the Enhancer of Trithorax and Polycomb family of cofactors, Corto is involved both in up and down regulation of target genes, through its interaction with Trithorax and Polycomb complexes. Further analysis showed that RpL12 and Corto bind the same *loci* on polytene chromosomes and regulate the same genes, mainly genes involved in ribosome biogenesis (Coléno-Costes et al., 2012, PLoS Genet 8, e1003006).

To determine the biological meaning of this interaction, we have created an RpL12 variant whose lysine 3 is mutated to an alanine (RpL12K3A), preventing its interaction with Corto. To confirm whether it retains the ability to participate in translation, we analyzed its distribution in polysomal fractions. We found that RpL12K3A was associated to actively translating ribosomes, showing that RpL12 methylation is not required for translation. RpL12K3A expression rescues the cell lethality induced by RNAi-mediated depletion of RpL12 in wing imaginal discs but not in adult wings, suggesting that it retains but a part of endogenous RpL12 functions. To test whether RpL12 methylation underlies a role in regulation of transcription, we overexpressed RpL12, RpL12K3A or the Corto chromodomain (CortoCD) in wing imaginal discs and sequenced their transcriptome. We found that ribosome biogenesis genes were repressed by RpL12 and CortoCD, yet insensitive to RpL12K3A overexpression, showing that they are specifically targeted by methylated RpL12. Altogether, these data suggest a role for RpL12 methylation in transcriptional regulation of ribosome biogenesis, possibly involving the epigenetic complexes associated with Corto.

D215 Specialized Ribosomes: eRpL22 paralogue-specific ribosomes translate specific mRNAs in the *Drosophila* testis. *Catherine M. Mageeney*, Vassie C. Ware. Lehigh University, Bethlehem, PA.

Most duplicated ribosomal protein (Rp) genes encode structurally similar or identical protein paralogues. The *Drosophila melanogaster* eRpL22 family is an exception in that encoded proteins eRpL22 and eRpL22-like are not only structurally divergent but are differentially expressed and post-translationally modified within the male germline, strongly favoring the hypothesis that each may have a functionally distinct role(s) in translation or function in an extra-ribosomal capacity in other cellular pathways within the testis. In early stage spermatogonia eRpL22 and eRpL22-like are nucleolar and cytoplasmic, consistent with a ribosomal role. In meiotic and post-meiotic spermatocytes, however, eRpL22 is nucleoplasmic, while eRpL22-like remains cytoplasmic, suggesting a change in role for eRpL22 that may

exclude the paralogue from a role in translation. That eRpL22 paralogues may contribute to ribosome heterogeneity within the male germline in early stages of spermatogenesis and contribute to translational specificity within germ cells is a compelling hypothesis. "Specialized ribosomes" (coined by Xue and Barna, 2012) may direct mRNA translation, or have a unique localization pattern in cells or developmental stages. Here we explore a direct test of this hypothesis by determining what, if any, differences in mRNA translational specificity may exist between ribosomes distinguished by eRpL22 paralogue content. Ribosomal profiling, followed by paralogue-specific affinity purification and mRNA analysis by RT-PCR or RNA sequencing, was performed to determine if eRpL22 paralogues specify a unique class of paralogue-specific "specialized ribosomes" that translate different mRNAs within *Drosophila* testes. We report the association of different mRNAs with eRpL22 paralogue-specific ribosomes, thereby defining different translatomes within the testis. Ubiquitous and early testis-specific transcripts are preferentially associated with eRpL22 ribosomes, found primarily in heavy polysome fractions. eRpL22-like ribosomes (abundant among lighter polysomes) translate some ubiquitous mRNAs and several testis-specific mRNAs, including multiple meiotic and post-meiotic transcripts. Collectively, these data provide compelling evidence for the existence of specialized ribosomes (based on eRpL22 paralogue content) within the male germline that translate different populations of testis- and spermatogenesis stage-specific mRNAs. These data highlight differences in ribosomal functions for eRpL22 paralogues and suggest a novel mechanism (dependent on ribosome composition) that regulates mRNA translation on paralogue-specific polysomes within the male germline.

D216 Translational compensation of segmental aneuploidy in *Drosophila melanogaster*. *Zhenguo Zhang*, Daven Presgraves. University of Rochester, Rochester, NY.

Segmental or chromosomal duplications and deletions, *i.e.*, aneuploidy, can cause aberrant gene expression due to altered gene dosage. Previous studies in Drosophila and yeasts showed that the expression of copy-number altered genes can be compensated at the mRNA level, though remaining different from wild type. In this study, we investigate gene dosage compensation in aneuploid S2 cells at the translational level using public data on mRNA translation rates measured with ribosome profiling. We find that on average copy-number increased (decreased) genes have lower (higher) translation rates than normal genes and that the compensation at the translational level is stronger than at the mRNA level. When combining the compensation effects at both levels, copy-number altered genes have protein synthesis rates (per gene) similar to normal genes— *i.e.*, they are fully compensated. Moreover, we do not find that copy-number altered genes are enriched or depleted in protein-complex genes except for X-linked copy-number-decreased genes, which are depleted in protein-complex genes. Taken together, our results suggest that gene dosage alteration in aneuploids can be further compensated at the translational level.

D217 When One Plus One Does Not Equal Two: Some Tandem Gene Duplicates are Overactive. *David W. Loehlin*, Sean B. Carroll. University of Wisconsin-Madison, Madison, WI.

Tandem gene duplication is an important mutational process in evolutionary adaptation and human disease. Hypothetically, two tandem gene copies should produce twice the output of a single gene, but this expectation has not been rigorously investigated. Here, we show that tandem duplication often results in more than double the gene activity. A naturally occurring tandem duplication of the *Alcohol dehydrogenase* (*Adh*) gene exhibits 2.6-fold greater expression than the single copy gene in transgenic *Drosophila*. This tandem duplication also exhibits greater activity than two copies of the gene in *trans,* demonstrating that it is the tandem arrangement and not copy number that is the cause of overactivity. We also show that tandem duplication of an unrelated synthetic reporter gene is overactive (2.3- to 5.1-fold) at all sites in the genome that we tested, suggesting that overactivity could be a general property of tandem gene duplicates. Overactivity occurs at the level of RNA transcription, and therefore tandem duplicate overactivity appears to be a novel form of position effect. The increment of surplus gene expression observed is comparable to many regulatory mutations fixed in nature, and if typical of other genomes, would broadly shape the fate of tandem duplicates in evolution.

D218 A double assurance mechanism controls enhancer-promoter specificity at the *hunchback* locus. *Jia Ling*[1], Theresa Apoznanski[2], Stephen Small[1]. 1) New York University, New York, NY; 2) New York College of Osteopathic Medicine.

Spatiotemporal gene expression is determined by enhancers, which interact with basal promoters to activate transcription. In genes where multiple enhancers and promoters co-exist at the same gene locus, individual enhancers target their specific promoters to activate. However, the underlying mechanism that regulates this enhancer-promoter interaction is unknown. In the current study, we focused on *hunchback* (*hb*), which contains two promoters: P1P, a maternal and late zygotic promoter, and P2P, an early zygotic promoter, which is activated by two *Bicoid* (*bcd*)-dependent enhancers: P2E and *shadow*. The *shadow* enhancer is located 4kb upstream of P2P but is close to P1P. By using a reporter system that mimics the endogenous *hb* locus and is capable of making a distinction between transcripts from two different promoters, we found that the *shadow* enhancer bypasses P1P and activates the more remote promoter P2P, and that P2E interacts with P2P, but not P1P. To investigate how these specific interactions between promoters and enhancers are regulated, we tested two hypothetical mechanisms: (i) motifs within the targeted promoter guide each enhancer to its specific promoter, and (ii) chromosome architecture, which facilitates contacts between *shadow* and P2E with P2P while blocking P1P. By manipulating TATA box sequences and spacing between regulatory elements in our reporter system and using single-molecule FISH (smFISH), we show that inserting or removing the TATA box of the basal promoters cause subtle changes in the relative RNA outputs of the two promoters, and that chromosome architecture plays a dominant role in preventing P1P from being activated. These results suggest that promoter motifs do affect a promoter's interaction specificity with an enhancer, but whether an interaction happens *in vivo* depends more on the chromosome environment. Overall, our experiments provided evidence for a double-assurance mechanism that provides robust expression of P2P to achieve normal thoracic development.

D219 Tailless repression sets pair-rule stripes and gap domains. *L. P. Andrioli*, G. Castro, F. Clava, W. dos Santos, F. Aguiar, J. Paciência, A. Ferrão-Neto, A. Machado-Lima, L. Digiampietri. EACH, USP, São Paulo, Brazil.

The segmentation cascade establishes the antero-posterior axis specification. The periodicity of the seven striped expression pattern of the segmentation pair rule genes is decisive to form the segments of the thorax and abdomen. However, in the presumptive anterior head region, no pair rule stripes are formed. Stripes are controlled by individual cis regulatory regions (CRMs), and we are working with the hypothesis that at least the CRMs of anterior pair-rule stripes are able to be activated but impeded to be expressed in more anterior regions of the blastoderm, due to the additive activity of several repressors such as *sloppy-paired 1* (*slp1*) and *huckebein* (*hkb*). Our goal is to identify other repressors and investigate transcription regulation mechanisms underlying this network. Here we investigated the activity of *tailless* (*tll*). To accomplish that, we integrated different approaches: genetic and misexpression assays, bioinformatics and biochemistry. We used an image computational tool to detect small perturbations of pair-rule striped patterns and gap domains in different genetic backgrounds. With that we were able to detect small deviations for anterior most pair-rule stripes of *hairy* (*h*) and *even-skipped* (*eve*). *h* 1 and *eve* 1 are anteriorly derepressed in *tll-* and increased effects were detected in *slp-;tll-* embryos, consistent with repression roles of Tll. We further confirmed that using reporter constructs under the control of *h* 1 and *eve* 1 CRMs in the genetic assays. Indeed, with a *tll* misexpression system we confirmed *tll* repression on these CRMs. We also investigated anterior gap genes in different genetic backgrounds and with the misexpression system. We did detect Tll effects on *empty-spiracles* (*ems*) and *buttonhead* (*btd*) in these assays, but not consistent with Tll indirect roles on *h* 1 and *eve* 1. These results point to Tll as being part of an anterior repression mechanism restricting anterior pair-rule stripes CRMs in the anterior blastoderm. Moreover, during our investigations with the misexpression system, we also detected repression effects for the majority of the other pair-rule stripes as well as for central gap genes, although we did not detected derepression for these putative targets in *tll-* or even in *slp-;tll-* double mutants. We discuss the physiological relevance of Tll also being a repressor to impede the expression of central pair-rule stripes and gap domains at the ends of the embryo. Financial support: FAPESP.

D220 Enzymatic modules of the SAGA chromatin-modifying complex play distinct roles in *Drosophila* gene expression and development. *Xuanying Li*[1,2], Christopher Seidel[1], Jerry Workman[1], Susan Abmayr[1,2]. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS.

Histone modifications are an important component of epigenetic control. Acetylation is generally associated with gene activation, whereas ubiquitination mediates both activation and repression. Histone modifying enzymes are often integrated into large multisubunit complexes. The modular SAGA chromatin-modifying complex interacts with transcription activators and the TATA-binding protein. It contains two enzymatic modules, with acetyltransferase (HAT) and deubiquitinase (DUB) activities. Using *Drosophila*, we sought to determine whether 1) SAGA is required for transcription of all genes, 2) SAGA dependent genes require both the HAT and DUB modules, and 3) individual modules can function independently of SAGA. We generated germline clone of genes encoding subunits of several modules to eliminate maternal gene product and examined the requirement for SAGA in oogenesis and early embryogenesis. Morphological analysis was used to determine the developmental stage at which mutants of different SAGA modules exhibit defects. Whole transcriptome profiling was used to determine which genes require different SAGA subunits. Lastly, ChIP-seq analysis was used to identify the direct targets of SAGA and its submodules in early embryos. We found that oogenesis is blocked upon loss of Ada2b, a subunit of the HAT module, whereas, germline clone females of Ataxin-7 and non-stop mutants, which are subunits of the DUB module, lay fertilized eggs with no overt defects until cellularization. These findings suggest that transcription essential for oogenesis requires the HAT activity but not the DUB activity of SAGA. Segmentation and D/V patterning appears to occur in embryos lacking the DUB module, as visualized by expression of eve and twi, but these embryos exhibit defects in cellularization. Consistent with this observation, some of the genes involved in cellularization are down regulated in Ataxin-7 GLCs. Whole genome profiling by RNA-seq shows that transcription of a subset of genes, primarily those involved in developmental, are affected by loss of Ataxin-7. ChIP-seq analysis of wild-type embryos revealed that SAGA (monitored by Ada2b) was bound at many, but not all sites bound by polII. Interestingly, ChIP-seq of DUB module proteins revealed their binding to SAGA-bound sites as well as novel sites. In summary, our data suggests that SAGA binds to only a subset of genes in the early embryos, and the DUB module is not required by all of these genes. Moreover, our data is the first to reveal that the DUB module can exist as a free form in wild type embryos that binds to novel locations in the genome.

D221 An ancient yet flexible *cis*-regulatory architecture allows localized Hedgehog tuning by *patched/Ptch1*. *S. Barolo*[1], D. Lorberbaum[1], A. Ramos[1], K. Peterson[2,3], B. Carpenter[1], D. Parker[4], S. De[5], L. Hillers[1], V. Blake[1,5], Y. Nishi[6], M. McFarlane[3], A. Chiang[1], J. Kassis[5], B. Allen[1], A. McMahon[3,6]. 1) University of Michigan Medical School, Ann Arbor, MI; 2) The Jackson Laboratory, Bar Harbor, ME; 3) Harvard University, Cambridge, MA; 4) Elon University, Elon, NC; 5) NICHD, NIH, Bethesda, MD; 6) University of Southern California Keck School of Medicine, Los Angeles, CA.

The Hedgehog signaling pathway is part of the ancient developmental-evolutionary animal toolkit. Frequently co-opted to pattern new structures, the pathway is conserved among eumetazoans yet flexible and pleiotropic in its effects. The Hedgehog receptor, Patched, is transcriptionally activated by Hedgehog, providing essential negative feedback in all tissues. Our locus-wide dissections of the *cis*-regulatory landscapes of fly *patched* and mouse *Ptch1* reveal abundant, diverse enhancers with stage- and tissue-specific expression patterns. The seemingly simple, constitutive Hedgehog response of *patched/Ptch1* is driven by a complex regulatory architecture, with batteries of context-specific enhancers engaged in promoter-specific interactions to tune signaling individually in each tissue, without disturbing patterning elsewhere. This structure—one of the oldest cis-regulatory features discovered in animal genomes—explains how *patched/Ptch1* can drive dramatic adaptations in animal morphology while maintaining its essential core function. It may also suggest a general model for the evolutionary flexibility of conserved regulators and pathways.

D222 Hemocytes as key regulators of respiratory system immunity in adult Drosophila melanogaster. *Katja Brückner*[1], Katrina S. Gold[1,5], Kalpana Makhijani[1,5], Rowan Baginsky[1], Brandy Alexander[1], Katie J. Woodcock[2], Leire Herboso[1], Elodie Ramond[3], Christa Rhiner[4], Eduardo Moreno[4], Bruno Lemaitre[3], Frédéric Geissmann[2], Katja Brückner[1]. 1) University of California San Francisco, San Francisco, CA; 2) King's College London, UK, and Memorial Sloan Kettering Cancer Center New York, NY; 3) EPFL Lausanne, Switzerland; 4) University of Bern, Switzerland; 5) equal contribution.

In adult Drosophila, the role and regulation of blood cells has been controversial and active hematopoietic sites have been proposed. Here we demonstrate that the main activity of the blood cell system in adult Drosophila lies in immunity. We show that the vast majority of blood cells, or hemocytes, are actively phagocytosing macrophages (plasmatocytes). As in many insects, hemocytes in Drosophila accumulate in clusters at the ostia of the adult heart. However, we find that the largest reservoir of adult hemocytes is the extensive respiratory epithelium of the thorax and head (tracheal air sacs), equivalent to the vertebrate lungs. Colonization of these areas depends on (1) developmental timing, in particular the completion of residual phagocytosis of larval tissue in the young adult, and (2) infection-induced localization following exposure to gram-negative or gram-positive bacteria. Hemocyte residence correlates with sites of particle accumulation after injection, illustrating connections of the respiratory tissues with the hemolymph through the open circulatory system. Using time- and tissue-specific permanent genetic lineage tracing, we show that more than half of the adult macrophages derive from the embryonic lineage of Drosophila hemocytes. Genetic and cell biological approaches for the detection of hemocyte proliferation, and the continuous decline of total hemocyte numbers over the course of adult life, do not provide any signs of active blood cell homeostasis or proliferation, both under unchallenged or immune challenged conditions. Consistently, the GATA factor Serpent (srp) marks actively phagocytosing macrophages in the adult animal, in contrast to its expression in prohemocytes during embryonic and larval development. To determine the role of hemocytes in the adult fly, we examined the immune response to bacterial challenge in genetically hemocyte-ablated animals. Interestingly, we find that hemocytes have a key role as sentinels of bacterial infection, promoting the induction of an antimicrobial peptide specifically in the respiratory tissue. We propose the respiratory epithelium of the tracheal air sacs as important immune tissue, which is directly linked with cellular immunity and the blood cell system in adult Drosophila.

D223 Modulation of occluding junctions alters the hematopoietic stem cell microenvironment to trigger immune activation in *Drosophila*. *Rohan Khadilkar*, Katharine Goodwin, Wayne Vogl, Guy Tanentzapf. Department of Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada.

The ability of stem cells to self-renew or differentiate is largely dictated by the signals derived from their local microenvironment where they reside, also known as the stem cell niche. During *Drosophila* larval hematopoiesis, the HSC (Hematopoietic stem cell) niche in the lymph gland regulates stem cell self-renewal and their capacity to rapidly differentiate into hemocyte lineages with immune function. Bacterial challenge is known to activate signalling pathways that initiate the humoral immune response in flies. Infection is likely to induce localized changes in the niche triggering the production of factors that promote HSC differentiation. However, the mechanisms by which the HSC's undergo this rapid switch towards the differentiation program is unknown. We show that septate junctions form a previously uncharacterized permeability barrier at the HSC niche regulating the accessibility of the HSC's to niche derived signals. We demonstrate that bacterial infection breaches this permeability barrier and leads to a dynamic outburst of differentiation. Also, genetic disruption of septate junctions at the niche mimics immune activation and provides immune-protection to the flies improving their survival post infection. Our results attribute a novel role for occluding junctions at the HSC niche in acting as a molecular switch between developmental and infection induced hematopoiesis.

D224 The TEAD family transcription factor Scalloped regulates blood progenitor maintenance and proliferation in *Drosophila* through PDGF/VEGFR receptor (Pvr) signaling. *J. A. Martinez-Agosto*, G. Ferguson. UCLA, Los Angeles, CA.

The *Drosophila* lymph gland has been characterized as the definitive hematopoietic organ in which a population of mulitpotent stem-like progenitors, defined by expression of the interleukin JAK-STAT receptor gene *domeless* (*dome*) and located within the Medullary Zone (MZ), is maintained both by a niche and an equilibrium signal emanating from nearby differentiated cells. However, it is not clear what the specific contribution of these progenitors is to the lymph gland during normal development or under immune challenge conditions. Here, we demonstrate a requirement for the TEAD family transcription factor Scalloped in the maintenance and proliferation of hematopoietic progenitors. We have identified a novel population of *domeless* negative progenitors in the early lymph gland that express Scalloped and the PVR ligand PVF2. In this unique population, Scalloped maintains PVF2 expression, which is required for progenitor proliferation and achieving normal lymph gland size. STAT activity marks actively proliferating early blood progenitors, and downregulating its activity causes decreased lymph gland growth similar to loss of Scalloped and PVF2, demonstrating a requirement for PVR/STAT signaling for determining lymph gland size. We further demonstrate that maintenance of *domeless*-expressing progenitors in the MZ is also dependent on a PVR-mediated equilibrium signal that originates from differentiating hemocytes. Pvr activation of STAT in hemocytes mediates expression of adenosine deaminase growth factor (ADGF), which maintains the balance between progenitors and differentiating hemocytes through regulation of extracellular adenosine levels that promote progenitor differentiation. Scalloped loss of function clones lack expression of PVR and depletion of Scalloped in differentiating hemocytes induces complete loss of progenitors, and this phenotype is rescued by overexpression of STAT and ADGF. Therefore, Scalloped function is required in hemocytes to maintain expression of PVR and maintain progenitors of the MZ. Additionally, the immune response to wasp parasitization is dependent on the presence of the pool of MZ progenitors to generate a cellular response to infestation. Scalloped mutants do not mount a lamellocyte mediated immune response to wasp parasitization, demonstrating that Scalloped is also required for the cellular response to infection. Scalloped is required to maintain the hematopoietic niche specifically during immune challenge conditions. These findings expand our mechanistic insight into the signals required to maintain hematopoietic progenitors and the underlying genetic requirements for niche competence essential for mediating the cellular immune response to infection.

D225 Microbial modulation of host lipid metabolism: lessons from *Drosophila*. *Chun Nin (Adam) Wong*[1,2], Wei Song[2,3], John Asara[2,4], Norbert Perrimon[2,3], Paula Watnick[1,2]. 1) Boston Children's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Howard Hughes Medical Institute, Boston, MA; 4) Beth Israel Deaconess Medical Center, Boston, MA.

Lipids are pivotal to animal metabolism, immunity and longevity. While the intestine plays an essential role in digestion, processing and assimilation of dietary lipids, less is known about how commensal microbiota and pathogens may integrate or interfere with host lipid metabolism. Using *Drosophila* as a model, our studies test the effects of the commensal microbiota and the intestinal pathogen *Vibrio cholerae* on host intestinal and systemic lipid metabolism. Confocal microscopy discovered remarkable changes in lipid distribution among tissues, including gut steatosis, when flies were made germ-free or orally infected with *V. cholerae*. Interestingly, this phenomenon is correlated with distinct but striking organ-specific changes in glycerolipid and phospholipid compositions, revealed by lipidomics. Further investigation by target gene expression analysis and the use of transgenic flies suggest commensal and pathogenic bacteria affect host lipid metabolism through different molecular mechanisms that involve enteroendrocine cell signaling and inter-organ communication, pathways that are highly conserved in mammals.

D226 A GWAS Analysis of Genetic Variation in *Drosophila melanogaster* Pathogen Susceptibility. *Jonathan Wang*, Hsiao-ling Lu, Raymond St. Leger. University of Maryland, College Park, MD.

The genetic basis of natural variation in disease resistance are not fully understood. To explore variation to pathogen infection, we used 188 *Drosophila* Genetic Reference Panel lines to perform a genome-wide association analysis for variation to infections with the fungus *Metarhzium anisopliae* (Ma549). We found substantial individual variation in within-host growth and host life span (LT_{50}'s ranged from 3.3 to 7.2 days), with males typically being more resistant than females, and resistant lines restraining fungal growth compared to susceptible lines suggesting that tolerance is less important than resistance in determining natural variation. We found Ma549 LT_{50}'s were moderately correlated with resistance to the bacterium *Pseudomonas aeruginosa*, In addition we found significant correlations with previously published phenotypes including oxidative stress sensitivity, sleep duration and number of "naps", and hemolymph glucose levels. The majority of polymorphisms affecting disease resistance had moderately large effects and were rare, suggesting that there is a general cost to defense involving trade-offs. Nevertheless, disease resistance was not correlated with longevity and fecundity. Many of the genes tagged by the top variants had plausible roles in host defense including components involved in hemocyte migration and phagocytosis, cuticle development, morphogenesis and tissue repair, glycerolipid metabolism, and protein phosphorylation. Several of the candidate genes have human homologs that were identified in studies of human disease, suggesting that genes affecting variation in susceptibility are conserved across species.

D227 Invasion dynamics in the fly gut microbiome. *B. Obadia*, T. Güvener, V. Zhang, W. Ludington. University of California, Berkeley, Berkeley, CA.

Recent studies have shown that the establishment and maintenance of the *Drosophila melanogaster* microbiota depend on constant ingestion of microorganisms, and that bacterial populations vary in richness and diversity over the lifespan of a fly. Animals are constantly faced with new potential invaders of their resident gut microbiota, but only some strains successfully establish themselves. We sought to understand to what extent this variability is probabilistic (*i.e.*, due to chance) *versus* determined by specific host and bacterial traits. We examined these questions in the context of a defined bacterial community of Acetobacter and Lactobacilli in gnotobiotic flies. Using a modified CAFE assay, we delivered a defined dose of bacteria to many hundreds of individual flies and measured their probability of successful colonization and their growth rate in the fruit fly gut. We focused on three different isolates of *Lactobacillus plantarum* (from human saliva, from Canton-S lab flies, and from wild flies). We quantified fitness and niche differences in vitro and in the fly gut, finding minor differences in both. High resolution microscopy indicates that Lactobacillus is capable of colonizing distinct regions of the fly gut. Notably, the wild fly strain had a greater colonization probability, a greater population size, and lower variation in population size than the other strains.

We are currently conducting experiments to determine the sources of the variation between individual flies (*e.g.*, immune activity) and between the different bacterial strains. In invasions of conventionally reared flies, we find that variance in the resident microbiota population increases with increasing input dose of commensal bacteria, indicating that invasion destabilizes the gut microbiota and suggesting that host-microbiota feedbacks may affect population dynamics.

Overall our data invalidate a simple lottery model and suggest a bistable system where invader-host feedbacks induce transitions between the two stable states (colonized *versus* uncolonized), with invasion facilitating more frequent state transitions. The results have implications for not only gut microbiome stability but also for macro-ecological invasions where large variation exists in the success of an invasion.

D228 Molecular analyses of immune-suppressive virus-like particles from a Drosophila parasitic wasp suggest cell-specific activities and a hybrid biotic particle nature. *M. E. Heavner*[1], J. Ramroop[1], G. Gueguen[2], G. Ramrattan[3], G. Dolios[4], M. Scarpati[5], W. Qiu[6], R. Wang[4], S. Singh[5], S. Govind[1]. 1) City College of NY & Graduate Center, CUNY, NY; 2) City College of NY, CUNY, NY; 3) Hunter College, CUNY, NY; 4) Icahn School of Medicine at Mount Sinai, NY; 5) Brooklyn College & Graduate Center, CUNY, NY; 6) Hunter College & Graduate Center, CUNY, NY.

The wasps *Leptopilina heterotoma (Lh)* and *L. boulardi (Lb)*, are obligate parasites of *Drosophila* species. *Lh*, a generalist parasite is successful on many fly species, while *Lb* is a melanogaster specialist. Remarkably, *Lh* silences both the humoral and cellular immunity of its fly hosts, while *Lb* activates humoral but suppresses cellular immunity. This difference in virulence has been attributed to their virus-like particles (VLPs). VLPs are produced in the wasp venom glands and enter the larval hemocoel during oviposition. Morphologically, *Lh* and *Lb* VLPs are similar, with spikes emerging from central particle cores.

Our sequence-based results have uncovered VLP protein classes that suggest an essentially non-viral, microvesicle-like structure with

eukaryotic and prokaryote-like proteins. We have also identified key proteins that might underlie the contrasting virulence strategies of the two wasps. p40, an abundant *Lh* VLP surface/spike tip protein is absent in *Lb.* Our previous work has shown that p40 is synthesized in venom glands and enters the larval hemocytes. Its surface localization and antibody inhibition experiments suggested that p40 mediates VLP interaction with host cell membranes and this interaction is pivotal to lamellocyte lysis.

Imaging studies show that in phagocytic hemocytes (plasmatocytes), VLPs require Rab5 to promote programmed cell death. However, they enter and kill encapsulating hemocytes (lamellocytes) by lysing cell membrane independently of Rab5. Domain analysis and *in silico* modeling of p40 suggests the presence of a fold from SipD/IpaD proteins of Gram negative type III secretion systems. These proteins reside at bacterial needle tips and regulate protein secretion into host cells.

VLPs are abundant in the larval dorsal vessel, within lymph gland progenitors, and around but not within posterior signaling center (PSC) cells. PSC cells are no longer tightly clustered but are instead dispersed in the body of the lobes. Lymph glands are non-responsive to wasps when PSCs are ablated and VLP staining in the lymph gland is low. These studies highlight the PSC's role in immunity and suggest that *Lh* VLPs may inactivate this function.

Our studies shed light on the biotic nature of VLPs and on novel mechanisms of immune suppression likely shared by many other wasp species and active in a variety of *Drosophila* hosts world-wide. (M.E. Heavner & J. Ramroop contributed equally.).

D229 Host-produced Eiger/TNF and the bacterial type 4 secretion system enable susceptibility of *Drosophila melanogaster* to *Coxiella burnetii* infection. *Alan G. Goodman*, Reginaldo G. Bastos. Washington State University, Pullman, WA.

Coxiella burnetii is the causative agent of Q fever, a zoonotic disease that threatens both human and animal health. Due to the paucity of experimental animal models, little is known about how host factors interface with bacterial components and affect pathogenesis. Here we used *Drosophila melanogaster*, in conjunction with the BSL2 Nine Mile phase II (NMII) clone 4 strain of *C. burnetii*, as a model to investigate host and bacterial components implicated in infection. We demonstrated that adult *Drosophila* are susceptible to infection with NMII clone 4 and that this bacterial strain, which activates the IMD pathway, is able to replicate and cause mortality in the animals. We show that in the absence of Eiger, the only known tumor necrosis factor (TNF) superfamily homolog in *Drosophila*, *Coxiella*-infected flies exhibit reduced mortality to infection. We also demonstrated that the *Coxiella* type 4 secretion system (T4SS) is critical for the formation of the *Coxiella*-containing vacuole and an establishment of infection in *Drosophila*. Altogether, our data revealed that the *Drosophila* TNF homolog Eiger and the *Coxiella* T4SS are implicated in the pathogenesis of *C. burnetii* NMII clone 4 in flies. The *Drosophila*/NMII clone 4 model mimics relevant aspects of the infection in mammals, such as a critical role of host TNF and the bacterial T4SS in pathogenesis. Our work also demonstrates the usefulness of this BSL2 model to investigate both host and *Coxiella* components implicated in infection.

D230 Postprandial sleep mechanics in *Drosophila*. *Keith R. Murphy*[1,2], Sonali A. Deshpande[1], James P. Quinn[1], Jennifer L. Weissbach[1], Alex C. Keene[2], Ken Dawson-Scully[2], Robert Huber[3], Seth M. Tomchik[1], William W. Ja[1]. 1) The Scripps Research Institute, Jupiter, FL; 2) Florida Atlantic University, Jupiter, FL; 3) Harvard University, Cambridge, MA; 6) L.

Sleep is regulated by a diversity of processes, such as circadian rhythm, homeostatic rebound, and dietary intake. While these inputs generally affect long-term sleep over hours or days, it is also thought that consuming food can induce an immediate, but transient, rise in sleep. Despite efforts to uncover mechanisms that drive postprandial sleep, little is known about how this behavior is regulated. Here, we developed a system for simultaneously measuring the sleep and food intake of individual *Drosophila* and found that animals experience a transient rise in sleep following meals. The effect of ingestion ranged from slightly arousing to strongly sleep inducing, depending on the amount of food consumed. While sucrose intake is primarily responsible for long-term sleep maintenance, we found that it had no effect on postprandial sleep. Rather, postprandial sleep was positively correlated with ingested volume, protein, and salt—revealing meal property-specific regulation. Silencing of Leucokinin receptor (Lkr) neurons, or Lkr knockdown in these cells, reduced postprandial sleep response specifically to protein ingestion. Paradoxically, silencing a subset of Leucokinin (Lk) neurons increased postprandial sleep, suggesting that Lk has waking outputs normally counterbalanced by Lkr circuitry. This circuitry has also recently been identified as a circadian output module, suggesting that these neurons may serve as an integration point for different types of sleep signaling. These findings reveal the dynamic nature of postprandial sleep and provide a novel system for studying the molecular and neuronal integration of sleep and feeding.

D231 A novel behavioural paradigm of interval timing in *Drosophila*. *W. J. Kim*[1], L. Y. Jan[2], Y. N. Jan[2]. 1) University of Ottawa, Ottawa, Ontario, CA; 2) University of California, San Francisco, San Francisco, USA; 3) University of California, San Francisco, San Francisco, USA.

Time is the fundamental dimension for animal's survival. The animal brain is the result of evolution to orchestrate temporal information across a wide spectrum of time scales. Especially, interval timing is a pivotal function of the human brain to support our cognitive ability such as memory, attention, and decision-making. Interval timing refers to the discrimination of durations in the seconds-to-minutes ranges. The genetic aspects of interval timing have not been vigorously investigated because of the lack of a genetically-traceable model organism. Here we present two novel behavioural paradigms of male *Drosophila* that fits the current 'internal clock model' of interval timing.

1. LMD, Rival induced prolonged mating: *Drosophila* males respond to the presence of rivals by prolonging mating duration to guard female and pass their genes. In previous studies, we examined the genetic network and neural circuits that regulate rival-induced longer mating duration (LMD). LMD can be induced solely via visual stimuli. LMD depends on the circadian clock genes *timeless* and *period*, but not *Clock* or *cycle*. LMD involves the memory circuit of the ellipsoid body (EB) (***Kim et al., Nat. neuroscience***). Further, we identified a small subset of clock neurons in the male brain that regulates LMD via neuropeptide signaling (***Kim et al., Neuron***).

2. SMD, Sexual experience-based shortened mating: *Drosophila* males exhibit a shortened mating duration for guarding female when sexually satiated, called 'Shorter-Mating-Duration (SMD)'. Both sexual experiences and contact-based chemoreception is necessary to induce SMD. SMD requires the sexually dimorphic Gr5a-positive neurons to detect female body pheromones, and can be induced by gustatory stimuli. The memory circuitry within the ellipsoid body (EB) and mushroom body (MB) brain regions are crucial to process this satiety state. SMD depends on the circadian clock genes *Clock* and *cycle*, but not *timeless* or *period*. SMD also relies on signaling via the neuropeptide sNPF, but not PDF or NPF. Sexual experience modifies the neuronal activity of a subset of sNPF-positive neurons involved in neuropeptide signaling, which modulates SMD (***Current Biol. in revision***).

Internal clock model (pacemaker-accumulator model PAM) constitutes of a pacemaker, mode switch, accumulator, memory circuit, and a comparator circuit. We have found that LMD/SMD provides all of these components, which are all identified in the fly brain. This presentation will provide 1) how the fly model of interval timing is similar with that of human and 2) what is the merit of this system.

D232 Scribble Scaffolds a signalosome for active forgetting. *I. Cervantes Sandoval*, R. L. Davis. The Scripps Research Institute, Jupiter, FL.

Forgetting, one part of brain's memory managment system, provides balance to the encoding and consolidation of new information by removing unused or unwanted memories or by suppresing their expression. Recent studies identified the Small G-protein, Rac1, as a key player of the mushroom bodies neurons (MBn), for active forgetting. We subsequently discovered that a few dopaminergic neurons (DAn) that innervate the MBn mediate forgetting. Here we show that Scribble, a scaffolding protein known primarly for its role as a cell polarity determinant, orchestrates the intracellular signaling for normal forgetting. Knocking down *scribble* expression in either MBn or DAn impairs normal memory loss. Scribble interacts physically and genetically with Rac1, Pak3 and Cofilin within the MBn, nucleating a forgetting signalosome that is downstream of dopaminergic inputs that regulates forgetting. These results bind disparate molecular players in active forgetting into a single signaling pathway: Dopamine→ Dopamine Receptor→ Scribble→ Rac→ Cofilin.

D233 The detection of bitter and sweet compounds by the evolutionarily conserved sweet clade in *Drosophila*. *Arun Kumar*, Erica Freeman, Adriana Lomeli, Anupama Dahanukar. University of California Riverside, Riverside, CA.

Detection of energy rich nutrients and potentially toxic compounds is crucial for the regulation of food choices. In *Drosophila*, sweet and bitter tastants are detected by members of a highly diverse family of 68 Gustatory receptors (Grs). Eight of these receptors are expressed in sweet taste neurons and are part of an evolutionarily conserved clade. In a previous study, we developed an *in vivo* functional expression system to individually express these receptors in a unique olfactory neuron that senses carbon dioxide. This neuron is unique because it expresses gustatory receptors, Gr63a and Gr21a, which detect carbon dioxide but do not respond to any known tastants. By expressing sweet taste receptors individually in this olfactory neuron, we found that each receptor responds to unique but overlapping subsets of sweet tastants. We also tested Gr43a, an internal fructose receptor, and its mosquito ortholog AgGr25, and discovered that both receptors respond to fructose as well as other sugars. Thus, all sweet receptors are directly involved in the detection of sweet compounds.

Drosophila have been shown to detect aversive compounds via two different cellular mechanisms – activation of deterrent neurons and inhibition of appetitive neurons. Recent studies have found that an odorant binding protein (Obp49a) is required for bitter tastant inhibition of sweet taste neurons. From a systematic analysis of the dynamics of sweet neuron inhibition, we found that sensitivity to bitter tastants is reduced but not abolished in Obp49a mutants, suggesting that other mechanisms are involved. Moreover, bitter tastants did not evoke the same degree of inhibition when combined with different sugar agonists, raising the possibility of receptor specific antagonist effects. We therefore investigated whether sweet taste receptors were directly inhibited by bitter tastants using the ectopic expression system. We tested seven receptors of the sweet clade individually against a panel of 21 bitter compounds at different concentrations and found that sweet Grs could be directly inhibited by bitter tastants in a dose dependent manner. Moreover, sweet Grs have different sensitivity to different bitter tastants, as well as unique inhibitory response profiles. Interestingly, this property is a distinguishing feature of the sweet Gr clade – neither Gr43a nor the Gr21a/Gr63a receptors are inhibited by bitter tastants. Thus, sweet Grs appear to have evolved to sense both appetitive and aversive compounds.

D234 A peptidergic pathway critical to satiety responses in Drosophila. *S. Min*[1], H. Ryu[2], J. Chung[3]. 1) Seoul National University, Seoul, Seoul, KR; 2) Seoul National University, Seoul, Seoul, KR; 3) Seoul National University, Seoul, Seoul, KR.

Although several neural pathways have been implicated in feeding behaviors in mammals, it remains unclear how the brain coordinates feeding motivations to maintain a constant body weight (BW). To gain insight into this issue, we sought to identify a neural pathway that functions critically in regulation of BW and food intake using Drosophila. To do so, we performed a genetic screen on a collection of neuropeptide GAL4 driver lines expressing a neural silencer using a series of quantitative assays measuring BW and food intake. In our recent study, we reported the identification of a neuropeptide pathway important for BW control via inducing satiety in Drosophila. Silencing of myoinhibitory peptide (MIP) neurons significantly increased BW through augmented food intake and fat storage. Likewise, the loss-of function mutation of *mip* also increased feeding and BW. Suppressing the MIP pathway induced satiated flies to behave like starved ones, with elevated sensitivity toward food. Conversely, activating MIP neurons greatly decreased food intake and BW and markedly blunted the sensitivity of starved flies toward food. Upon terminating the activation protocol of MIP neurons, the decreased BW reverts rapidly to the normal level through a strong feeding rebound, indicating the switch-like role of MIP pathway in feeding. Surprisingly, the MIP-mediated BW decrease occurred independently of sex peptide receptor (SPR), the only known receptor for MIP, suggesting the presence of a yet-unknown MIP

receptor. Together, our results reveal a novel anorexigenic pathway that controls satiety in Drosophila and provide a new avenue to study how the brain actively maintains a constant BW.

D235 A genetically tractable platform for identifying regulators of acute and chronic pain. *S. Im*[1], P. Huang[2], M. Galko[1]. 1) UT MD Anderson Cancer Center, Houston, TX; 2) Rice University, Houston, TX.

Nociceptive (pain) sensitization occurs when tissue damage leads to an adaptive lowering of the threshold for pain behavior. We have developed a platform for studying the genetic basis of adaptive and maladaptive pain modulation. Our unique platform combines UV radiation with quantitative behavioral assessment of thermal or mechanical nociception. To identify conserved genetic players underlying nociceptive sensitization we used tissue-specific RNAi, electrophysiology, cell-based assays, and behavior analysis to discover that *Drosophila* Substance P (Tachykinin) signaling modulates injury-induced nociceptive sensitization by controlling Hedgehog signaling (Im et al., *eLife*, 2015). This is a novel genetic interaction regulating acute sensitization.

While injury-induced nociceptive sensitization is an adaptive modulation of pain, when misregulated it can lead to maladaptive, debilitating chronic pain. To identify regulators of the transition from acute to chronic pain, we performed a tissue-specific RNAi screen looking for genes that led to persistent thermal hypersensitivity when specifically knocked down in multidendritic sensory neurons. Among 150 RNAi lines targeting conserved kinase-encoding genes, we found multiple hits that represent potential regulators of the elusive acute-chronic pain transition. The most striking candidate to emerge from this pilot screen was the *insulin receptor* (*InR*). *InR* knockdown caused prolonged hypersensitivity toward thermal and mechanical stimulation that was not accompanied by defects in baseline nociception (no injury) or acute adaptive sensitization to injury. Thus, the defect in *InR* knockdown larvae is specific to the transition from acute to chronic pain. The identification of *InR* as a regulator of the acute to chronic pain transition prompted us to model painful diabetic neuropathy (PDN), one of the most prevalent complications of diabetes with disabling pain syndromes. Similar to knockdown of *InR*, *Drosophila* models of both type I and type II diabetes exhibited prolonged PDN-like thermal pain hypersensitivity. This data suggest that diabetic conditions in *Drosophila* lead to pathological pain hypersensitivity similar to that experienced by diabetic patients, advocating that our genetically tractable platform is useful for dissecting genetic/molecular mechanisms of PDN pain syndromes. This discovery suggests a completely novel hypothesis not yet investigated in the field, namely that Insulin signaling is required within nociceptive sensory neurons to prevent PDN. We are currently investigating how this PDN-like chronic pain is achieved.

D236 The molecular and cellular basis of pharyngeal taste in *Drosophila*. *Yu-Chieh Chen*, Anupama Dahanukar. UC Riverside, Riverside, CA.

In *Drosophila*, sweet and bitter taste sensation is mediated mainly by gustatory receptors (Grs) expressed in gustatory receptor neurons (GRNs). GRNs are housed within taste sensilla that are distributed in different body parts, including the labellum, pharynx, distal segments of the legs (tarsi), wing margins, and ovipositor. Despite extensive studies that have described taste coding in the main taste organs (labellum and tarsi), little is known about the molecular and cellular basis of pharyngeal taste. Given that the pharynx lies in an anatomical position where it may serve as the last checkpoint for food ingestion, our aim is to explore pharyngeal taste coding for a better understanding how gustatory inputs from different taste organs are weighed and translated into appropriate feeding behaviors.

We first systematically analyzed the expression patterns of *Gr-Gal4* lines representing all Grs via the Gal4/UAS system in three pharyngeal sense organs: labral sense organ, and ventral and dorsal cibarial sense organs.The Gr-to-neuron mapping results reveal distinct pharyngeal sensilla organization as compared to external taste organs.

To specifically study the function of pharyngeal GRNs in feeding behaviors, we took advantage of pox-neuro (*poxn*) mutants, in which the external taste bristles are transformed into mechanosensory bristles. Previously, we showed that *poxn* mutants have intact pharyngeal GRNs, arguing that *poxn* mutants serve as a good tool for dissecting the functional role of pharyngeal GRNs without other confounding taste inputs (e.g. labellar and tarsal gustatory inputs). Using binary choice assays, we found that *poxn* mutants retain similar feeding preferences for most sweet, bitter, salt, amino acid, and acid tastants as compared to wild-type flies. Moreover, we have identified classes of pharyngeal GRNs in mediating both food acceptance and avoidance behaviors, respectively by activating or silencing these pharyngeal GRNs in the *poxn* mutant background. Currently, we are investigating response profiles of pharyngeal GRNs to various tastants by Ca^{2+} imaging, which will allow comparisons of ligand specificity and sensitivity between taste organs.

D237 Ionotropic Receptors mediate thermo- and hygro-sensation in Drosophila. *P. A. Garrity*[1], Z. Knecht[1], L. Ni[1], M. Klein[2], A. Silbering[3], G. Budelli[1], K. Svec[1], A. Samuel[4], R. Benton[3]. 1) Brandeis Univ, Waltham, MA; 2) University of Miami, Coral Gables, FL; 3) University of Lausanne, Lausanne, Switzerland; 4) Harvard University, Cambridge, MA.

The detection of temperature and moisture is critical for survival. Flies must avoid thermal extremes and maintain appropriate body temperatures and hydration levels. The mechanisms by which thermo- and hygro-sensory stimuli are detected remain poorly understood. We find that members of the Ionotropic Receptor (IR) family of invertebrate sensory receptors have crucial roles in both of these sensory modalities. IRs are a large family of receptors (66 IRs in *Drosophila melanogaster*) that have been extensively studied as chemoreceptors. In the olfactory system, broadly expressed IR co-receptors like IR25a combine with more selectively expressed odor-specific IRs to create receptors conferring sensitivity to distinct chemicals. We now show that IR25a combines with members of a distinct group of “orphan” IRs (IRs not previously associated with chemical ligands) to confer sensitivity to thermo- and hygro-sensory stimuli and to drive thermo- and hygro-sensory behaviors. Analogous to IR25a-mediated chemosensing, we find that different IR combinations act in different neurons to mediate physiological and behavioral responses to distinct thermo- and hygro-sensory stimuli. Our data define a new set of combinatorial mediators of

thermo- and hygro-sensation in *Drosophila*, and provide a new view of the relationship between these sensory modalities at a mechanistic level.

D238 A Potential Role for DNA Replication in Establishing Distinct Epigenomes. *M. I. Wooten*[1], V. Tran[3], J. Snedeker[1], R. Ranjan[1], X. Yang[2], J. Buss[2], X. Chen[1]. 1) Johns Hopkins University, Baltimore, MD; 2) Johns Hopkins University School of Medicine, Baltimore, MD; 3) Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington.

Epigenetic mechanisms specify cell fate decisions by altering chromatin structure and gene expression patterns while preserving primary DNA sequences. Epigenetic mechanisms play a key role in specifying and maintaining stem cell identity throughout multiple cell divisions. Many types of stem cells have the ability to asymmetrically divide to give rise to one daughter cell capable of self-renewal and another daughter capable of differentiating. Previously, we discovered that the preexisting H3 is segregated to the male germline stem cell (GSC) whereas newly synthesized H3 is enriched toward the differentiating daughter cell in *Drosophila melanogaster*. Since post-translational histone modifications are a key component of the epigenome, our studies provide the first direct evidence suggesting that stem cells may selectively retain preexisting histones that define their stem cell identity. We have demonstrated that this asymmetric pattern is specific to the canonical H3, but not for histone variant H3.3. Because H3 is incorporated during DNA replication whereas H3.3 is incorporated in a replication-independent manner, our findings suggest a potential role for DNA replication in establishing epigenetic information.

Using super-resolution microscopy, I have been able to study the localization patterns of preexisting vs. newly synthesized histones for histones H3 and H3.3. Whereas histone H3.3 shows no separation between preexisting and newly synthesized histones during DNA replication, histone H3 shows regions of separation approximately 200nm in size. Furthermore, using a technique known as a proximity ligation assay (PLA), I have been able to test whether different histone populations show a strand preference (leading vs. lagging) during their incorporation onto nascent chromatin. By testing the proximity of preexisting and newly synthesized histones to proteins known to be enriched upon the lagging strand, I have generated preliminary data suggesting that newly synthesized histones are preferentially incorporated onto the lagging strand. In addition, I have been able to nucleotide analogues (EdU, BrdU) to observe regions of DNA replication in the *Drosophila* germline. By comparing the replication patterns observed in asymmetrically dividing GSCs to the replication patterns observed in symmetrically dividing progenitor germ cells, I have been able to generate preliminary data suggesting that GSCs coordinate DNA replication in a manner distinct from symmetrically dividing progenitor cells. Based on these data, we propose that GSCs may regulate DNA replication in such a manner that sister chromatids may be built primarily via leading-strand synthesis or lagging-strand synthesis, thereby coordinating the differential deposition of preexisting vs. newly synthesized histones onto distinct sister chromatids.

D239 Sensing Respiratory Gases for the Control of the Hematopoietic System. *B. Cho*[1], C. Spratford[2], F. Chi[2]. 1) Hanyang University, Seoul, KR; 2) University of California, Los Angeles, LA, CA, USA.

Aerobic respiration is the fundamental process in which cells generate energy by consuming oxygen and producing carbon dioxide. Organisms elaborately check the levels of respiratory gases from both internal and external systems for survival. Here, we find a novel link in between the external CO2 sensing cascade and the *Drosophila* hematopoietic system. In the brain, CO2 receptor neuron directly interacts with a small subset of O2 sensing neurons that systemically modulate physiological status according to the respiratory gases. Loss of the CO2 receptor neuron triggers the O2 sensing neuron to lead a hypoxia-like stress through changes in neuropeptides. Changes in the neuropeptide stimulates Notch-mediated cell to cell interaction in the hematopoietic organ, which induces expansion of crystal cells, a hallmark of hypoxic blood. This study provides a mechanistic and novel paradigm for the sensory perceptions of the CO2 gases and their effects on the hematopoietic system that may be conserved in mammals.

D240 Opposite temporal gradients of Imp and Syp govern senescence of neural stem cells via distinct effectors. *Ching-Po Yang*, Tzumin Lee. Howard Hughes Medical Institute, Ashburn, VA.

Senescence of neural stem cells is exquisitely regulated to ensure production of appropriate numbers of neurons that can vary drastically in different neuronal lineages. For instance, while neurogenesis ends in most *Drosophila* brain regions around pupation, the four pairs of mushroom body (MB) neuroblasts incessantly make neurons throughout pupal development. It is unclear what intrinsic and/or extrinsic mechanisms underlie the differential senescence of neural stem cells. We have uncovered that most, if not all, cerebral neuroblasts express the Imp and Syp RNA-binding proteins in opposite temporal gradients that show distinct lineage-characteristic temporal dynamics. The reciprocal Imp/Syp temporal gradients are steep and progress rapidly in non-MB neuroblasts, but get stretched into very shallow profiles in those long-lived MB neuroblasts. Here we report that the lineage-characteristic temporal profiles of Imp and Syp control when particular neuroblasts end in a lineage-autonomous manner. We further demonstrate that Imp and Syp act sequentially through distinct effectors to stop neurogenesis. Taken together with their known roles in specifying serially derived neuronal types, we propose that the opposing Imp/Syp temporal gradients constitute an evolutionally conserved stem cell aging program that govern both the length of neuronal lineages and the diversification of neuronal temporal fates.

D241 A Transcriptional Network Specifies The Intestinal Stem Cell Fate In *Drosophila* Adult Midgut. *Qing Lan*, Min Cao, Rahul K. Kollipara, Jeffrey B. Rosa, Ralf Kittler, Huaqi Jiang. UT Southwestern Medical Center, Dallas, TX.

Increasing evidence indicate that transcription factors (TFs) play an essential role in specifying cell identities. Here, we report the identification of a transcriptional network that maintains intestinal stem cells (ISCs) in *Drosophila* adult midgut. The core module of the network consists of FoxA TF Fkh, E-protein TF Da and SoxE TF dSox9. Our genetic analyses indicate that the ISCs defective in these TFs rapidly

lose their progenitor fates. On the other hand, their ectopic inductions in the progenitors prevent them from differentiating into mature gut cells. We mapped their binding sites in the progenitors and revealed that they bind to many of the same genomic loci and likely regulate a common set of target genes including themselves. In addition, we showed that TFs downstream of several key ISC niche pathways and epigenetic regulators also bind to these same genomic loci. And these shared genomic loci likely function as active enhancers and mediate the induction of progenitor genes. Finally, we explored the interaction between the core module and niche pathways and demonstrated that dSox9 primes Jak-Stat pathway for activation in the progenitors, which in turn induces Emc to suppress Da to promote midgut differentiation.

D242 Niche Appropriation by *Drosophila* Intestinal Stem Cell Tumors. *P. H. Patel*[1,2], D. Dutta[1,2], M. Roca[1,2], B. A. Edgar[1,2]. 1) German Cancer Research Center (DKFZ), Heidelberg, Germany; 2) Center for Molecular Biology, University of Heidelberg (ZMBH), Heidelberg, Germany.

Mutations that inhibit differentiation in stem cell lineages are a common early step in cancer development, but precisely how a loss of differentiation initiates tumorigenesis is unclear. We investigated *Drosophila* intestinal stem cell (ISC) tumors generated by suppressing *Notch* (*N*) signaling, which blocks differentiation. *Notch*-defective ISCs require stress-induced divisions for tumor initiation and an autocrine EGFR ligand, Spitz, during early tumor growth. Upon achieving a critical mass these tumors displace surrounding enterocytes, competing with them for basement membrane space and causing their detachment, extrusion and apoptosis. This loss of epithelial integrity induces JNK and Yki/YAP activity in enterocytes and, consequently, their expression of stress-dependent cytokines (Upd2, Upd3). These paracrine signals, normally used within the stem cell niche to trigger regeneration, propel tumor growth without the need for secondary mutations in growth signaling pathways. Interestingly, we have recently found that tumor growth additionally induces p38 signaling in surrounding epithelial cells. The appropriation of niche signaling by differentiation-defective stem cells may be a common mechanism of early tumorigenesis.

D243 The niche ligand-receptor directly orients the spindle in *Drosophila* male germline stem cells. *C. Chen*[1,2], Y. Yamashita[1,2]. 1) University of Michigan, Ann Arbor, MI; 2) Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI.

Asymmetric cell division is a critical aspect of many stem cells in balancing self-renewal and differentiation. For those stem cells residing in the niche, spindle orientation with respect to the niche is crucial to achieve asymmetric stem cell division. Despite this critical relationship between the stem cell niche and spindle orientation, the niches' role in spindle orientation is poorly understood. Here we show that, in Drosophila male germline stem cell (GSC) niche, niche ligand Upd and its receptor Dome, but not their downstream JAK-STAT pathway, is required for spindle orientation. We found that the receptor Dome directly interacts with a plus-end microtubule binding protein Eb1 to orient the spindle toward the niche. Live imaging experiments suggest that Dome and Eb1 function to pull the spindle toward the niche. Photoconversion experiments demonstrate that microtubule dynamics is asymmetric between apical and basal half of the spindle, possibly explaining the mechanism by which spindle is pulled toward the niche. Our study reveals a novel mechanism by which the niche directly governs the asymmetric stem cell division via direct regulation of microtubule cytoskeleton.

D244 Somatic cell encystment promotes abscission in germline stem cells after a regulated block in cytokinesis. *K. Lenhart*, S. DiNardo. University of Pennsylvania, Philadelphia, PA.

Stem cell behavior, from rates of cell division to the capacity for self-renewal, is regulated by the specialized microenvironment in which those stem cells reside. A critical function of such niches in many tissues is the coordination of behavior across multiple stem cell lineages. However, the means by which this coordination is achieved are largely unknown. We have identified delayed completion of cytokinesis in germline stem cells (GSCs) as a novel mechanism that regulates the production of stem cell daughters within the testis niche. Through live imaging, we have characterized the delay within GSCs and identified two regulatory mechanisms layered on top of cytokinesis. Following contractile ring disassembly, a novel F-actin ring is formed exclusively in the stem cell population and only in male, but not female, GSCs. This ring is regulated by Cofilin activity and serves to block cytokinesis progress. The duration of this block is controlled by Aurora B activity. Additionally, we have identified a critical requirement for somatic cell encystment of the germline in promoting the final step of GSC cytokinesis. We suggest that this non-autonomous role exists to promote the coordination necessary between stem cell lineages within this niche to achieve robust production of sperm. Together, these findings shed significant insight into a niche-imposed block and reinitiation of cytokinesis in GSCs and why such complex regulation might exist within a stem cell niche.

D245 An intercellular E-cadherin-EGFR relay maintains organ size during renewal by coupling cell division and death. *J. Liang*, S. Balachandra, L. E. O'Brien. Stanford, Stanford, CA.

Self-renewing organs require balanced rates of cell production and loss to maintain a constant number of cells. Cellular imbalance leads to organ hyperplasia or degeneration, but how cellular balance is enforced during normal renewal remains poorly understood. Examining intestinal renewal in Drosophila, we find that feedback inhibition from mature enterocytes to stem cells serves to couple cell death and division and maintain constant cell number. In enterocytes, the adhesion receptor E-cadherin (E-cad) inhibits stem cell divisions not by binding stem cell E-cad, but by repressing the EGF maturation factor *rhomboid* to limit secretion of enterocyte EGFs. Conversely, loss of E-cad upon physiological apoptosis derepresses *rhomboid*, triggering local activation of EGFR in nearby stem cells. EGFR activation induces stem cells to divide, generating new cells to replace apoptotic enterocytes. When enterocyte apoptosis is blocked, stem cells compensate by slowing their divisions. Disrupting E-cad-EGFR feedback inhibition impairs this compensatory response and leads to organ hyperplasia. Our results show that global inhibition of stem cell EGFR is locally relieved when an enterocyte apoptoses. This mechanism couples division and death, ensuring zero-sum cell replacement and constant organ size during renewal.

D246 Poster Awards Presentation. *Nancy Bonini*. The Icahn School of Medicine at Mount Sinai, New York.
no abstract submitted

D247 Coordination of neuroepithelial specification and neurogenesis modes in the *Drosophila* visual system. *I. Salecker*, H. Apitz. The Francis Crick Institute, London, England, UK.

Vertebrate and invertebrate brains are organized into different areas with distinct neuron subtypes and functions. While we have some insights into the role of region-specific patterning mechanisms, it is not well understood whether alterations in neurogenesis strategies also contribute to circuit diversification.

In the *Drosophila* visual system, neurons in four ganglia originate from two neuroepithelia, the outer (OPC) and inner (IPC) proliferation centers. Neuroepithelial cells in the medial OPC directly convert into neural stem cell equivalents, the neuroblasts, to generate medulla neurons. One IPC subdomain, the proximal (p)-IPC, is dedicated to generating two neuron populations, distal cells and lobula plate neurons that include the motion-detecting T4 and T5 neurons. By contrast, we observed that the p-IPC produces offspring using a different mode of neurogenesis. p-IPC neuroepithelial cells gradually convert into progenitors that migrate within cell streams and acquire neuroblast properties in a second domain, the distal (d)-IPC. Progenitors emerge by an epithelial-mesenchymal transition-like mechanism that in part depends on the Snail transcription factor Escargot and Decapentaplegic signaling. Furthermore, we found that the proneural bHLH proteins Lethal of scute and Asense differentially control the supply rate and maturation of neuroblasts, respectively. Neuroblasts switch expression from Asense to Atonal, a third proneural protein. Cross-regulatory interactions of the Sox protein Dichaete and the orphan nuclear receptor Tailless are essential for the transition from Asense to Atonal expression, and the generation of distal cells to lobula plate neurons. These factors thus act as switching factors of neuroblast competence. Finally, we provide evidence that the neuroepithelial default-state is IPC-like and that retinal determination gene network members play a central role in conferring neuroepithelial identity to the OPC, including its neurogenesis mode by direct conversion into neuroblasts.

We propose that the novel neurogenesis mode in the p-IPC und the underlying regulatory mechanisms could represent general strategies for setting-up a new proliferative zone to facilitate spatio-temporal matching of neurogenesis and connectivity across ganglia.

D248 Growth coordination mechanisms during Drosophila development. *P. Leopold*. Univ Nice / CNRS /Inserm, IBV, Nice, FR.

Body size is an intrinsic property of organisms linked to their developmental program to produce fit individuals with proper proportions. Final size is the result of genetic determinants as well as sophisticated mechanisms adapting size to available resources. Classical regeneration and transplantation experiments have established that different body parts grow according to autonomous programs, challenging the concept of systemic, harmonious growth. Therefore, coordination mechanisms must ensure that all parts have reached an appropriate final size before animals stop growing. Recent advances making use of physiological and genetic approaches have started unravelling some of the cross talks contributing to body growth coordination. In flies, the “coordination hormone” Dilp8 plays a major role in these processes. This relaxin-like hormone is produced by tissues upon growth perturbation. It couples the program of developmental transitions with organ growth by acting on its membrane receptor Lgr3, activating a neural circuitry that is only partially elucidated. Interestingly, animals lacking Dilp8 or Lgr3 present defects in bilateral symmetry, a sign that this novel hormonal system participates in the developmental control of organ growth coordination. In this presentation, I will discuss our recent research aimed at understanding the mechanisms allowing organ/organ growth coordination both in response to abnormal growth (injury/neoplasm) and in the course of normal development (control of developmental stability).

D249 Networking at the nuclear periphery: Contributions of Drosophila LEM domain proteins. *P. K. Geyer*, L. J. Barton, K. E. Lovander, C. Jensen-Cody, W. Ke. Univ Iowa, Iowa City, IA.

Nuclear architecture changes as cell fate specification occurs. During development, chromatin domains adopt distinct spatial distributions, with repressed domains directed to the nuclear periphery. Such positioning depends on the extensive protein network that lines the inner nuclear envelope, known as the nuclear lamina. Included in this network are the LAP2-emerin-MAN1-domain (LEM-D) proteins. This family of proteins binds lamins and tethers repressive chromatin to the nuclear periphery. The importance of the human family of LEM-D proteins is underscored by the fact that loss of individual proteins causes progressive, tissue-restricted diseases, including muscular dystrophy, cardiomyopathy and bone density disorders. Mechanisms by which LEM-D proteins contribute to these diseases are poorly understood. To understand the function of LEM-D proteins, our laboratory has investigated three members of the Drosophila family, Otefin, Bocksbeutel and dMAN1. Genetic studies revealed that each protein has unique and over-lapping functions during development, with loss of individual proteins causing age-enhanced phenotypes. As an example of these development contributions, the LEM-D protein Otefin is uniquely required for oogenesis. Loss of Otefin causes premature death of adult germline stem cells (GSCs) wherein mutant GSCs display features of aging, including altered nuclear structure and chromatin organization. GSC death results from activation of the DNA damage signaling kinases ATR and Chk2. Surprisingly, ATR/Chk2 activation occurs without evidence of DNA damage or transposon transcription. Together, these findings reveal that GSCs have a novel survival checkpoint that depends on nuclear architecture. Our studies suggest that human pathologies result from progressive dysfunction of adult stem cell populations that lead to defects in tissue homeostasis.

D250 Molecular genetics of sex-specific evolutionary innovations. *A. Kopp*. University California, Davis, Davis, CA.

One of the most intriguing features of animal development and evolution is the rapid turnover of sex-specific traits. Most animal species are sexually dimorphic, yet the characters distinguishing males from females are different in every case. This simple observation implies that new sexual characters are gained, and old ones are lost, during the evolution of every animal lineage. The molecular mechanisms of this turnover are poorly understood. We are using the "sex comb" of *Drosophila*, a recently evolved and rapidly diversifying male-specific structure, to elucidate how new sexually dimorphic traits originate and evolve. Key roles in this process are played by the HOX gene *Sex combs reduced* (*Scr*) and by the *doublesex* (*dsx*) transcription factor, the main effector of the *Drosophila* sex determination pathway. Only some cells in *Drosophila* express *dsx*, resulting in a complex mosaic of "sex-aware" and "sex-naive" cells. In *D. melanogaster*, which has sex combs, *dsx* expression in the presumptive sex comb region is activated by *Scr*, and the male-specific isoform of *dsx* up-regulates *Scr* so that both genes become expressed at high levels in this region in males but not in females. Precise spatial regulation of both *dsx* and *Scr* is essential for defining sex comb position and structure. Comparative analysis of *Scr* and *dsx* expression reveals a tight correlation between sex comb size, position, and morphology and the expression patterns of both genes. In *Drosophila* species that primitively lack sex combs, no *dsx* expression is observed in the homologous region while *Scr* shows no male-specific upregulation, suggesting that the origin and diversification of sex combs were linked to the gain of a new, *Scr*-dependent expression domain of *dsx*, and to the evolution of the *Scr*/*dsx* autoregulatory loop. At the molecular level, these changes were caused by the origin of a new *cis*-regulatory element that drives *dsx* expression in the sex comb, and by changes in the spatial activity of *Scr* enhancers. Two other, distantly related fly lineages that independently evolved novel male-specific structures show convergent evolutionary gains of *dsx* expression in the corresponding tissues. Thus, changes in the spatial regulation of sex-determining genes may be a key mechanism that enables the evolution of new sex-specific traits, contributing to some of the most dramatic examples of phenotypic diversification in nature.

MOUSE GENETICS 2016 CONFERENCE

Plenary and Platform Session Abstracts

Mouse Genetics 2016

SCHEDULE AT-A-GLANCE

Wednesday, July 13		
2:00pm-9:30pm	Speaker Ready Room Open	Hall of Cities - Anaheim
7:00pm-9:00pm	Scientific Session: International Resources	Crystal Ballroom G1
9:00pm-11:00pm	Opening Mixer with Exhibits	Cypress Ballroom
Thursday, July 14		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities -Anaheim
7:45am-10:00am	Genetics and Determinants of Health Joint Plenary Session	Palms Ballroom
8:00am-4:00pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:30am-12:30pm	Scientific Session: Comparative Genomics, Computational Methods and Evolution	Crystal Ballroom G1
12:30pm-1:30pm	Mentoring Roundtables #1	North Tower - Harbor Beach
12:30pm-1:30pm	Speaking Up for Genetics and Model Organism Research	Crystal Ballroom H
1:30pm-3:30pm	Poster Presentations 1:30pm-2:30pm: Even-numbered poster 2:30pm-3:30pm: Odd-numbered posters	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center and Job Fair	Cypress Ballroom 1C
4:00pm-6:00pm	Scientific Session: Development	Crystal Ballroom G1
4:00pm-6:00pm	Plenary Session and Workshop for Undergraduate Researchers	North Tower - Sawgrass
7:45pm-9:45pm	Scientific Session: Translational and Systems Genetics	Crystal Ballroom G1
10:00pm-11:30pm	*Science Cafe Event	Palms Ballroom Sabal
Friday, July 15		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-9:30am	Scientific Session: Technological Innovations	Crystal Ballroom G1
8:00am-4:30pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:00am-12:00pm	Scientific Session: Human Disease Models 1	Crystal Ballroom G1
12:00pm-1:30pm	*Editor's Panel Discussion and Roundtable	North Tower - Harbor Beach
1:30pm-3:30pm	Poster Presentations 1:30pm-2:10pm: "A" poster authors present 2:10pm-2:50pm: "B" poster authors present 2:50pm-3:30pm: "C" poster authors present	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center	Cypress Ballroom 1C

** Ticketed Event*

#TAGC16

Friday, July 15 (continued)		
2:00pm-2:45pm	GeneticsCareers Workshop - Nailing the Job Talk	Cypress Ballroom 1B
4:00pm-6:00pm	Scientific Session: Epigenetics	Crystal Ballroom G1
6:00pm-7:30pm	*Women in Genetics Panel and Networking	North Tower - Harbor Beach
7:30pm-9:30pm	Development and Evolution Joint Plenary Session	Palms Ballroom
Saturday, July 16		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-10:00am	Workshops: See topics and descriptions in the Workshop Section	Multiple locations
8:00am-12:00pm	Exhibits Open	Cypress Ballroom
8:00am-9:00am	Trainee Bootcamp Workshops: Session 1	North Tower
9:00am-10:00am	Trainee Bootcamp Workshops: Session 2	North Tower
10:00am-12:00pm	Poster Presentations 10:00am-11:00am Odd-numbered posters 11:00am-12:00pm Even-numbered posters	Cypress Ballroom *(Posters must be removed by 1pm)*
10:00am-12:00pm	GeneticsCareers Center	Cypress Ballroom 1C
10:30am-11:15am	GeneticsCareers Workshop	Cypress Ballroom 1B
12:15pm-1:45pm	*Mentoring Roundtables #2	North Tower - Harbor Beach
1:45pm-3:45pm	Scientific Session: Cancer and Immunology	Crystal Ballroom G1
4:00pm-6:00pm	Scientific Session: Rosa Beddington Lecture Stem Cells	Crystal Ballroom G1
6:00pm-6:30pm	IMGS Business Meeting	Crystal Ballroom G1
Sunday, July 17		
8:00am-10:00am	Scientific Session: Human Disease Models II	Crystal Ballroom G1
10:30am-12:30pm	Technology and its Application Joint Plenary Session	Palms Ballroom

** Ticketed Event*

M251 Building the first comprehensive functional catalogue of a mammalian genome. *M. Hrabé de Angelis*, International Mouse Phenotyping Consortium, www.impc.org. Institute of Experimental Genetics, Neuherberg, Bavaria, DE.

A major challenge facing mammalian genetics over the next decade is the systematic and comprehensive annotation of mammalian gene function. As part of the International Knockout Mouse Consortium, several programmes are ongoing to generate conditional mutants for all mouse genes. An even greater challenge will be the determination of phenotypic outcomes for each mutation and the identification of disease models. The International Mouse Phenotyping Consortium (IMPC, www.impc.org) is undertaking the development of a comprehensive Catalogue of Mammalian Gene Function. The IMPC incorporates 20 major mouse centres around the world that undertake mouse production and phenotyping. The IMPC programme has two phases: Phase 1, 2011-2016, is approaching completion and will deliver the phenotypes of around 5000 mouse mutant lines; Phase 2 from 2016-2021 will undertake the analysis of the remaining genome. IMPC centres operate a core, standardised, broad-based adult phenotyping pipeline encompassing the major biological and disease systems, including gross pathology and tissue collection as a mandatory requirement. Many centres have also begun to employ a standardised embryonic phenotyping pipeline to analyse the many homozygous lethals, incorporating an assessment of time of lethality and morphological defects. In addition, lacZ expression data is being collected for adult organs and E12.5 embryos. All data from each production and phenotyping centre is uploaded to a central Data Coordination Centre (DCC), and following QC and analysis is archived and disseminated to the wider biomedical sciences community along with appropriate annotation tools. In the first 4 years of the programme, nearly 8000 ES cell lines have been injected, over 5000 mouse mutant lines generated and phenotype data from nearly 3500 mutants collected at the DCC. We will describe many new insights into the genetic and molecular bases of disease, report the generation of numerous novel disease models, and elaborate a fundamental appraisal of the pleiotropic landscape of mammalian gene function.

M252 Large-scale discovery of embryonic lethal phenotypes in mice. *S. A. Murray*[1], A. M. Flenniken[2,13], X. Ji[3], L. Teboul[4], M. D. Wong[2,11], J. K. White[5], T. F. Meehan[6], H. Westerberg[4], M. Justice[7,14], M. Hrabe de Angelis[8,17,18], Y. Herault[9], T. Mohun[10], R. M. Henkelman[2,11], S. D. Brown[4], K. C. Lloyd[12], C. McKerlie[2,13], D. Adams[5], A. L. Beaudet[14], M. Bucan[15], M. E. Dickinson[16], The International Mouse Phenotyping Consortium. 1) The Jackson Laboratory, Bar Harbor, ME; 2) Toronto Centre for Phenogenomics, Toronto, Ontario, Canada; 3) Genomics and Computational Biology Program, Perelman School of Medicine, University of Pennsylvania, Philadelphia PA 19104; 4) Medical Research Council Harwell (Mammalian Genetics Unit and Mary Lyon Centre), Harwell, Oxfordshire, UK; 5) The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK; 6) European Molecular Biology Laboratory- European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK; 7) The Hospital for Sick Children, Toronto Ontario, Canada; 8) Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Experimental Genetics and German Mouse Clinic, Neuherberg, Germany; 9) Institut Clinique de la Souris (ICS), PHENOMIN, Illkirch, Cedex, France; 10) The Francis Crick Institute Mill Hill Laboratory, The Ridgeway, Mill Hill, London A, UK; 11) Mouse Imaging Centre, The Hospital for Sick Children, Toronto, Ontario, M5T 3H7, Canada; 12) Mouse Biology Program, University of California, Davis; 13) Mount Sinai Hospital, Toronto, Ontario, Canada; 14) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX USA; 15) Departments of Genetics and Psychiatry, Perlman School of Medicine, University of Pennsylvania, Philadelphia PA 19104; 16) Department of Molecular Physiology and Biophysics, Houston, Texas, USA; 17) Chair of Experimental Genetics, School of Life Science Weihenstephan, Technische Universität München, Freising; 18) German Center for Diabetes Research (DZD), Neuherberg, Germany.

Nearly one third of all mammalian genes are essential. Embryonic lethal genes identified and characterized through mouse knockouts (KO) have greatly furthered our understanding of gene and pathway function. The overarching goal of the Knockout Mouse Phenotyping Program (KOMP2) and its partners in the IMPC International Mouse Phenotyping Consortium (IMPC) is to generate an encyclopedia of gene function through genome-wide generation and phenotyping of knockout mice. Collectively, the consortium has produced over 5000 knockout strains and identified over 400 embryonic lethal genes. To characterize the lethal lines, we have built and implemented a high-throughput embryo phenotyping pipeline, which includes the use of high-resolution 3D imaging for the generation of rich datasets that are distributed to the scientific community. The screen has revealed numerous phenotypes in genes with no previously ascribed function, and added new annotations to a subset of genes with prior knockout data. Unexpectedly, our analysis reveals frequent incomplete penetrance and variable expressivity of developmental phenotypes despite a defined genetic background. In addition, we found that human disease genes are highly enriched for in the essential genes identified in our screen, thus providing a novel dataset that facilitates prioritization and validation of mutations identified in clinical sequencing efforts.

M253 3D image analysis of embryonic lethal mutations: An IMPC/KOMP2 resource. *M. E. Dickinson*[1], A. Flenniken[2,3], X. Ji[4], L. Teboul[5], M. D. Wong[2,3], J. K. White[6], T. Meehan[7], W. J. Weninger[8], H. Westerberg[5], C.-W. Hsu[1], M. J. Justice[1,3], Y. Herault[9], T. Mohun[10], A.-M. Mallon[5], R. M. Henkelman[2,3], S. D. Brown[5], K. C. Lloyd[11], A. L. Beaudet[1], M. Bucan[4], S. A. Murray[12], The International Mouse Phenotyping Consortium. 1) Baylor College of Medicine, Houston, TX; 2) Toronto Centre for Phenogenomics, Toronto, Ontario, M5T 3H7, Canada; 3) The Hospital for Sick Children, Toronto Ontario, M5G 1X8, Canada; 4) Genomics and Computational Biology Program, Perelman School of Medicine, University of Pennsylvania, Philadelphia PA 19104; 5) Medical Research Council Harwell (Mammalian Genetics Unit and Mary Lyon Centre), Harwell, Oxfordshire, UK; 6) The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK; 7) European Molecular Biology Laboratory- European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK; 8) Centre for Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria; 9) Institut Clinique de la Souris (ICS), PHENOMIN, Illkirch, Cedex, France; 10) The Francis Crick Institute Mill Hill Laboratory, The Ridgeway, Mill Hill, London A, UK; 11) Mouse Biology Program, University of

California, Davis; 12) The Jackson Laboratory, Bar Harbor, Maine, USA.

Mutant mice provide a rich resource to identify and understand gene function. The overall goal of the International Mouse Phenotype Consortium (IMPC) and the Knockout Mouse Project (KOMP2) is to utilize comprehensive, broad-based, high-throughput phenotyping to characterize phenotypes resulting from null alleles in all ~21,000 functional genes within the mouse genome. To date, over 5000 mutant mice have been generated and consistent with other large-scale mutant screens, 35% of these genes are essential for embryonic and neonatal development. To describe the embryonic and neonatal phenotypes in recessive lethal mutations, a high-throughput pipeline has been established. The pipeline is being used to define the window (embryonic/postnatal) stage of lethality, to assess gross morphology and to generate 3D imaging data. The imaging data is used to identify structural defects and then it is curated and made available to the scientific community. Here, we will describe the imaging technologies, resources to visualize the data, unique phenotypes revealed with 3D imaging and future directions to enhance this resource.

M254 The DMDD programme: an online database of embryonic lethal mouse gene mutations. *T. Mohun*[1], and the DMDD Consortium[1,2,3,4,5,6,7,8]. 1) The Francis Crick Institute, London, UK; 2) Wellcome Trust Sanger Institute, Cambridge, UK; 3) Babraham Institute, Cambridge, UK; 4) King's College London, London, UK; 5) University of Oxford, Oxford, UK; 6) MRC Human Genetics Unit, Edinburgh, UK; 7) Medizinische Universität Wien, Vienna, Austria; 8) UCL, London, UK.

The DMDD programme (Deciphering the Mechanisms of Developmental Disorders) provides a free online database of embryonic-lethal mouse gene knockouts and their related phenotypes. This Wellcome Trust-funded research programme is a unique and expanding resource for both developmental biologists and clinicians, offering a novel way to study embryo development and the possible aetiology of human developmental disorders.

Embryos are comprehensively imaged using high-resolution episcopic microscopy (HREM), allowing them to be examined in 3D at an unprecedented resolution. A team of expert anatomists then scores structural abnormalities in tissue organisation and organ structure using a standardised phenotype ontology. Embryo placentas are examined by histology to assess the impact of gene mutation on placental development, while for perinatal deaths the neural tissue in the brain and spinal cord are examined using immunohistochemistry.

The DMDD database (http://dmdd.org.uk) is continually updated with new results and currently holds data from over 300 embryos, comprising nearly 3 million images. Each embryo can be viewed in a stack viewer at full (3 micron) resolution and tools are provided to allow rapid navigation in all 3 orthogonal planes. All phenotype information is available in tabular form and is also identified within the appropriate images. Data is presented both by embryo and by mouse line, enabling both the severity and penetrance of each phenotype to be assessed. Image data from different embryos can be viewed side by side and a reference library of datasets from normal embryos at different stages of development is also available for comparison.

Powerful search functions increase the potential of the database, enabling users to identify relevant data by gene, tissue or phenotype. All data is available for download on request, while mouse lines of interest can be obtained from the European Mutant Mouse Archive (EMMA) for further study.

DMDD data has already suggested links between specific genes and abnormal embryonic heart structure, and shed light on the importance of gene mutations on the developing placenta. The database offers a wealth of information to be explored by those researching specific genes or developmental disorders, and has the potential to identify previously unknown links between groups of genes or phenotypes.

M255 GENCODE: using new technologies to improve reference mouse genome annotation. *Mark Thomas*, Jennifer Harrow, GENCODE Consortium. Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom.

Understanding transcriptional complexity is important for the study of disease, especially now that CRISPR-Cas9 technologies are driving a genome-editing revolution. The GENCODE resource is now the default human gene set in the UCSC and Ensembl genome browsers, and we are currently working to improve reference mouse genome annotation. With an emphasis on alternative splicing, our current release (M9) contains 115,125 transcripts from 21,971 protein coding and 9,436 long non-coding genes. The increasing availability of next-generation sequencing data from RNAseq, CAGE and PolyAseq allows us to define transcribed regions with ever increasing accuracy; adding to the transcriptional complexity of the genome. Identifying functional transcripts is particularly important, so as to differentiate them from transcripts arising via stochastic events or spliceosomal errors. The function of most protein coding transcripts is evident from the encoded protein, where as the function of long non-coding transcripts is more difficult to determine.

In an effort to improve our functional understanding of transcripts, we are combining phyloCSF comparative data with advances in ribosome profiling and mass spectrometry to assess the coding potential of transcripts. Combining these approaches not only allows us to improve protein coding gene annotation, it also highlights how differences in the precise TSS can influence the translational start of proteins. This has actually resulted in a decrease in the total number of mouse protein coding genes, while the number of pseudogenes has increased. The number of long non-coding RNA transcripts is also increasing, with longer reads from PacBio RNAseq and Capture-Seq experiments improving transcript annotation. With the increased effort, we now have regular releases of the mouse GENCODE gene set for the C57BL/6 reference genome. We are also collaborating with the Sanger Mouse Genomes project to extend annotation to *de novo* genome assemblies for the other laboratory mouse strains.

M258 Beyond the spreadsheet. *K. F. Manly*. UNC Chapel Hill, Chapel Hill, NC.

The development of widely-available computers, growing each year more powerful and less expensive, has helped make genetics an information science. This computer revolution, beginning 40 years ago, also offered many scientists an alternative career. Since about 1988 I have been developing software to support aspects of mouse genetics. The Map Manager programs supported mapping of Mendelian and quantitative trait loci with mouse crosses and recombinant inbred lines. They offered a graphical user interface, unusual at that time for scientific software. As the potential of the Web became apparent, Rob Williams and I developed WebQTL, which brought this type of analysis online. A few years later, an international consortium started the Collaborative Cross (CC) to produce a set of recombinant inbred mouse lines with more genetic diversity. Breeding began in Israel, Australia, and at the Oak Ridge National Laboratory in the US. As breeding began, I began writing software, now called CCDB, to guide and document the development of the ORNL lines. During inbreeding of these lines, we discovered that a program for display of human pedigrees, CraneFoot, could be adapted for mouse pedigrees. As some lines approached inbred status, I wrote two database programs: (1) RXDB to document production of RIX hybrid lines from CC inbreds, and (2) BOTA to maintain records of tissue and DNA samples from many stages of breeding. The most recent project, now replacing CCDB and RXDB, is BOBS, a program to guide and document the production and distribution of CC lines and RIX hybrids to the mouse genetics community. The database programs for CC have had two goals: first, to ensure accurate data entry, secure storage and flexible information retrieval; second, to minimize data entry time by adapting data entry to laboratory and mouseroom procedures.

M259 Good dad, bad dad: the genetic basis of parental care *Hopi Hoekstra*, Andres Bendesky, Young-Mi Kwon, Jean-Marc Lassance, Caitlin Lewarch, Shenquin Yau, Brant Peterson, Meng-Xiao He, Catherine Dulac. HHMI/Harvard University, Cambridge, MA.

Parental care is critical to offspring survival, yet we know little about how genetic differences affect the brain to modify parental care. Using a monogamous and a promiscuous species of deer mice (genus *Peromyscus*), which we show have large differences in parental behaviour, we perform a genetic screen to identify loci that affect parental care. We find that some loci have a specific effect on single parental traits whereas other loci affect modules of traits; moreover, some loci act in a sex-specific manner whereas others have similar effects in both sexes. Importantly, we also drilled down into one locus associated with parental nest building that contains the vasopressin gene and find that vasopressin is expressed at higher levels in the hypothalamus of the promiscuous species, which builds less sophisticated nests. Using chemogenetic studies in laboratory mice, we demonstrate that exciting or repressing vasopressin neurons can suppress or promote nest building, respectively. Together, our genetic dissection of parental behaviour opens exciting new avenues of research for the neurobiological understanding of complex social behaviour such as parenting.

M260 Accumulation and detection of germline spontaneous mutations in C57BL/6JJcl inbred mouse strain. *Y. Gondo*[1], R. Fukumura[1], K. Mori[2], A. Toyoda[3], Y. Ishitsuka[1], S. Makino[1], H. Kotaki[1], Y. Nakai[1], S. Kuhara[2], A. Fujiyama[3]. 1) Riken BioResource Ctr, Tsukuba, JP; 2) Kyushu University Fac Agr, Fukuoka, JP; 3) Ctr Info Biol, Comp Genomics Lab, Mishima, JP.

Spontaneous mutations originate evolution. Kimura's neutral theory elucidated the fixation rate of neutral mutations to be equal to the mutation rate of $1X10^{-8}$/yr. On the other hand, mutation rate, encompassing neutral mutation rate, varies depending on gender, genetic background, environmental factors and so forth. The estimation of mutation rate is thus critical for the studies on evolution as well as for the assessments of environmental risk factors. The advancement of high-throughput sequencing technologies have made it possible to comprehensively detect and analyze very rare mutations even in mammalian genomes. We here designed a mating scheme to accumulate germline spontaneous mutations, which is universally applicable to any sexually reproductive species. Starting from twelve sets of G1 mating pairs of C57BL/6JJcl inbred mice, we obtained eight G5 females from eight independent pedigrees in which *de novo* germline spontaneous mutations were accumulated by complete outbreeding. The genomic DNA samples of the eight G5 female mice were subjected to whole genome sequencing (WGS). The single nucleotide variation (SNV)-call informatics pipeline subjected to the WGS dataset primarily gave rise to a total of 11,984 SNV candidates. These SNV calls consist of not only *de novo* spontaneous germline mutations that arose somewhere between G1 gametogenesis and G5 fertilization but also SNVs/SNPs predisposed in the twelve sets of the original G1 genomes. For instance, 4,601 primary SNV calls were found to be homozygous in all the eight G5 females; thus, they are the fixed SNPs between C57BL/6J and C57BL/6JJcl strains. The two inbred strains were branched in 1989 and the current mouse genome reference sequence (GRCm38.4) of C57BL/6J is 2.8 Gbps; thus, ~1.6 base substitutions and fixation per Mb on average have occurred in a total of 50 yrs (= 25 yrs X 2) or $3.3X10^{-8}$/bp/yr. One thousand nine hundreds and fifty six SNV calls were found in two or more of the eight G5 females but not completely fixed yet; thus, they are recently-arisen polymorphic SNVs predisposed to the breeder's colony. The remaining 5,427 SNV candidates were identified in only one G5 female as heterozygotes; thus, they are good candidates for the *de novo* germline spontaneous mutations accumulated in the four generations between G1 and G5. We are currently focusing on the validation and identification of the origin of the 5,427 SNV candidates in the eight outbred pedigrees.

M261 Discovery, assembly, and annotation of subspecies specific haplotypes in classical and wild-derived mouse strains. *J. Li*, Keane Thomas. Wellcome Trust Sanger Institute, Cambridge, GB.

The vast majority of modern mouse strains are derived from three *M. Musculus* subspecies: *M. m. domesticus*, *Mus musculus musculus*, and *Mus musculus castaneus*. Currently, the only mouse strain with a fully assembled and annotated genome, C57BL/6J, is primarily *M. m. domesticus* in origin. Apart from a small number of well-studied loci, this has meant the sequence and coding alleles in regions of the genome containing subspecies specific haplotypes are largely unknown. In this project, we have used the whole-genome assemblies produced by the Mouse Genomes Project to create the first genome-wide catalog subspecies specific haplotypes and alleles across 16 laboratory mouse strains,

including wild-derived strains representatives of *Mus musculus musculus*, *Mus musculus castaneus*, and *Mus spretus*.

In this project, we have discovered more than one hundred subspecies specific haplotypes by identifying dense clusters of heterozygous SNPs in the reference genome as a marker and examining the corresponding assembled sequence in the strains. We find many more of these highly polymorphic loci in the wild derived laboratory strains and observe significant enrichment for genes involved in immunity, programmed cell death, kin recognition, neuron development and sensory functions. We successfully reassembled and annotated four immune related loci: *Ifi47* (IRG), *schlafen* and *Nlrp1* loci in four wild derived mouse strains. The results show a startling amount of structural variation compared to other loci in the genome reflecting the remnants of balancing selection and repeated host-pathogen co-evolution. We have identified new allelic forms of these genes, gene shuffling, large translocations, and incorporation of open reading frames (ORFs) from other parts of the genome, including several cases of gene disruption caused by transposable elements, and rearrangement between promoter regions and ORF of gene family members, which significantly alter gene expression levels. The genome structure of these regions will provide the basis for understanding different infection phenotypic responses observed in genetic reference panels such as the Collaborative Cross and the Diversity Outbred Cross.

M262 Post-translational mechanisms buffer protein abundance against transcriptional variation. *G. A. Churchill*[1], S. C. Munger[1], J. M. Chick[2], S. P. Gygi[2]. 1) Jackson Lab, Bar Harbor, ME; 2) Harvard Medical School, Cambridge, MA.

Recent studies have reported low correlation among transcript and protein levels in mouse tissues and human lymphoblastoid cell populations. Here we describe the largest combined RNA-seq and shotgun proteomics study to date, in liver samples from genetically heterogeneous Diversity Outbred (DO) mice. We observe two classes of gene regulation that act on protein levels. Most protein abundance QTL (pQTL) map locally to the gene region and act in cis to affect both message and protein, resulting in high concordance between transcript and protein levels. In stark contrast, proteins with distant pQTL appear largely uncoupled from transcript abundance. We apply a novel conditioning approach to identify causal regulatory proteins and transcripts underlying distant pQTL. We discover that stoichiometric buffering of protein complexes and metabolic pathways is the predominant trans-acting post-translational mechanism that buffers protein levels against cis genetic variation in the protein-coding gene.

M265 Multiple mouse reference genomes defines subspecies specific haplotypes and novel coding sequences. *T. M. Keane*, Mouse Genomes Project consortium. Wellcome Trust Sanger Institute, Cambridge, GB.

The Mouse Genomes Project is nearing the completion of the first draft assembled genome sequences and strain specific gene annotation for 16 laboratory and wild-derived mouse strains. The sequence accuracy of these draft genomes compares favourably to the first release of the mouse genome (MGSCv3), with increased base pair accuracy and comparable structural accuracy. For genome annotation, we have used a hybrid approach that combines evidence from the C57BL/6J Gencode annotation and strain specific transcript evidence (RNA-Seq and Pacbio cDNA) to identify and refine strain specific gene structures and alleles. The strain gene sets have provided many updates to the C57BL/6J genes, including genes that were previously missing or mis-annotated. We observe the largest number of novel gene structures in the wild derived strains. We can now determine the underlying sequence and coding alleles in the subspecies specific haplotype regions of the genome. We have identified over a hundred of these loci to date, finding enrichment in genes related to immunity, olfaction, and sensory function. We highlight examples of some well characterised QTLs that are located in these regions, which will enable more precise targeting and functional studies of these alleles. We show how these new reference genomes can be used to improve the accuracy of gene expression analysis of RNA-Seq from heterogenous mice.

The draft genome sequences and annotation can be viewed through our development UCSC genome browser (http://hgwdev-mus-strain.sdsc.edu/cgi-bin/hgGateway) and the gEVAL assembly browser (http://mice-geval.sanger.ac.uk/index.html), with full Ensembl and UCSC genome browser support coming in 2016.

M266 Symmetry breaking and self-organization in mouse development. *T. Hiiragi*. EMBL, Heidelberg, DE.

A fundamental question in biology is the mechanism by which the embryonic polarity is established during development. Unlike many organisms, mammalian eggs lack polarity and symmetry among cells has to be broken during early embryogenesis. This symmetry breaking results in formation of the blastocyst, consisting of two major cell types, the inner cell mass and trophectoderm, which are distinct in their position and gene expression. Recent studies unexpectedly revealed that morphogenesis and gene expression is highly dynamic and stochastic during this process. What signal breaks the initial symmetry and how stochastic gene expression leads to the reproducibly patterned blastocyst remain open questions about the beginning of mammalian life. We have developed an experimental system to monitor early mouse embryogenesis by live-imaging at unprecedented spatio-temporal resolution. This provides us with a basis for investigating the cellular and molecular mechanism of symmetry breaking and self-organisation in early mammalian development.

M267 A SUMO-Ubiquitin Relay Recruits Proteasomes to Chromosome Axes to Regulate Meiotic Recombination. *Neil Hunter*[1], Prasada Rao[1], Huanyu Qiao[1], Shubhang Bhatt[1], Logan Bailey[1], Hung Tran[1], Sarah Bourne[1], Wendy Qiu[1], Anusha Deshpande[1], Ajay Sharma[1], Connor Beebout[1], Roberto Pezza[2]. 1) Univ California, Davis, Davis, CA and the Howard Hughes Medical Institute; 2) Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma.

Meiosis produces haploid gametes through a succession of chromosomal events that include pairing, synapsis and recombination. These events are highly orchestrated, but the mechanisms that regulate them are poorly understood. We demonstrate that SUMO, ubiquitin and

proteasomes specifically localize along chromosome axes in mouse and mediate major events of meiotic prophase including synapsis and recombination. Axis-localized conjugation of SUMO and ubiquitin and the ensuing recruitment of proteasomes are dependent on just two E3 ligases, RNF212 and CCNB1IP1 (HEI10), previously shown to be essential for crossing over. These proteins mediate a checkpoint-like process in which RNF212-dependent SUMO conjugation stalls recombination by rendering the turnover of a subset of recombination factors dependent on CCNB1IP1-mediated ubiquitylation. We propose that SUMO conjugation establishes a precondition for the regulation of crossing over via selective protein stabilization. Chromosome axes are thus revealed as hubs for regulated protein degradation via SUMO-dependent control of ubiquitin-mediated proteolysis.

M268 SMC5/6 complex is required for the formation of bivalent chromosomes capable of segregation during meiosis I in oocytes. *G. H. Hwang*[1], M. O'Brien[2], F. Sun[2], J. Eppig[2], MA Handel[2], P. Jordan[1]. 1) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 2) The Jackson Laboratory, Bar Harbor, ME.

The frequency of chromosome segregation errors in oocytes increases as women age, especially after the age of ~35, and results in dramatically increased incidence of miscarriage and developmental abnormalities. Despite the correlation between age and chromosome segregation errors, in most cases the causes remain unknown. Recent work using mouse oocytes has linked the structural maintenance of chromosomes (SMC) complexes to being important for accurate chromosome segregation following meiotic resumption. SMC complexes include three major classes: cohesin, condensin, and SMC5/6. During the first meiotic division cohesin is required to ensure sister chromatids remain associated with one another, and condensin is required for the structural integrity and resolution of bivalents. Until our recent work, the functions of the SMC5/6 complex during oogenesis had not been examined. We show that the SMC5/6 complex is enriched at the pericentromeric heterochromatin, and also localizes along chromosome arms during meiosis. To determine the role of the SMC5/6 complex during meiotic resumption, we constructed a conditional knockout (cKO) allele of mouse *Smc5*. Using the Tg(Zp3-cre)93Knw (Zp3-Cre) transgene we deleted *Smc5* in growing *Smc5*-condition ready ($Smc5^{tm1c(KOMP)Wtsi}$) oocytes prior to meiotic resumption. The *Smc5* cKO mutant mice are infertile. After *in vitro* fertilization of MII oocytes obtained from 3 month-old mice, markedly reduced percentages of 2-cell embryos from *Smc5* cKO were observed, and blastocysts were nearly absent. These *Smc5* cKO mutant oocytes fail to accurately separate homologous chromosomes during meiosis I, and resulted in aneuploid MII oocytes. Despite what appears to be an inability to resolve concatenation between chromosomes during meiosis we did not observe topoisomerase II alpha mislocalization. However, we did observe defects in condensin localization along the chromosome axes. Remarkably, these phenotypes are less prominent when analyzing oocytes isolated from younger mice. We have obtained data to suggest that the SMC5/6 complex remains stable for weeks following ZP3-Cre-mediated deletion of *Smc5*, but levels present prior to deletion diminish with age. Taken together, our results demonstrate that the SMC5/6 complex is essential for the formation of bivalents that are capable of accurate segregation during meiosis I, and age-related meiotic aberrancies may be directly related to a gradual reduction in SMC5/6 protein levels.

M269 Imaging how Transcription Factors Bind DNA to Control Cell Fate in Living Mouse Embryos. *N. Plachta*. A*STAR, Singapore, SG.

Transcription factor (TF) binding to DNA is fundamental for gene regulation. However, it remains unknown how the dynamics of TF–DNA interactions change during cell fate determination *in vivo*. We established imaging techniques to quantify TF–DNA binding in single cells of developing mouse embryos. We show how POU5F1 (OCT4) and SOX2 re-partition between specific and non-specific DNA sites as cells decide their fate *in vivo*. Furthermore, we discover heterogeneities in SOX2-DNA binding appearing as early as the 4-cell stage of development, and regulated by histone methylation, which predict cell fate. Finally, we develop new techniques to show how cells change their shape and position in the embryo to form the pluripotent inner mass.

References: White et al (2016) **Cell**; Samarage et al (2015) **Dev. Cell**; Fierro-Gonzalez et al (2013) **Nat. Cell Bio.**; Kaur et al (2013) **Nat. Comm..**

M270 Maternally provided KDM1A enables the maternal-to-zygotic transition and prevents defects that manifest postnatally. *J. A. Wasson*[1*], A. K. Simon[1], D. A. Myrick[1], G. Wolf[2], S. Driscoll[3], S. L. Pfaff[3], T. S. Macfarlan[2], D. J. Katz[1]. 1) Emory University School of Medicine, Atlanta, GA; 2) Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; 3) The Salk Institute for Biological Studies, La Jolla, CA.

Somatic cell nuclear transfer has established that the oocyte contains maternal factors with epigenetic reprogramming capacity. Yet the identity and function of these maternal factors during the gamete to embryo transition remains poorly understood. In *C. elegans*, KDM1A (AKA LSD1) enables this transition by removing H3K4me2 and preventing the transgenerational inheritance of transcription patterns. Here we show that loss of maternal KDM1A in mice results in embryonic arrest at the 1-2 cell stage, with arrested embryos failing to undergo the maternal-to-zygotic transition. This suggests that KDM1A maternal reprogramming is conserved. Moreover, partial loss of maternal KDM1A results in striking phenotypes weeks after fertilization; including perinatal lethality and abnormal behavior in surviving adults. These maternal effect hypomorphic phenotypes are associated with alterations in DNA methylation and expression at imprinted genes. These results establish a novel mammalian paradigm where defects in early epigenetic reprogramming can lead to defects that manifest later in development.

M271 A Forward Genetics Approach to Discover Modifiers of Developmental Phenotypes. *K. A. Geister*[1], A. E. Timms[1], S. Ha[1], R. J. Lipinski[2], D. R. Beier[1]. 1) Seattle Children's Research Institute, Seattle, WA; 2) University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI.

The discovery of modifier genes in human and mouse has seen slow progress. In mice, this is largely due to the reliance on inherent variability among inbred strains, which makes identifying the causal variant, even in highly resolved mapped loci, quite challenging. We have

developed a fast and cost-effective method for genetic mapping of causal ENU-induced mutations using Next Generation Sequencing that combines single nucleotide polymorphism (SNP) discovery, mutation localization, and identification of causal sequence variants. This approach precludes the need for an outcross to facilitate mapping, as we use the ENU-induced variants as SNP markers. This strategy allows us to perform both primary screens and modifier screens on fully inbred lines. We have successfully mapped causal mutations using this approach, and we are currently testing the efficacy of this strategy with regard to modifier discovery using two C57BL/6 congenic strains as our sensitized lines. One is a loss-of-function allele of the SHH transcriptional effector *Gli2*, which can be modified by prenatal ethanol exposure to generate holoprosencephaly-like phenotypes. The other is an ENU-induced loss-of-function allele of *Pibf1* that causes cleft lip/palate and ciliopathy-like phenotypes. Both alleles could reveal modifiers of genes involved in craniofacial, brain, and skeletal development as well as those involved in the formation and function of the primary cilium. In the simplest cases, whole-genome sequencing will reveal areas of homozygosity shared between mutants with a consistent "modified" phenotype, and allow us to narrow our focus to the induced variants included in these homozygous regions. We anticipate that we may ascertain more complex genetic interactions as well as new ENU-induced mutant phenotypes that model human birth defects. In our first screen we discovered a mutation in *Colgalt1*(collagen beta (1-O) galactosyltransferase type 1), which is required for proper galactosylation of hydroxylysine residues in a number of collagens. Mutant embryos exhibit skeletal and muscular defects, and we hypothesize that the *Colgalt1* mutant could serve as a model of a human connective tissue disorder and/or congenital muscular dystrophy or myopathy. The mutants also show variable expressivity of cleft palate and exencephaly. Examination of the sequencing data appears to exclude the likelihood that these are due to an unlinked ENU-induced variant, illustrating that stochastic effects remain an important source of variation in developmental phenotypes. Funding: NIH R01 HD36404.

M272 ER stress-induced remodeling of placental mRNA and small RNA expression networks. *Clement Y. Chow*[1], Kevin Klatt[2], Jessica Brown[2], Andrew G. Clark[2], Mark S. Roberson[2]. 1) University of Utah, Salt Lake City, UT; 2) Cornell University, Ithaca, NY.

Placental dysfunction can lead to a variety of pregnancy pathologies, including intrauterine growth restriction (IUGR) and pre-eclampsia (PE). While these two conditions are distinct and can have differing outcomes for the mother and fetus, they share common molecular signatures. High levels of endoplasmic reticulum (ER) stress have been found in placentas from human cases and mouse models of IUGR and PE. The ER is a large organelle responsible for synthesis, maturation, and delivery of proteins essential for cellular function. ER stress occurs when misfolded proteins accumulate in the ER lumen. The cell responds with the conserved unfolded protein response and returns the ER to homeostasis by attenuating protein synthesis, activating transcriptional signaling cascades, and refolding or degrading misfolded proteins. ER stress can result in inflammation, cell death, and disease. The appearance of ER stress in the placenta does not occur in isolation and is accompanied by a variety of changes in maternal physiology. Inhibition of protein synthesis, reduced cell proliferation, or activation of pro-inflammatory pathways are all thought to be associated with differing levels of placental ER stress. This complex milieu makes it difficult to identify the direct effects of ER stress on placental and fetal health. To study the effects of the isolated ER stress response on the placenta, we used Tunicamycin to induce robust ER stress in E10.5 and E14.5 C57BL/6J mouse placentas. Because the response to ER stress involves thousands of gene expression changes, we used RNA-seq to measure the mRNA and small RNA changes associated with ER stress. We find that at each developmental time point, the placenta has a unique response to ER stress, with a specific associated ER stress response profile. We also find that the small RNA network reorganizes in ways that depend on both ER stress and on developmental time. Finally, small RNA expression changes appear to correlate with their targets under ER stress, and this correlation changes with development. Our data demonstrate that there is a complex regulation of mRNA and small RNA expression in the developing placenta, and ER stress impacts this regulatory network in profound ways. These results have important implications for potential roles of ER stress response in the pathophysiology of a variety of placental conditions.

M273 Verne Chapman Lecture: Mendel 2.0, revisiting the determinants of inheritance and the origins of phenotypic variation. *J. H. Nadeau*. Pacific Northwest Research Institute, Seattle, WA.

Mendel's discoveries were a breakthrough in understanding the inherited relations between genotype and phenotype for simple traits. Extending these principles to more complex traits has led to a model of genetic and phenotypic architecture that involves large numbers of genes, each with small additive effects. But considerable evidence suggests that circumstances are more complex. Mendelian traits often depend on strong interactions with genetic background. More recent work with complex traits in genetic reference populations shows that epistasis is pervasive. The strong dependence of both simple and complex traits on genetic background also argues that gene action is conditional rather than constant, although the basis for conditional functions is largely unknown. Strong epistasis implies that a revised picture of genetic architectures is needed.

Phenotypic variation can also result from environmental exposures and genetic variants in ancestral generations. These inherited epigenetic effects, which are independent of DNA sequence variants, can be as common and strong as conventional genetic effects and they can persist for tens of generations. Their significant contribution to phenotypic variation calls for a rethinking of our concept of inheritance and the formulation of heritability.

Finally, a fundamental tenet of Mendelian inheritance is that, with few exceptions, fertilization results from a random union of gametes. However, reanalysis of many reports with mutant genes shows that both specific genetic variants and gene-diet interactions can lead to a strong bias in gamete preferences at fertilization. These cases are unusual because they depend on genetically-determined interactions between specific egg and sperm, unlike conventional forms of transmission distortion. Fertilization bias would have a profound impact not only on measures of inherited disease risk, but also on inherited gene variation.

Together these discoveries involving epistasis, transgenerational epigenetic effects, and fertilization bias argue that genomes are composed of specific combinations of interacting genetic variants that confer viability and fertility as well as resilience to mutational perturbations, that

generations are more continuous than discrete with inherited memories of ancestral genetics and environments, and that fertilization bias helps drive genetic architecture and phenotypic variation across generations.

M274 A suppressor screen in *Mecp2* mice reveals pathways for Rett syndrome pathogenesis. *M. J. Justice*, S. M. Kyle, J. Ruston, R. Zeng. SickKids Research Institute, Toronto, Ontario, Canada.

Mutations in methyl CpG binding protein 2 (*MECP2*) cause Rett syndrome, an X-linked neurological disorder with autistic features and developmental regression. Mutation of *MECP2* causes widespread gene dysregulation, making the syndrome difficult to treat. *Mecp2* mouse mutants provide an excellent animal model to identify molecules that are important for disease pathogenesis. Studies of the mouse model have shown that the symptoms can be reversed by introduction of *Mecp2* and partially rescued by other factors, providing evidence that therapeutic intervention is possible in humans. *MECP2* mutation impacts many biological pathways, but it is unclear which are relevant to symptom onset and progression. A modifier screen is a forward-genetic approach to find mutations that suppress or enhance a phenotype of interest, allowing the organism to reveal important pathways for morbidity. Here, random unbiased mutagenesis with *N*-ethyl-*N*-nitrosourea (ENU) was employed to isolate mutations that suppressed clinical signs and improved overall health in a *Mecp2* mouse model. Strikingly, the lesions identified by whole exome sequencing and segregation in 46 suppressing lines reveal 32 candidate genes that lie in only four pathways. One modifying mutation showed that cholesterol synthesis was abnormal in *Mecp2* mice, and revealed the importance of brain lipid homeostasis to neurological function. Subsequent data show that MECP2 is required to link a repressor complex that regulates lipid homeostasis to DNA. Because MECP2 is ubiquitously expressed, metabolic syndrome also develops in *Mecp2* mice. Many of the suppressor mutations lie in the pathway that regulates lipid homeostasis, and metabolic modulators such as statin drugs improve health. Lipid homeostasis thus represents a potential new therapeutic target for Rett syndrome treatment. Altogether, our data suggest that multiple factors will be required to reverse disease entirely, requiring combination therapies. A similar genetic approach could be exploited to identify targetable pathways involved in other “untreatable” diseases, opening a new field for translational discovery.

M275 Systems Genetics Approach toward Understanding Regulation of MECP2 Expression in the Brain. *Lucy H. Williams*, Rachel C. Mcmullan, Joshua Starmer, Tim A. Bell, Darla R. Miller, Terry R. Magnuson, Fernando Pardo Manuel de Villena. University of North Carolina, Chapel Hill, NC.

Rett syndrome (RTT) is a pediatric neurological disorder that results from dysfunction of X-linked Methyl-CpG-binding protein 2 (*MECP2*). Treatment strategies that are under-development for RTT either target *MECP2* itself or target pathways downstream of *MeCP2*. In the first category, the goal is to normalize *MECP2* expression through molecular techniques such as gene therapy. Targeting *MECP2* is particularly attractive because it may lead to curing RTT yet special attention must be paid to MECP2 dosage. The brain is highly sensitive to perturbation of *MECP2* as over-expression causes MECP2 Duplication syndrome and even subtle fluctuations are associated with a spectrum of neurological disorders, such as autism. We are taking advantage of genetically diverse mouse populations to integrate genetic, genomic (mRNA), proteomic (protein) and epigenetic (X inactivation) level data to address questions: (1) how are *MECP2* transcript and protein levels controlled in the brain? And (2) how does genetic background influence the spectrum and severity of RTT-related phenotypes?

We have quantified *Mecp2* transcript levels in the brain from three different but genetically related mouse populations: 65 Collaborative Cross (CC) strains, 27 F1 hybrid crosses of two different CC strains, and 223 Diversity Outbred (DO) mice. In all three populations, heritability is well over 0.50, demonstrating that expression is under genetic control and is segregating within our reference populations. *Mecp2* expression in the inbred CC strains far exceeds the range of levels quantified between the three most diverse founders of the CC (CAST/EiJ, PWK/PhJ, and WSB/EiJ), demonstrating that *Mecp2* expression is under the control of trans acting factor(s). Using the 223 Diversity Outbred mice, we have mapped trans eQTL. To establish *Mecp2* expression thresholds in the brain, we are quantifying the correlation between steady-state transcript levels and protein abundance. And, we describe here strategies that we will employ to test how outlier levels are associated with clinical features. This work will produce new mouse lines and molecular and behavioral datasets will be valuable tools to map modifiers of phenotypes, inform on the molecular mechanisms that define transcript and protein levels, and develop and test more effective treatment strategies.

M276 Conserved and tissue-specific effects of natural genetic variation on transcript and protein abundance. *S. C. Munger*[1], J. M. Chick[2], P. Simecek[1], K. Choi[1], E. L. Huttlin[2], D. M. Gatti[1], N. Raghupathy[1], K. L. Svenson[1], S. P. Gygi[2], R. Korstanje[1], G. A. Churchill[1]. 1) The Jackson Laboratory, Bar Harbor, ME; 2) Harvard Medical School, Boston, MA.

Genetic variation can influence protein expression through transcriptional and post-transcriptional mechanisms, and these effects may be conserved across tissues or specific to one. To characterize the shared and tissue-specific effects of natural genetic diversity on the proteome, we combined RNA-seq and multiplexed, quantitative mass spectrometry with a genetically diverse mouse population, the Diversity Outbred (DO) heterogeneous stock. We measured genome-wide transcript and protein abundance in livers and kidneys from 192 DO mice, and mapped quantitative trait loci that influenced transcript (eQTL) and protein (pQTL) expression. We identified nearly 3,000 pQTL in each of the liver and kidney, divided equally between local and distant variants. Local pQTL generally had larger effects on protein abundance, these effects were conferred primarily through transcriptional mechanisms, and half showed conserved protein responses in both tissues. In contrast, distant pQTL influenced protein abundance nearly exclusively through post-transcriptional mechanisms and most were observed to be specific to the liver or kidney. We applied mediation analysis and identified a second protein or transcript as the causal mediator for half of the significant distant pQTL. Furthermore, we identified groups of proteins within known pathways that shared coincident subthreshold distant pQTL for which we could identify a single causal protein intermediate from the same pathway, demonstrating the power of integrating ontology and mediation analyses to tease out subtle but real genetic effects from mapping populations with modest sample sizes. Overall, our analysis

revealed extensive tissue-specific networks of direct protein-to-protein interactions that act to achieve stoichiometric balance of functionally related enzymes and subunits of multimeric complexes.

M277 Discovering novel susceptibility genes for aggressive prostate cancer using an integrated, systems-based cross-species strategy. *Jean M. Winter*[1], Derek Gildea[2], Jonathan Andreas[1], Kendra A. Williams[1], Ying Hu[3], Minnkyong Lee[1], Daniel Gatti[4], Tyra Wolfsberg[2], Gary Churchill[4], Nigel Crawford[1], NISC Comparative Sequencing Program. 1) Genetics and Molecular Biology Branch, NHGRI, National Institute of Health, Bethesda, MD; 2) Computational and Statistical Genomics Branch, NHGRI, NIH, Bethesda, MD; 3) Center for Biomedical Informatics and Information Technology, NCI, National Institute of Health, Rockville, MD; 4) The Jackson Laboratory, Bar Harbor, ME.

Prostate cancer (PC) is the most commonly diagnosed cancer in men. However, relatively few (<13%) cases result in death, with many men undergoing unnecessary and invasive treatments. New approaches for assessing patients at higher risk of developing metastatic disease are vital for preventing over-treatment and improving outcomes. We aimed to identify aggressive PC susceptibility genes using the C57BL/6-Tg(TRAMP)8247Ng/J (TRAMP) mouse model of neuroendocrine PC, which demonstrates a particularly aggressive form of oncogenesis. TRAMP mice were bred to Diversity Outbred (J:DO) mice and a cohort of 498 TRAMP x J:DO males was developed in order to identify genetic regions associated with susceptibility to aggressive disease. Modifier locus mapping revealed one locus spanning ~4Mb on Chromosome 8 associated with distant metastasis free survival (LOD=8.42, p=5.33e-6). Candidate genes within this locus were identified by RNA-seq analysis of 195 TRAMP x J:DO tumors. Trait-correlation and expression quantitative trait locus data for transcripts within this locus were integrated with strain-specific SNP data to distinguish eleven candidate metastasis susceptibility genes. Relevance of these genes to aggressive human PC was investigated via in silico validation, in a cohort size over 5,550 prostate cancer patients, which encompassed three independent human PC tumor gene expression datasets and two human PC genome-wide association studies. This identified two novel aggressive PC susceptibility genes: *CENPU (MLF1IP)* and *RWDD4.* Interestingly, *CENPU* acts as a transcriptional suppressor and chromosome segregation protein, and has recently been the focus as a potential gene therapy target for human prostate cancer. The data presented here demonstrate how systems genetics approaches centered on the use of a mouse model of PC can help us understand how hereditary variation influences disease outcomes of aggressive PC. Ongoing work is focusing on *in vitro* and *in vivo* functional characterization of these two genes to better understand their role in metastatic PC.

M278 Confirming Functional Genomics with Optoacoustic and Raman Imaging. *Vasillis Ntziachristos*. Technical University, Munich, Germany.

no abstract submitted

M279 A Cross-Species Novel Genetic Cell Ablation Technology Involving CD59 and Intermedilysin. *E. C. Bryda*[1], Marina Hanson[1], Suman Gurung[1], Fengming Liu[2], Shen Dai[2], Alison Kearnes[2], Dechun Feng[3], Bin Gao[3], Anand Chandrasekhar[1], Xuebin Qin[2]. 1) University of Missouri, Columbia, MO; 2) Temple University School of Medicine, Philadelphia, PA; 3) National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD.

The ability to selectively ablate cell types in model organisms is a powerful tool for understanding cell lineage, cell function, developmental processes and disease mechanisms. Current methodologies have limitations including lack of cell-type specificity, off–target effects, narrow pharmacological windows, and relatively slow onset of cell death. Our novel system utilizes human CD59 (hCD59), a membrane receptor, and intermedilysin (ILY), a bacterial toxin that binds hCD59 selectively. We have generated multiple rodent models that express hCD59 in a cell-specific manner. We demonstrated 1) ablation of circulating cells in a rat anemia model which expresses hCD59 on erythrocytes for the study of the pathogenesis of hemolysis-associated complications, 2) ablation of cells in a solid organ by generating a mouse model in which epithelial cells in the liver were ablated for the study of liver damage and regeneration, and 3) ablation of neuronal cells in an inducible rat model carrying a transgene with constitutive expression of ZsGreen and hCD59 in the presence of Cre recombinase. The Cre-inducible model can be bred with any conditional Cre recombinase-expressing rat to generate animals with expression of hCD59 and ZsGreen in the cell type/tissue of choice. These models have shown that cell ablation is dose-dependent, rapid, and has a large pharmacological window. Extending these experiments to zebrafish, we performed dose-response studies with *hCD59* RNA-injected and control zebrafish embryos treated with ILY and found that ILY rapidly induced extensive tissue lysis in the embryos expressing hCD59. Current efforts are focused on further characterization of the effectiveness of hCD59-ILY-mediated cell ablation in zebrafish. Together, these studies demonstrate that the hCD59-ILY cell ablation technology has wide utility and can be applied to study virtually any tissue or cell type in the animal species of choice.

M280 RNAi and CRISPR/Cas9 based *In Vivo* Models for Drug Discovery. *P. Premsrirut*[1*], G. Martin[2], L. Dow[3], S. Kim[4], J. Zuber[5], S. Lowe[6], G. Hannon[7]. 1) Mirimus, Inc., Woodbury, NY; 2) Charles River Laboratories, Wilmington, MA; 3) Weill Cornell Medical College, New York, NY; 4) New York University, New York, NY; 5) Research Institute of Molecular Pathology, Vienna, Austria; 6) Memorial Sloan Kettering Cancer Center, New York, NY; 7) Cancer Research UK, Cambridge, Cambridge.

With the advent of CRISPR-Cas9 technology, the speed and precision in which genetically engineered mouse models can be created is unprecedented. We now have at our disposal a genetic toolbox that will enable the rapid generation of sophisticated mouse models of human disease. Recently, an inducible CRISPR-Cas9 (iCRISPR) system was described that enables doxycycline-regulated Cas9 induction of widespread gene mutagenesis in multiple tissues. Previously, we also demonstrated how inducible RNA interference (RNAi) can be exploited experimentally to effectively and reversibly silence nearly any gene target not only *in vitro* but also in live mice. Here, we take advantage of these powerful technologies and combine both tet-inducible CRISPR-Cas9 and inducible RNAi-mediated gene silencing to develop animal models in which both

de novo disease pathogenesis can be induced by Cas9-mediated genome editing and therapeutic strategies assessed downstream via RNA interference-mediated gene silencing. By using this combination of CRISPR/Cas9 and RNAi technologies, we are able to not only model disease, but also mimic drug therapy in the same mice, giving us advanced capabilities to perform preclinical studies *in vivo*. Using our robust flexible system, we have created a cost-effective and scalable platform for the production of complex genetically engineered mouse models with RNAi silencing of nearly any gene - mice with enormous predictive power that will shape our development of better tolerated therapies.

M281 CRISPR/Cas9 Genome Editing Pipeline for Mice and Rats. *Thom Saunders*, Wanda Filipiak, Galina Gavrilina, Anna LaForest, Corey Ziebell, Michael Zeidler, Elizabeth Hughes. University of Michigan, Ann Arbor, MI.

CRISPR/Cas9 is a RNA guided nuclease that produces double strand breaks in DNA molecules and chromosomes. Mouse and rat zygotes with chromosome breaks may repair breaks with non-homologous endjoining (NHEJ) or homology directed repair (HDR) pathways. Repair NHEJ by often produces deletions and insertions in critical regions for gene expression that causes gene knockouts as a result. When the zygote copies new DNA sequence from a DNA donor template during HDR a new DNA sequence will be expressed from the targeted locus. Genes for the CRISPR/Cas9 pipeline are identified by investigators and the Transgenic Core designs sgRNA targets in critical regions with online algorithms. High scoring sgRNAs are cloned for *in vitro* sgRNA transcription. Activity of sgRNA is validated in two steps: 1) cleavage of PCR templates *in vitro* after mixture with Cas9 protein and 2) microinjection into mouse zygotes and testing blastocysts for NHEJ repair. Rat gene sgRNAs are tested by transfection of rat cells and identification of NHEJ mutations. Results will be presented on 1) producing gene knockouts in mouse and rat strains, 2) introducing coding SNPs knockins with oligonucleotide in mice and rats, 3) producing reporter gene knockins in mice and rats, 4) producing floxed genes with a novel one-cut strategy in mice, and 5) reporter gene knockins into the *Gt(ROSA)26Sor* locus. Analysis shows that mosaic founders occur frequently. Mutations observed in founders vary from deletion/insertion of a few nucleotides to the deletion of several hundred base pairs. These patterns are observed in gene knockouts in both mouse and rat models. The efficiency of CRISPR/Cas9 targeting was lower in inbred C57BL/6J mice than in mixed genetic backgrounds (C57BL/6 and SJL). NHEJ inhibitors such as SCR7 were ineffective in a series of reporter knockin experiments. Experiments are underway to determine if the high fidelity Cas9 system (Cas9HF1) is as efficient as the wild type Cas9 endonuclease. Gene targeting with CRISPR/Cas9 is highly efficient, we guarantee the production of mouse and rat gene model knockouts with reagents designed and cloned in our Core facility. The efficiency of oligonucleotide knockins is lower, the majority of SNPs succeed. ROSA26 knockins are also efficient. The introduction of complex alleles such as multi-reporter knockins (e.g. iCre-P2A-mCherry) are least efficient and cannot always be guaranteed. Compared to preceding technologies, CRISPR/Cas9 technology has significantly increased access to mouse and rat genomes for the generation of biomedical research models.

M282 *Easy*-(*Isi*)-CRISPR; a method to efficiently knock-in long DNA inserts. *CB Gurumurthy*[1], Rolen Quadros[1], Don Harms[1], Guy Richardson[2], Suzanne Mansour[3], Masato Ohtsuka[4]. 1) University of Nebraska Medical Center, Omaha, NE; 2) University of Sussex, UK; 3) University of Utah; 4) Tokai University, Japan.

CRISPR/Cas9 technology efficiently produces *indels* (*in*sertions or *del*etions) and inserts short sequences when single stranded oligonucleotides are used as repair templates. However, insertion of longer stretches using double stranded DNA (dsDNA) templates is proven to be less efficient and challenging. There are a few reports that employed strategies to enhance homology directed repair mechanism of longer dsDNA templates. Here we describe a novel strategy called Isi-CRISPR (***I***vTRT-***s***sDNA-***i***nsertion-CRISPR; pronounced *Easy*-CRISPR) that uses *in vitro* synthesized ssDNAs up to 1.6 kb and show that they get inserted at the Cas9 cut sites very efficiently.

M283 Modeling the Gene: Maternal environment interaction in neurodevelopmental disorders. *Freda Miller*. The Hospital for Sick Children, Toronoto, Canada.

no abstract submitted

M284 An inducible dominant negative allele of *Sox10* models neurocristopathy deficits characteristic of PCWH patients. *M. Southard-Smith*[1], M. Halaka[1], N. J. Lawler[1], J. M. DeKeyser[1], J. Corpening Rosebrock[1], S. Arpag[1], J. Li[1], A. Economides[2], A. Braun[3], K. H. Schäfer[3], K. Boyd[1]. 1) Vanderbilt University Medical Center, Nashville, TN; 2) Regeneron Pharmaceuticals Inc., Tarrytown, NY; 3) University of Applied Sciences Kaiserslautern, Zweibrücken, RLP, GER.

Sox10 is a transcription factor that is essential for development of a wide range of cell fates including oligodendrocytes and neural crest-derived neurons and glia of the peripheral and enteric nervous systems, melanocytes in the skin, craniofacial bones, teeth, and the adrenal medulla. Investigating *Sox10* function in these distinct lineages has been difficult because simple gene knockouts cause complete loss of gene expression in early neural crest leading to embryonic lethality. Efforts to temporally ablate *Sox10* through gene knockout strategies have been hampered by kinetics of mRNA and protein decay. We have generated and characterized a COnditional INducible ("COIN") dominant negative allele of *Sox10* in mice as a novel tool for temporal and cell type specific analysis of gene function. Mice bearing a COIN cassette in the *Sox10* locus appear normal until Cre action flips a fluorescently tagged dominant negative *Sox10* isoform into the coding frame of exon four. Our characterization of this new allele indicates that the COIN cassette functions in a dominant negative fashion and fluorescently labels neural crest derivatives following Cre action. Crosses of *Sox10-COIN* mice with Cre drivers produce mutant progeny (B6J.B6N-*Sox10*$^{tm1.1Sout}$ or *Sox10-COIN*$^{INV+}$) that exhibit hypopigmentation, deficits in central and peripheral glia as well as gastrointestinal phenotypes. *Sox10-COIN*$^{INV+}$ pups show spotting accompanied by coat color variegation. We have named this new allele *"Merle"* based on the coat color variegation similarity to blue merle dogs. At P10 *Merle* pups exhibit peripheral limb weakness and instability in tracking. By P15 *Merle* mutants are severely affected with motor deficits, circling, and difficulty in righting. Histological and functional analysis of *Merle* tissues has identified central and peripheral

neuropathy that is consistent with phenotypes reported in patients afflicted by the complex neurocristopathy called Peripheral Demyelinating Neuropathy, Central Dysmyelinating Leukodystrophy, Waardenburg Syndrome, and Hirschsprung Disease (PCWH). The availability of this new allele will provide mechanistic insight into the pathology suffered by PCWH patients and permit manipulation of distinct neural crest lineages.

M285 Alpha-synuclein, the cause of Parkinson's disease, has a vital function in aged mice. *D. E. Cabin*, Dan Zou. McLaughlin Research Institute, Great Falls.

Parkinson's disease is the second most common neurodegenerative disease in humans.

It is slowly progressive, affecting many regions of the nervous system, including nigral dopaminergic neurons, and can eventually lead to dementia in addition to the well known motor symptoms. Alpha-synuclein (SNCA) is the protein responsible for Parkinson's disease. SNCA can convert to a toxic form and that toxic form propagates through the nervous system in a templated manner, dependent on endogenous production of SNCA. While the precise nature of the toxic form is not known, this mechanism indicates that SNCA is a prion. One potential therapeutic strategy would be reduction of endogenous SNCA production. Lowering the endogenous levels of SNCA would likely slow disease progression. However, the normal role of SNCA is not completely understood, so reducing SNCA levels might have unintended consequences. We have recently found that mice null for *Snca*(129S6-*Scna*^{tm1Nbm}/*Snca*^{tm1Nbm}) have a significantly shorter lifespan than WT mice, indicating that SNCA function is important in aging. This difference in lifespan appears at about 400 days and shortens overall lifespan by about 200 days. Conversion of SNCA to a toxic form in humans may prevent it from performing some needed role in aging, thus contributing to disease.

We performed a sensitized ENU mutagenesis screen using *Snca* null mice to better understand the protein's normal function. One sensitized mutation was identified after screening 125 pedigrees. This mutation occurred in the gene for ATP7A, a trans-Golgi copper transporter. Female mice carrying the X-linked mutation have a significantly higher rate of early death when they lack *Snca* than when the *Atp7a* mutation is on the *Snca* WT background. In humans, mutations in *ATP7A* cause Menkes disease. Linking SNCA and ATP7A genetically indicates that SNCA has a role in the trans-Golgi compartment. Immunofluorescence on both brain sections and on primary neurons derived from a) 129S6;B6J-*Atp7a*^{m1Decn}/*Y Snca+/+*, b) 129S6;B6J-*Atp7a*^{m1Decn}/*Y Snca-/-*, c) 129S6;B6J-*Atp7a+/Y Snca+/+*, d) 129S6;B6J-*Atp7a+/Y Snca-/-* mice indicates that SNCA is found in the trans-Golgi compartment, co-localizes with ATP7A, and seems to play a role in proper localization of ATP7A to axons. We are now examining brains of aged mice to determine aging affects SNCA and ATP7A subcellular localization, and whether lack of SNCA affects the trans-Golgi compartment during aging. Golgi fragmentation has been observed in Parkinson's disease, but whether this is due to SNCA toxicity or whether the loss of SNCA function plays a role is unknown.

M286 Aberrant DNA binding by mutant (E339D) KLF1 induces upregulation of embryonic β-globin in adult mice. *D. M. Nebor*[1], R. R. Robledo[1], A. C. Perkins[2], K. Gillinder[2], J. H. Graber[1], D. Gatti[1], V. Philip[1], J. J. Bieker[3]. 1) The Jackson Laboratory, Bar Harbor, ME; 2) University of Queensland, Woolloongabba, Australia; 3) Mount Sinai School of Medicine, New York, NY.

The KLF1 transcription factor regulates nearly all aspects of erythroid differentiation including heme synthesis and globin regulation. Previously we showed that the inbred mouse model *Nan* (neonatal anemia) carries a missense mutation (E339D) in the second zinc finger of KLF1 that causes severe lifelong anemia in heterozygotes accompanied by a striking failure of hemoglobin switching. Embryonic *Hbb-bh1* globin expression is significantly upregulated in *Nan* E14.5 fetal liver (FL) and in adult spleen. In humans elevation of fetal hemoglobin (HbF) ameliorates the severity of sickle cell disease and b-thalassemia. To examine the mechanisms of *Hbb-bh1* regulation in adult *Nan*, we analyzed the global erythroid transcriptome by RNA-seq in wild type (WT) and *Nan* E14.5 FLs. Only 18% of upregulated and 52% of downregulated genes in *Nan* overlap known KLF1 target genes. Moreover, just 3% of upregulated *Nan* genes overlap genes normally activated by KLF1. We also analyzed the transcriptome of sorted erythroid precursors (pro, basophilic, polychromatophilic and orthochromatic erythroblasts) in adult spleens. Together the RNA-seq data show that extensive ectopic gene expression occurs in *Nan.* To analyze the DNA binding of KLF1^{Nan} vs. WT, we performed ChIP-seq in K1-ER and K1-NanER cell lines. Results show aberrant DNA binding, both absent and ectopic, by KLF1^{Nan}. To identify potential genetic modifiers of *Hbb-bh1* expression, we performed expression QTL analysis of *Hbb-bh1* in two *Nan* F2 intercrosses and in an outbred high resolution mapping population, the diversity outcross (DO). Statistical analyses reveal multiple significant and suggestive *Hbb-bh1* eQTL. Among the genes present in these eQTL confidence intervals, those showing abnormal expression in *Nan* with dysregulated DNA binding by KLF1^{Nan} represent strong candidate genes for future functional anaylses as novel regulators of *Hbb-bh1* expression. A better understanding of the genetic regulation of the switch of hemoglobin is key to the identification of new drug targets for the treatment of sickle cell disease and β-thalassemia.

M287 Mutations in beta spectrin protect mice from malaria by increasing parasite susceptibility to clearance. *G. R. Burgio*[1], P. M. Lelliott[1], H. M. Huang[1], M. Dixon[2], L. Tilley[2], B. J. McMorran[1], S. J. Foote[1]. 1) The Australian National University, Canberra, ACT, AU; 2) University of Melbourne, Melbourne, VIC, AU.

Malarial parasite resistance to all known antimalarial drugs is now the norm. Parasites develop resistance through modification of both target and intra-parasitic drug concentrations. We have developed a strategy to develop new therapies that will bypass both these mechanisms of resistance. ENU mutagenesis is used to introduce mutations into the germline of mice that are otherwise susceptible to murine malaria. Mice carrying protective mutations will survive a malarial challenge whereas all other mice will succumb. The genes harbouring the mutations are identified and assessed as potential antimalarial drug targets. At present we have identified over 40 mutations conferring resistance to malarial infection and have 100 resistant lines. Here, we propose novel mechanisms of host resistance to malaria infection. Through our dominant large scale ENU screen for abnormal red blood cell count, 2 novel mutant alleles were identified in spectrin beta (*Sptb*) gene. All of the mutants

exhibit an abnormal red blood cell count, an increase osmotic fragility and a shorter red blood cell (RBC) half-life responsible for hereditary spherocytosis disease. When challenged with the rodent malaria parasite, *P. chabaudi* and *P.berghei*, *Sptb* mutant mice were resistant showing a dramatic increase in survival. We propose that the malaria resistance of *Sptb* lines is mediated by the deformability and the increase susceptibility to red blood cells clearance from splenic macrophages. This study provides the first evidence that a mutation in *Sptb* can lead to malaria resistance in mice, and shows that splenic clearance is essential for eliminating *Plasmodium* spp within Red Blood cells.

M288 Interaction of BRCA2 and PALB2 is essential for genome stability. *S. A. Hartford*[1], R. Chittela[1], X. Ding[1], A. Vyas[1], B. Martin[2], S. Burkett[1], D. Haines[2], E. Southon[1,2], L. Tessarollo[1], S. K. Sharan[1]. 1) Mouse Cancer Genetics Program Center for Cancer Research National Cancer Institute, Frederick, MD; 2) Leidos Biomedical Inc, National Cancer Institute Frederick, MD.

Efficient DNA repair, DNA replication, and regulating cell cycle checkpoints are all essential factors in protection from genomic instability. Breast cancer 2 (*Brca2*) and partner and localizer of BRCA2 (*Palb2*) are well-known factors associated with increased risk of breast and ovarian cancers. They both have essential roles involved in DNA repair, protection of stalled replication forks, and maintenance of the cell cycle checkpoint. While these two proteins interact and have similar roles in protection of the genome, what hasn't been shown is if the interaction is critical for full functionality *in vivo*. BRCA2 and PALB2 each have their own DNA binding motifs and both can bind to RAD51, so can the protection of the genome occur even if this interaction is perturbed? To investigate the functional importance of the interaction between BRCA2 and PALB2 we created a knock-in mouse model of BRCA2. The single amino acid change (Gly25Arg) is in the highly conserved N-terminal region that interacts with PALB2. This particular mutation was previously shown to cause a decrease but not abolish this interaction. These mice vary in phenotype and tumor latency base on whether the mutation is in homozygous or hemizygous state. An additional heterozygous loss of *Palb2*$^{Gt(CG0691)Wtsi}$ results in earlier tumor formation and more severe phenotypes in the mice. At the cellular level there is an increasing amount of genomic instability with the decreasing amount of interaction between BRCA2 and PALB2. This also holds true when looking at the ability of RAD51 foci formation after damage and the protection of the DNA replication fork in times of replication stress. This series of phenotypically distinct mouse models shows the critical need for proper interaction between these two genes for protection of the genome. In addition this series of animal models will also distinguish between the protection of the replication fork verses the ability for efficient HR in terms of being able to protect against genomic instability.

M289 Pathophysiological responses to dietary patterns differ with genetic backgrounds. *William T. Barrington*[1,2], Daniel Pomp[3], Brian Bennett[3], Carolina Mantilla Rojas[1], Selene Howe[1], David Threadgill[1]. 1) Texas A&M University, College Station, TX; 2) North Carolina State University, Raleigh, NC; 3) University of North Carolina, Chapel Hill, NC.

Dietary patterns have repeatedly been shown to have profound effects on health when studied at the population level. For example, Japanese and Mediterranean diets are associated with longevity and low rates of various chronic diseases. Western diets are associated with increased risk of heart disease and certain types of cancer. However, studies evaluating dietary interventions in individuals find significant variations in responses. Much of this variation is likely because of underlying genetic differences among individuals. To determine how diet affects pathophysiological responses in different genetic backgrounds, we examined cardiometabolic-related effects of five diets (current Western diet, traditional Mediterranean diet, traditional Japanese diet, a ketogenic diet and standard mouse chow) in each sex of four inbred mouse strains (A/J, C57BL/6J, FVB/NJ, and NOD/ShiltJ) selected for their known disparate susceptibilities to cancer, diabetes, and other diseases. Mice were fed diets ad libitum for six months while undergoing a variety of clinical analyses. We found that many health-related diet responses are dependent upon genetic background including: adiposity, glucose tolerance, blood chemistry profiles, liver triglyceride storage, liver mitochondrial function, and metabolic rate. The severity and directionality of many of these diet responses differ depending on the genetic background of the individual. For example, C57BL/6J mice became obese and showed signs of cardiometabolic distress on a Western diet but maintained good health on a ketogenic diet; FVB/NJ mice had minimal fat gain and were relatively healthy on a Western diet but became obese and suffered cardiometabolic distress on a ketogenic diet. Most strains had positive health profiles on the Japanese diet, while the health of A/J mice differed little across diets. Mouse strains differed as to which diets were optimal or suboptimal, suggesting that human individuals likely have a specific diet for optimal health based upon their unique genetic makeup. These results call into question the categorization of diets as good or bad and emphasize the need to evaluate dietary efficacy on an individual level. Follow-up studies are investigating the genetic factors underlying the different diet responses with the ultimate goal of allowing accurate prediction of diet response in genetically diverse human individuals.

M290 *Xist* RNA, its interactome, and consequences of their disruption in vivo. *Jeannie Lee*[1*], Lin Yang[1], James Kirby[2]. 1) HHMI, Harvard Medical School, Massachusetts Gen Hosp, Boston, MA; 2) Beth Israel - Deaconess Medical Center, Harvard Medical Schoo, Boston, MA.

Epigenetic regulation often occurs at the interface between RNA and chromatin. Long noncoding RNAs (lncRNAs) have a special role in epigenetic regulation because they are able to serve as barcodes that target protein complexes to specific genomic addresses. The inactive X chromosome serves as an excellent model by which to investigate the function of lncRNA. My presentation will focus on how lncRNAs collaborate with protein factors to regulate X-chromosome counting, allelic choice, and the spread of silencing along the inactive X chromosome. I will also discuss the consequences of losing those interactions in the context of development.

M291 Allelic imbalance is a prevalent and tissue-specific feature of autosomal and X-linked genes in F1 hybrid mice. *S. F. Pinter*[1,2,3,7], D. Colognori[2,3,4,7], B. J. Beliveau[3], R. I. Sadreyev[2,3], B. Payer[2,3,5], E. Yildirim[2,3,6], C. T. Wu[3], J. T. Lee[2,3,4,8]. 1) UConn Health, Institute for Systems Genomics, Farmington, CT; 2) Massachusetts General Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) Howard Hughes Medical

Institute, Chevy Chase, MD; 5) Centre for Genomic Regulation, Barcelona, Spain; 6) Duke University, School of Medicine, Durham, NC; 7) co-first authors; 8) corresponding author.

In mammals, several classes of monoallelic genes have been identified, including those subject to X-chromosome inactivation (XCI), genomic imprinting, and random monoallelic expression (RMAE). However, the extent to which these epigenetic phenomena are influenced by underlying genetic variation is unknown. We performed a systematic classification of allelic imbalance in mouse hybrids derived from reciprocal crosses of divergent strains. We observe that deviation from balanced biallelic expression is common, occurring in ~20% of the mouse transcriptome in a given tissue. Allelic imbalance attributed to genotypic variation is by far the most prevalent class and typically is tissue-specific. However, some genotype-based imbalance is maintained across tissues and is associated with greater genetic variation, especially in 5' and 3' termini of transcripts. We further identify novel random monoallelic and imprinted genes and find that genotype can modify penetrance of parental origin even in the setting of large imprinted regions. Examination of nascent transcripts in single cells from inbred parental strains reveals that genes showing genotype-based imbalance in hybrids can also exhibit monoallelic expression in isogenic backgrounds. This surprising observation may suggest a competition between alleles and/or reflect the combined impact of *cis*- and *trans*-acting variation on expression of a given gene. We also identify tissue-specific genes that escape XCI, and share some surprising and speculative insights from integrating these allele-specific expression results with other epigenomic data.

M292 *Vive la difference*: zooming in on sex-specific differences in mouse embryonic stem cells. *N. Engel*, R. Werner, B. Schultz, J. Madzo, J. Jelinek. Temple University, Philadelphia, PA.

Mortality rates in males are higher than in females at every age, including as newborns. Although sexual dimorphisms are usually attributed to hormonal influences, differences observed at early embryonic stages can't be accounted for in this way. For example, male embryos have a higher growth rate at preimplantation stages, before overt sexual differentiation and exposure to sex hormones. X inactivation, a drastic epigenetic event exclusive to females, likely affects the embryonic transcriptome and epigenome in a sex-specific manner. Thus, male and female genomes are epigenetically poised for their divergent pathways early on. To determine whether there is differential expression at preimplantation stages, we derived male and female mouse embryonic stem (ES) cell lines and performed RNA-sequencing. When XY and XX cell lines were compared, over 400 coding genes were differentially expressed ($\alpha < 0.01$). A substantial number of these are transcription factors and epigenetic enzymes that are predicted to be dosage sensitive, indicating that there are regulatory differences between male and female embryos that depend solely on their chromosomal composition. In addition, we found more than 300 non-coding RNAs that were sex-biased ($\alpha < 0.01$). RNA-seq results were validated by qPCR and effects of differentially expressed transcription factors were confirmed with luciferase assays. To determine whether these expression differences translated into epigenomic differences, we conducted focused chromatin immunoprecipitation analyses and observed significant sex-dependent variation in chromatin accessibility in specific genes. We are exploring whether these sexual dimorphisms predict distinctions in response to environmental signals and whether they foreshadow sex-specific health-related outcomes after birth. Our results will have implications in understanding the developmental origins of disease, will impact disease treatment and stratification and, importantly, may have significance in the field of regenerative medicine. Furthermore, understanding sex biases in transcription and epigenetic status will provide insights into sex differences in susceptibilities to and protection from diseases.

M293 Genetic control of the epigenetic landscape. *Christopher L. Baker*[1], Guruprasad Ananda[1], Michael Walker[1], Catrina Spruce[1], Karl W. Broman[2], Petko Petkov[1], Greg Carter[1], Kenneth Paigen[1]. 1) The Jackson Laboratory, Bar Harbor, ME, USA; 2) The University of Wisconsin-Madison, Madison, WI, USA.

Cellular differentiation and numerous biological functions are controlled by a wide variety of regulatory elements associated with characteristic epigenetic modifications. Although a variety of proteins are known to act as writers, readers, and erasers of these marks, we know little about the organizing principles behind the systems regulating the establishment and maintenance of this epigenetic landscape. Understanding how these marks are controlled, and ultimately the ability of cells to differentiate and regulate their functions, is a fundamental and open question in biology. As a model to explore these issues, we have leveraged the strength of mouse genetics to understand how natural genetic variation impacts these processes. Studying levels of H3K4me3, an epigenetic mark associated with promoters, enhancers and recombination hotspots, we compared the genomes of C57BL6/J and DBA2/J mice finding considerable differences in activity of individual H3K4me3 sites in spermatocytes. Haplotype-specific analysis in F1 hybrids showed evidence of trans-control at numerous sites where the phenotype at the site did not coincide with the genotype of the parent. To identify the underlying genetic control, we measured H3K4me3 levels at 72,859 sites in a mapping population of 32 BXD recombinant inbred lines, identifying 6,868 QTL at FDR < 0.1. Most QTL map proximally, indicating control of H3K4me3 levels in cis. There were also1,262 trans-regulated sites, with five major QTL controlling greater than 55 sites each, collectively regulating 62% of all trans-regulated sites. The largest trans QTL on Chr 13 controls 452 sites. Importantly, trans-regulated H3K4me3 sites are clustered in domains of 1-10 Mb (R-scan statistic $p < 0.001$), indicating that trans control involves regional higher order chromatin organization rather than acting on individual H3K4me3 sites. In agreement with this, we find that the same QTL can affect both recombination hotspots where H3K4me3 is deposited by the enzyme PRDM9, and at promoters and enhancers, which are methylated by distinct enzyme systems. Finally, comparing spermatocytes with hepatocytes, we find evidence that these systems are cell and tissue specific, suggesting different systems may play key roles in establishing or maintaining differentiation. Together, these QTL constitute an integrated system controlling the epigenetic landscape in which each QTL controls multiple domains scattered across the genome. Their existence obviously raises questions of the identity of the genes underlying these QTL, their mechanism of action and their roles in regulating cellular differentiation and function.

M294 Genetic Variation Mediates the Epigenetic Response to Corticosteroids in Mice. *Gregory W. Carter*, Catrina Spruce, Robyn Ball, Wendy Pitman, Narayanan Raghupathy, Anna L. Tyler, Michael Walker, Kenneth Paigen, Petko M. Petkov. The Jackson Laboratory, Bar Harbor, ME.

Epigenetic changes such as histone modifications and DNA methylation can modulate transcript expression through chromatin state reorganization. Natural genetic variation potentially alters these modifications, providing a molecular mechanism for many of the abundant cis-acting gene expression quantitative trait loci (eQTL) that associate local genetic variation with differences in transcript levels. Importantly, such variation may mediate the individual response to drug treatments. However, the effects of genetic variation on regulatory regions and the extent to which alterations in DNA methylation and local chromatin configuration play a role in them have not been extensively studied. We use purified hepatocytes from a set of nine diverse mouse strains, including the founders of the Collaborative Cross and DBA/2J, to study how genetic variation affects baseline epigenetic marks as well as the response to the glucocorticoid dexamethasone. By combining RNA-seq, bisulfite DNA-seq, and ChIP-seq assays for H3K4me1, H3K4me3, H3K27me3, and H3K27ac, we obtained a detailed view of the impact of genetic variation on both epigenetic marks and transcript abundances. We found that strain-specific variation in both gene expression and epigenetic marks is abundant, and is much greater than the consequences of dexamethasone treatment in terms of both number of loci affected and the magnitude of effects. Furthermore, the effect of treatment varies substantially across mouse strains, both at the genetic and epigenetic level, providing evidence of coordinated variation in molecular responses to a common immunosuppressant. Finally, our results provide insights on the extent to which local eQTL detected in mouse intercross populations such as the Diversity Outbred and BxD may be due to cis-acting genetic variation in promoter and enhancer activities.

M295 EZH2 Isoforms Differentially Regulate the Function of Polycomb Repressive Complex 2. *W. Mu*, J. Starmer, D. Yee, T. Magnson. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Polycomb-repressive complex 2 (PRC2) catalyzes the methylation of histone H3 Lys27 (H3K27) and functions as a critical epigenetic regulator of both stem cell pluripotency and somatic differentiation. EZH1 and EZH2 are enzymatic subunits of PRC2, the latter of which is encoded by alternative splice isoforms. To elucidate how EZH variants regulate PRC2 in establishment of H3K27 methylaiton during cell differentiation, we analyzed the alteration of di- versus tri-methylation of H3K27 in spermatocytes during meiotic prophase I. We found that H3K27me2 levels were strikingly elevated across all nuclei during the zygotene-to-pachytene stage transition, which occurs along with upregulation of expression of *Ezh2*. In contrast, there was no significant increase in H3K27me3 during meiotic progression. Interestingly, a new *Ezh2* isoform, where exon 14 is deleted due to alternative splicing, was dramatically increased in meiocytes compared to mitotic germ cells. However, the *Ezh2* isoform containing exon 14 (*Ezh2- ex14*) downreguated as meiosis was initiated. We observed that expression of *Ezh2-ex14* isoform upregulated during mitosis in rapidly dividing mouse embryonic fibroblasts. These results suggest that EZH2 isoforms may function differently in non-dividing cells such as spermatocytes undergoing differentiation during meiosis versus mitotically replicating cells such as mouse embryonic fibroblasts and that these distinct isoforms may determine the choice of di- versus tri-methylation of H3K27.

M296 The function of the histone demethylase KDM1A (LSD1) in Tau mediated neurodegeneration. *David Katz*, Michael Christopher, Dexter Myrick, Amanda Engstrom, Rohitha Moudgal. Dept. of Cell Biology, Emory University, Atlanta, GA.

Alzheimer's disease (AD) is the most common form of dementia that occurs during aging. AD is associated with the pathological aggregation of Ab and pTau. Nevertheless, it remains unclear how these protein aggregates lead to neuronal cell death in AD. Here we implicate a potential epigenetic step in AD. Data from our lab demonstrates that the histone demethylase KDM1A/LSD1 is mislocalized with aggregated pTau in AD cases. In addition, we find that loss of KDM1A systemically in adult mice is sufficient to recapitulate many aspects of AD, including widespread neuronal cell death in the hippocampus and cortex, learning and memory defects, and global gene expression changes that match AD cases. Surprisingly, in degenerating hippocampal neurons, we also detect the inappropriate re-expression of stem cell genes. These data raise the possibility that aggregated pTau leads to neuronal cell death in AD by interfering with the continuous requirement for KDM1A to epigenetically repress transcription associated with alternative cell fates. To further investigate the potential involvement of KDM1A in the AD pathway, we also removed one copy of *Kdm1a* from Tg(Prnp-MAPT*P301S)PS19Vle (P301S Tau) mice, which contain a transgene overexpressing an aggregation prone form of MTAP (Tau). If pTau leads to neurodegeneration by interfering with KDM1A, we would expect these mice to exhibit a faster, more severe neurodegeneration phenotype. Remarkably, our preliminary results indicate that partial loss of KDM1A enhances the Tau-mediated neurodegeneration in this mouse model. These results indicate that it may be possible to target KDM1A therapeutically to block the progression of AD in patients.

M297 Engineering the Cancer Genome. *Tyler Jacks*. Koch Institute for Integrative Cancer Research, HHMI, MIT, Cambridge, MA.

no abstract submitted

M298 Genetic inhibition of MTOR during thymic Pre-T LBL development delays tumorigenesis and points to the IRF4-CDK6 pathway as a potential target in the treatment of T-ALL/LBL. *B. A. Mock*[1*], J. M. Gary[1,2], J. K. Simmons[1], J. Xu[3], S. Zhang[1], N. Watson[1], B. Gamache[1], K. Zhang[1], A. L. Kovalchuk[4], A. M. Michalowski[1], M. Kiupel[2], S. Gaikwad[1], W. Dubois[1], J. Testa[1]. 1) CCR, NCI, NIH, Bethesda, MD; 2) Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI; 3) Fox Chase Cancer Center, Philadelphia, PA; 4) NIAID, NIH, Rockville, MD, USA.

The PI3K/AKT/MTOR pathway is frequently activated in T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL). To model inhibition of this pathway in lymphoma, mice with T-lymphocyte-specific, constitutively-active AKT (Tg(Lck-Akt2*)#Test, Lck-MyrAkt2) were crossed to mice

with genetically reduced MTOR expression (*Mtor*^{tm1Lgm} knock-down, KD). Mice with genetic reduction of MTOR had increased survival relative to wild type MTOR mice (average survival of 24 versus 14 weeks, respectively), though both groups ultimately developed thymic pre-T-cell lymphoblastic leukemia/lymphoma (pre-T LBL). A similar increase in survival was observed when MTOR wild type Lck-MyrAkt2 mice were treated for 8 weeks with the rapamycin analog, everolimus, an inhibitor of the mTORC1 complex. Transcriptional profiling of wildtype (WT) and MTOR KD thymic lymphomas identified cyclin dependent kinase, *Cdk6*, as one of the most down-regulated genes during tumorigenesis in the Lck-AKT, MTOR KD tumors; its expression was the same in WT and KD pre-tumor thymocytes. The interferon regulatory transcription factor, *Irf4*, was identified as an upstream regulator; protein levels of IRF4 were down-modulated in both pre-tumor thymocytes and pre-T LBL cells in the KD mice, compared to levels in WT littermates. Rescue experiments have confirmed the relationship between IRF4 and CDK6. Pharmacologic inhibition of MTOR in tumor cells also decreased CDK6 protein levels, further suggesting a mechanistic relationship in this tumor type. Combination treatment with the MTOR inhibitor rapamycin and the CDK4/6 inhibitor palbociclib cooperatively decreased the overall viability and signaling downstream of drug targets in human T-ALL/LBL cell lines. Further, the combination of palbociclib and rapamycin decreased tumor size and proliferation in nude mice with Lck-Akt/MTOR WT tumor flank transplants, and significantly increased survival in an intravenous transplant model of disseminated leukemia compared to single agent treatments, suggesting the potential for this drug combination in treating T-ALL/LBL.

M299 Adenoma Susceptibility Modulated by Variable Complex Gut Microbiota in a Rat Model of Familial Colon Cancer. *Susheel Busi*, Aaron Ericsson, Taybor Parker, Craig Franklin, Elizabeth Bryda, James Amos-Landgraf. University of Missouri-Columbia, Columbia, MO.

Mechanisms of colonic tumor initiation suggest that cancer can develop through multiple genetic and epigenetic pathways. Human epidemiologic studies have shown the presence of cancer is associated with changes in the complex gut microbiota (GM). To determine if differences in the complex GM directly cause differences in cancer susceptibility, we used complex microbiota targeted rederivation (CMTR) to rederive isogenic embryos of the F344/NTac-*Apc*^{Pirc}*/+ (Pirc)* rat model of familial adenomatous polyposis, using three different strains of surrogate dams (F344/NHsd (*F344*), LEW/SsNHsd (*LEW*), and Crl:SD (*SD*)) each harboring distinct gut microbiota. Fecal samples from the rederived pups were collected from weaning up to 180 days. The GM composition was characterized to the operational taxonomic unit (OTU) level by extracting bacterial DNA from fecal material and sequencing the 16S rRNA gene V4 hypervariable region on the Illumina MiSeq platform. Adenoma development was also monitored longitudinally via colonoscopy and tumor multiplicity was determined at 6 months.

We found that the GM varied between dams, and that the pups' microbiota resembled their surrogates by 1.5 months. Two rats with the LEW GM did not develop any colonic tumors, decreasing the Pirc phenotype penetrance from 100% to 87%. All rats with this GM also had a significantly reduced colonic tumor burden compared to both F344 GM ($p<0.001$) and SD GM ($p<0.05$). The relative abundance of certain OTUs including family Peptococcaceae and *Akkermansia muciniphila* were positively correlated with tumor multiplicity in the F344 GM ($p<0.05$). A previously reported sex bias in colonic tumor burden was also confirmed with males showing more adenomas than females ($p<0.05$). To assess functional changes associated with variation in GM, PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) was used and showed elevated expression of H_2S-related bacterial genes in the LEW GM.

To assess whether tumors from rats with diverse GM differed in loss of heterozygosity (LOH), we used a quantitative allele-specific pyrosequencing assay to determine the allelic ratio of the genomic *Apc*^{Pirc} loci in colonic tumors. Approximately 70% of tumors from rats with F344 GM underwent LOH with 30% tumors maintaining heterozygosity of the *Apc* allele, consistent with previous reports. Interestingly, rats with SD and LEW GM showed 100% and 96% LOH respectively, suggesting a possible link between differing complex microbiota and loss of the wildtype Apc allele. Our study provides new insight into the role of gut microbiota as a modulator and predictor of disease phenotype in this rat model.

M300 The aggressive prostate cancer susceptibility gene *HIST1H1A* is a modulator of androgen receptor signaling and epithelial to mesenchymal transition. *K. Williams*[1], M. Lee[1], Y. Hu[2], J. Andreas[1], S. Zhang[1], N. Crawford[1]. 1) NHGRI/NIH, Bethesda, MD; 2) NCI/NIH, Rockville, MD.

In 2016, approximately 190,000 new prostate cancer (PC) cases will be diagnosed, and over 26,000 men will succumb to PC. Presently, there is an urgent need to more precisely identify men at risk for aggressive PC. This study aims to characterize how germline variation modulates susceptibility to 'neuroendocrine (NE) differentiation', which is a marker of aggressive PC. Our earlier study identified aggressive PC susceptibility genes using the C57BL/6-Tg(TRAMP)8247Ng/J (TRAMP) mouse model of NE PC. We performed quantitative trait locus (QTL) mapping in transgene-positive (TRAMPxNOD/ShiLtJ) F2 intercross males (n=228), and expression QTL mapping using primary tumor microarray data (n=126) identified 35 aggressive PC candidate genes. Furthermore, *in silico* analysis identified *HIST1H1A*, which encodes the linker histone H1.1, as having an expression level associated with patient outcome in a human PC gene expression dataset, and harboring a SNP associated with lymph node metastasis in the PC genome-wide association study. Ectopic expression of *HIST1H1A* in the aggressive, androgen receptor (AR)-negative human prostate PC3-Luc cell line suppressed tumor growth in a xenograft flank assay (avg. tumor vol. = 311+/-69 mm^3 vs. 565+/-193 mm^3 for controls; P=1.01x10^{-4}), and reduced dissemination in a systemic metastasis assay (avg. flux=4.45x10^8 vs. 1.01x10^9 for controls; P=0.035). Conversely, in the AR-positive LNCaP cell line, ectopic expression of *HIST1H1A* enhanced tumor growth. Microarray analysis of the latter cell lines revealed that over 2,600 transcripts were significantly dysregulated in response to *HIST1H1A* over-expression (fold change +/-1.5, false discovery rate < 0.05). Ingenuity Pathway Analysis revealed that both AR signaling and epithelial to mesenchymal transition (EMT) were significantly impacted. Quantitative RT-PCR indicated that higher *HIST1H1A* levels were associated with suppression of epithelial gene expression (e.g., *KRT18*, *TJP1*) and activation of mesenchymal genes (e.g., *SNAI2*, *VIM*). Immunoblot analysis revealed suppression of long-form AR expression and activation of the aggressive disease associated AR-V7 splice variant. Collectively, these data indicate that *HIST1H1A* affects aggressive PC by modulating AR signaling and EMT. Ongoing work involves ATAC-seq analysis to determine how *HIST1H1A* dysregulation

impacts PC-specific chromatin structure, as well as CRISPR/Cas9 knockout of *Hist1h1a* in mice. These data demonstrate the use of systems genetics to provide insights into how hereditary variation influences aggressive PC susceptibility.

M301 Polymorphisms in the *Arntl2* promoter affect metastatic susceptibility in estrogen-receptor negative breast cancer. *K. W. Hunter*, N.-H. Ha. NCI, Bethesda, MD.

Breast cancer is the leading incident of cancer and the second-leading cause of cancer-related deaths in women with the majority dying from metastatic disease. Previous studies have demonstrated the importance of inherited polymorphisms in the progression of breast cancer metastasis. In this study, we used a mouse cross between the metastatic mammary tumor model MMTV-PyMT and MOLF/EiJ to identify the circadian transcription factor *Arntl2* as a metastasis modifier in estrogen receptor (ER)-negative breast cancer. Sequence analysis of MOLF/EiJ demonstrated that non-synonomous substitutions of *Arntl2* existed between MOLF/EiJ and MMTV-PyMT mice. However, modulation of expression levels of *Arntl2* signifcantly affected metastastic capacity in multiple mammary tumor cell lines indicating that transcriptional levels play an important role in metastatic progression. Furthermore, implantation of wild type tumor cells into *Arntl2* knockout mice demosntrated that the effect of *Arntl2* on metastasis is a tumor cell intrinsic phenotype. To identify putative causative eQTL variants that modulate *Arntl2* expression levels DNase hypersensitivity site (DHS) analysis was performed. This analysis revealed 13 single nucleotide polymorphisms (SNP) in the proximal promoter region that potentially affect transcriptional activity. CRISPR/Cas9-mediated substitution of the MOLF/EiJ polymorphic promoter allele reduced *Arntl2* expression and metastatic capacity of the 4T1 metastatic mammary tumor cell line, as predicted. Taken in toto, these results provide strong evidence that the expression levels of the circadian rhythm gene *Arntl2* plays an important role in the predisposition to the development of mammary tumor pulmonary metastases.

M302 Glioma modeling with MADM, a mouse genetic mosaic system, revealed cell competition as the mechanism that enables inevitable malignant progression. *Hui Zong*, Phil Gonzalez, Jungeun Kim, Guoxin Zhang. University of Virginia, Charlottesville, VA.

Understanding tumor initiation is critical for developing early detection and cancer prevention strategies. However, this problem has been virtually impossible to study because pre-malignant mutant cells are indistinguishable from their normal counterparts. To overcome this hurdle, our lab uses a mouse genetic system termed Mosaic Analysis of Double Markers (MADM) that, from a non-labeled mouse heterozygous for a tumor suppressor gene (TSG), generates GFP-labeled TSG-null cells and RFP-labeled sibling wildtype cells. The sparseness of mutant cells due to the low frequency of *inter*-chromosomal recombination closely mimics the clonal origin of human cancer, and the definitive correlation between color and genotype greatly facilitate the analysis of the earliest tumorigenic events. In particular, **increased ratio of green to red cell numbers (G/R ratio) serves as the clear indication of initial expansion of mutant cells**. Malignant glioma is the deadliest type of cancer in the brain. One devastating fact is the inevitable progression of benign grade II tumors into full malignancy within a few years. Previously our lab identified oligodendrocyte precursor cells (OPCs) as its cell-of-origin because MADM revealed a massive expansion of pre-malignant mutant OPCs (G/R ratio > 100) long before malignant transformation. Surprisingly, the overall OPC density barely changed in the mutant brain, suggesting that **the pre-malignant expansion of mutant OPCs was achieved at the cost of WT OPCs**. Using *in vivo* time course analysis and *in vitro* co-culture experiments, we determined that the competition is mediated via active killing of WT OPCs by mutant ones. These observations well explain the inevitable progression of glioma since any individual OPC gaining an advantageous mutation would outcompete less fit ones. By repeating this process, a mutant OPC would eventually gather all necessary mutations for the full-blown transformation. This finding also predicts the unavoidable failures with cytotoxic treatment methods because they merely provide selective pressure on the evolutionary process of the tumor mass, which eventually lead to the enrichment of most resistant tumor cells. Therefore, an effectiveness treatment must deal with cell competition between mutant and WT OPCs. **In a proof-of-principle experiment, we used a genetic model to remove the competitiveness of mutant OPCs, and successfully blocked glioma formation.** Different from conventional studies that are mainly focused on cell intrinsic gene functions during tumorigenesis, MADM enabled our discovery of non-cell-autonomous mechanism for glioma progression, which should lead to completely novel therapeutic strategies.

M303 Host-pathogen genetic interactions drive outcome to tuberculosis in the Collaborative Cross. *Clare Smith*[1], Martin Ferris[2], Fernando Pardo Manuel de Villena[2], Robert Williams[3], Richard Baker[1], Christopher Sassetti[1]. 1) UMASS Medical School, Worcester, MA, USA; 2) Department of genetics, UNC Chapel Hill, NC, USA; 3) 3Department of Anatomy and Neurobiology, University of Tennessee Health Sciences Center, Memphis, TN, USA.

The complex interplay between host and pathogen determines if an individual controls infection or progresses to disease. While abundant evidence suggests that genetic diversity contributes to the variety of outcomes, the combined effect of variation in the host and pathogen remains unclear. We developed a “dual-genome” system to unravel genetic interactions between *Mycobacterium tuberculosis (Mtb)* and its mammalian host that drive outcome to infection. Host variation was modeled using a collection of ~100 recombinant inbred mouse strains, including the Collaborative Cross panel, the BxD recombinant inbred panel and a series of targeted single-gene knockouts. Bacterial variation was concurrently generated using whole-genome knockout libraries and panels of diverse *Mtb* clinical isolates, each of which contained a molecular barcode to allow parallel assessment of multiple *Mtb* variants in each of the diverse host backgrounds.

The disease spectrum of CC and BXD mice infected with *Mtb* libraries exceeded that seen in parental strains and standard inbred lines. Metrics of disease that are tightly linked in the standard C57BL/6J resistant model such as bacterial burden, dissemination, weight loss and inflammation were genetically separable in the diverse strains. We identified individual polymorphic host genome regions (QTLs) underlying lung and spleen bacterial load and host control of infection independently in the CC and BXD panels.

We additionally separated the clinical disease traits into intermediate phenotypes by determining the relative fitness of thousands of

bacterial mutants in the mouse panels. QTLs underlying differential bacterial fitness modules were identified, many of which mapped to the same host region as the clinical disease metrics. In addition to these overlapping "master loci", we identified further QTLs impacting the requirement of various bacterial genes, including virulence factors, nutrient acquisition and oxidative radical generation.

Overall, the strategy of using bacterial fitness profiles as reporters of the underlying host microenvironment is a sensitive and specific method for identifying disease-modifying host polymorphisms, demonstrating the power of a dual-genome systems genetics approach to understand the fundamental drivers of susceptibility to infection.

M304 Rosa Beddington Lecture: Single cells get together: cell lineage specification & tissue morphogenesis in the early mouse embryo. *AK. Hadjantonakis*. Memorial Sloan Kettering Cancer Center, New York, NY.

The last twenty years have seen major advances in our understanding of animal development. Genes regulating particular processes have been identified and, in many instances, they have been linked to human diseases, but how genetic information is transformed into three-dimensional tissues and organs remains an open question. We are seeking to fill this critical gap in knowledge by exploiting mouse genetics, high resolution quantitative imaging and gene expression profiling to investigate mechanisms of cell lineage specification, tissue patterning and morphogenesis, using the mouse as a model system. I will provide an overview of our interests and discuss some of our studies. My talk will reflect our focus on two distinct but interrelated events; blastocyst development and gastrulation.

M305 *Snai1* is required for stem cell maintenance in the mouse intestinal epithelium. *Helen Abud*[1*], Katja Horvay[1], Thierry Jarde[1], Franca Casagranda[2], Katharina Haigh[3,6], Christian Nefzger[4], Reyhan Akhtar[1], Thomas Gridley[5], Geert Berx[6,7], Jody Haigh[3,6], Jose Polo[4], Gary Hime[2]. 1) Cancer Program, Monash Biomedicine Discovery Institute, Clayton, Victoria, Australia; 2) Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Australia,; 3) Australian Centre for Blood Diseases, Monash University, Clayton, Australia; 4) Australian Regenerative Medicine Institute, Monash University, Clayton, Australia; 5) Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, Maine, USA; 6) Molecular and Cellular Oncology, Inflammation Research Center, VIB, Ghent, Belgium; 7) Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium.

The Snail family of transcription factors are well known for mediating epithelial to mesenchymal transitions and cell motility during both embryonic development and tumour invasion. Although they are generally regarded as markers of mesenchymal cells, Snail proteins have recently been implicated in regulating stem cell populations in several organs. *Snai1* is normally expressed in the intestinal epithelium within the crypt base columnar (CBC) stem cells and transit amplifying cell populations. Our studies have shown that both the expression and cellular localisation of *Snai1* is dependent on canonical Wnt signalling, a key regulatory pathway of intestinal stem cells. *Snai1* is up-regulated in polyps from *Apc*$^{min/+}$ mice indicating that *Snai1* may also play a part in the early stages of tumorigenesis in addition to promoting invasion of intestinal tumours. We investigated *Snai1* function in the mouse intestinal epithelium using an inducible conditional knockout approach and found that *Snai1* is required for maintenance of CBC stem cells and the appropriate balance of differentiated cell types. Further analysis of the effects of *Snai1* loss on the CBC stem cell population using a combination of Fluorescent Activated Cell Sorting (FACS), lineage tracing and *in vitro* organoid culture showed that loss of *Snai1* results in a decrease in cell proliferation and apoptotic loss of stem cells. In contrast, ectopic expression of *Snai1* using a conditional transgenic approach results in an increase in cell proliferation at the crypt base which correlates with elevated levels of expression of CBC stem cell markers. In addition, a decrease in secretory Paneth and enteroendocrine cells is observed. Our functional studies show that intestinal epithelium where *Snai1* is depleted fails to produce a proliferative response following radiation induced damage. These studies demonstrate a critical role for *Snai1* in survival of CBC cells and regeneration of the epithelial cell layer following damage. In conclusion, this suggests that *Snai1* has a key role in stem cell maintenance and control of lineage choice during cellular differentiation in the intestinal epithelium.

M306 Muscle fiber signaling scales the myogenic stem cell pool. *Christoph Lepper Lepper*[1], Sheryl Southard[1], Ju-Ryoung Kim[2], Richard Tsika[2]. 1) Carnegie Institution for Science, Baltimore, MD; 2) University of Missouri, Columbia, MO.

When unperturbed, quiescent somatic stem cells are poised to affect immediate tissue restoration upon trauma. Yet, little is known regarding the mechanistic basis controlling initial and homeostatic 'scaling' of adult stem cell pool sizes relative to their target tissues for effective regeneration. Here, we show that transgenic TEAD1 (TgTg(Ckm-Tead1)#Tsik)-expressing skeletal muscle features a dramatic hyperplasia of muscle stem cells (i.e. the satellite cells, SCs) but surprisingly without affecting muscle tissue size. Such super-numeral SCs attain a 'normal' quiescent state, accelerate regeneration, and maintain regenerative capacity over several injury-induced regeneration bouts. In dystrophic muscle, the TEAD1 transgene also ameliorated the pathology. We further demonstrate that hyperplastic SCs accumulate non-cell-autonomously via signal(s) from the TEAD1- expressing myofiber, suggesting that myofiber-specific TEAD1 overexpression activates a physiological signaling pathway(s) that determine initial and homeostatic SC pool size. We propose that TEAD1 and its downstream effectors are medically relevant targets for enhancing muscle regeneration.

M307 Plasticity, self-renewal and transcriptional dynamics – How embryonic stem cells stall for time in the decision making process? *J. M. Brickman*[1], William Hamilton[1], Yaron Mosesson[2], Naama Barkai[2], Chiara Francavilla[1], Jesper Olsen[1], Rob Illingworth[3], Jurriaan Hölzenspies[1], Wendy Bickmore[3]. 1) University of Copenhagen, Copenhagen, DK; 2) Weizmann institute of Science, Rehovot, Israel; 3) University of Edinburgh, Edinburgh, UK.

Embryonic stem cells (ESCs) are immortal cell lines derived from the peri-implantation mammalian embryo. Both ESCs and the embryos from which they are derived are remarkable, in that individual cells retrain the capacity to begin developmental anew, despite having **undergone a**

degree of patterning or differentiation. What is the basis for this plasticity at a transcriptional level? We have identified two new mechanisms regulating this process. The first involves a protective activity of Polycomb co-repressor complexes, that enables transcriptional stimulation in the absence of commitment. The second relies on the reversible disengagement of RNA Polymerase II from specific promoters in response to signaling. I will discuss these mechanisms and how they impact on the capacity of ESCs to retain their plasticity while they prime for differentiation but continue in self-renewal.

M308 Comparative Mendelian genomics and disease modeling in mice. *L. Reinholdt*[1], H. Fairfield[3], A. Srivastava[1], G. Ananda[2], R. Liu[2], A. Lakshminarayana[2], B. Harris[1], S. Y. Karst[1], L. Dionne[1], C. Kane[1], M. Berry[1], C. Byers[1], A. Czechanski[1], W. Martin[1], D. Berstrom[1]. 1) The Jackson Laboratory, Bar Harbor, ME; 2) The Jackson Laboratory for Genomic Medicine, Farmington, CT; 3) Maine Medical Research Institute.

Large consanguineous pedigrees, controlled environments, and genetically defined inbred strain backgrounds afforded by the laboratory mouse provide significant advantages for the discovery of Mendelian disease genes. The mouse as a disease model also offers the advantages of highly orthologous protein coding genes and mammalian physiology. Until the advent of high throughput sequencing, the rate of Mendelian disease gene discovery in both the human and mouse genomes was comparatively slow. Taking advantage of the world's largest collection of mouse strains with naturally occurring Mendelian disease phenotypes, we are using whole exome sequencing (WES) to discover naturally occurring disease mutations at unprecedented rates. To date, we have found over 90 new disease causing alleles. Over 80% of these alleles are in genes that have been implicated with Mendelian disease in the human population and an additional 10% are in genes that are novel, and, until recently, had yet to be associated with a mouse phenotype or human disease. These data demonstrate that even after nearly 100 years of research, spontaneously arising Mendelian mutations in the mouse genome continue to yield new discoveries. Despite our unprecedented rate of spontaneous mutation discovery, 50% of our cases remain unsolved using a standard exome sequencing analytics pipeline. Using a combination of approaches, we have found evidence that a large fraction of unsolved exome cases likely involve structural mutations that span exons but escape detection by standard exome pipelines. This result directly informs efforts to investigate the similar proportion of apparently Mendelian human phenotypes that are recalcitrant to exome sequencing. To advance our Mendelian disease modeling efforts we are integrating our disease allele discovery with parallel human Mendelian disease efforts by the Baylor-Hopkins Center for Mendelian Genomics (BHCMG). This integrated approach not only informs unsolved human exome cases, it also provides clinical information that we are using to implement disease relevant phenotyping and in some cases, the development of precise models through gene editing. We will provide several examples of new disease gene associations and mouse models including Diamond Blackfan anemia, Charcot-Marie-Tooth disease, Maffucci syndrome and idiopathic learning disability.

M309 A New Mouse Model for Costello Syndrome. *T. Sorg*[1], M.-C. Birling[1], G. Bou About[1], M.-F. Champy[1], H. Jacobs[1], H. Meziane[1], G. Pavlovic[1], A. Ayadi[1], Y. Herault[1], R. Rossignol[2], D. Lacombe[2]. 1) PHENOMIN - ICS, Illkirch, FR; 2) MRGM, Bordeaux, FR.

Costello Syndrome (CS) is a distinctive rare multisystem disorder comprising characteristic prenatally increased growth retardation, coarse facial features, redundant skin with deep palmar, plantar creases and papillomata of later onset. CS patients present also laxity of small joints, tight Achilles tendons, cardiac malformations, and developmental delay. The primary cause of CS was associated to the germ line activation of *HRAS* oncogene, with a common missense mutation G12S in 80% of the patients. Here we describe the generation and the consequent phenotypic characterization of a genetically engineered mouse model of CS, by introducing the oncogenic G12S mutation by homologous recombination into the mouse *Hras* gene, *Hras*$^{tm1.1Ics}$. The effect of the HRAS G12S mutation was evaluated on behavioral, visual, metabolic, cardiac and histological traits in young adult animals. The behavioral analysis revealed that HRAS^{G12S} mutant males displayed reduced locomotor activity, accompanied by decreased muscle strength and altered motor coordination performance. In addition, the cardiac exploration revealed that HRAS^{G12S} mutants exhibit a hypertensive phenotype combined with tachycardia. In conclusion, the HRAS^{G12S} mutant mice showed a polysyndromic phenotype reproducing some of the CS features observed in patients. The future study of the here-described CS mouse model should have a significant impact of our understanding of CS disease. The use of HRAS^{G12S} mutant mice as a CS mouse model opens up new fields of investigation to better understand the pathophysiology of the disease and to evaluate drugs dedicated to the reduction of the disease associated symptoms.

M310 A genetic epistasis analysis of an ENU-induced *Reln* mutant reveals that the C-terminal domain of RELN is required for binding to the receptor VLDLR but not to LRP8 (APOER2). *D. R. Beier*[1,2], S. Ha[1]. 1) Seattle Children's Research Institute, Seattle, WA; 2) University of Washington, Seattle, WA.

We have previously described a forward genetic screen for brain lamination defects using ENU-mutagenesis of a reporter line that marked specific layers of the cerebral cortex (Ha et al., Cereb Cortex, 2015). In this study we identified a novel hypomorphic allele of *reelin*, named *Reln*^{CTRdel}, which carries a splice-site mutation that results in a truncation of the C-terminal region (CTR) domain of the RELN protein product. The mutant secretes RELN, distinguishing *Reln*^{CTRdel} from *Orleans reeler,* which has a larger C-terminal truncation that completely prevents RELN secretion and phenocopies *reeler*. In contrast, *Reln*^{CTRdel} displays remarkably distinct phenotypes from *reeler*. First, the size and foliation of the cerebellum is normal, and this mutant is not ataxic. Second, the mutant does not have the inversion of cortical layers; however, both superficial and deep layer neurons migrate beyond normal boundaries. Third, the dentate gyrus morphology of the hippocampus is severely disturbed; most of the infrapyramidal blade is absent, while the suprapyramidal blade is present and better laminated when compared with *reeler*. Genetic epistasis analysis showed that *Reln*^{CTRdel}/*Lpr8*^{tm1Her} (AKA *Apoer2*^{null}) double homozygotes have phenotypes akin to *reeler*, while *Reln*^{CTRdel}/*Vldlr*^{tm1Her} mice do not. Given that *Apoer2*^{null}/*Vldlr*^{tm1Her} double-knockout mice resemble *reeler* and single-knockout mice are hypomorphic, we infer that *Reln*^{CTRdel}/*Apoer2*^{null} double homozygotes have both receptor pathways disrupted. Therefore, the epistasis results

suggest that CTR-truncation disrupts an interaction with VLDLR, while the APOER2 signaling pathway remains active, which accounts for the hypomorphic phenotype in *Reln*^{CTRdel} mice. A receptor binding assay confirms that CTR-truncation of RELN significantly decreases binding to VLDLR, but not to APOER2. Taken together, the *in vitro* and *in vivo* results demonstrate that the absence of the CTR domain can limit receptor-binding specificity of RELN. Our discovery, using a focused neurodevelopmental analysis, of an allele of *reeler* that would likely not otherwise be identified (as it is not ataxic) illustrates the utility of unbiased forward genetic approaches in the mouse.

M311 Inhibition of activin A stops the regrowth of surgically resected heterotopic bone in a mouse model of Fibrodysplasia Ossificans Progressiva and indicates a new potential path to therapy. *A. N. Economides*[1,2*], L. Huang[1], L. Q. Xie[1], N. Das[1], X. Wen[1], L. Wang[1], A. J. Murphy[1], V. Idone[1], S. J. Hatsell[1]. 1) Regeneron Pharmaceuticals, Tarrytown, NY; 2) Regeneron Genetics Center, Tarrytown, NY.

Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disorder characterized by episodic yet cumulative heterotopic ossification (HO), which develops connections to the normal skeleton, resulting in progressive immobility. The heterotopic bone cannot be resected because such procedures result in regrowth of the resected lesions and induction or exacerbation of additional HO. FOP is caused by mutations in ACVR1, with the most common mutation altering arginine 206 to histidine (ACVR1^{R206H}). Using a genetically accurate mouse model of FOP – 129;B6N-*Acvr1*^{tm2Vlcg} (aka *Acvr1*$^{[R206H]FlEx}$)/+ *Gt(ROSA26)Sor*$^{tm3.1(cre/ERT2)Vlcg}$/+ – we have demonstrated that unlike wild type ACVR1, ACVR1^{R206H} perceives activin A as an agonistic ligand, and that it is this new property of ACVR1^{R206H} that drives HO. Furthermore, using neutralizing anti-activin A antibodies we have shown that there is an absolute requirement for activin A to initiate HO (Hatsell, Idone et al; PMID 26333933). Here we extend our findings and demonstrate that inhibition of activin A in a delayed dosing setting (where the mice are allowed to form HO over three weeks and then dosing with the antibody is initiated), as well as in a surgery setting (where an HO lesion is resected while the mice are administered antibody) provides 'therapeutic' benefit. In both settings, inhibition of activin A stops further growth of existing or partially resected HO lesions. Importantly, whereas surgery (in the absence of anti-activin A) induces 'explosive' growth of the resected lesion as well as nearby lesions, this effect is greatly inhibited in the presence of the antibody. Given that the majority of FOP patients already present with ongoing or existing HO at the time of diagnosis (and also the number of patients with developed disease), our results not only demonstrate that activin A remains a key HO-promoting factor even after initiation of HO but also indicate a new potential path to therapy, and indeed one where surgical intervention may be incorporated.

M312 Driving discovery and characterisation of novel genes important for bone biology by combining high-throughput mouse phenotyping and a tissue-based deep phenotyping platform. *C. J. Lelliott*[1], J. Logan[2], S. Beck-Cormier[3], D. Lafont[1], A. Swiatkowska[1], H. Protheroe[2], S. Pearson[1], S. Maguire[1], A. Gogakos[2], N. Butterfield[2], V. Leitch[2], J. K. White[1], L. Beck[3], G. R. Williams[2], J. H. Bassett[2], Sanger Mouse Pipelines. 1) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 2) Molecular Endocrinology Group, Department of Medicine, Imperial College London, London, UK; 3) Institut National de la Sante et de la recherche Medicale, U791, LIOAD, Nantes, France.

Bone diseases causing abnormal development or maintenance of structure and function are an area of intense scientific investigation. Bone mineralization measurements by dual-energy X-ray absorptiometry (DEXA) are performed by many International Mouse Phenotyping Consortium members and could identify new candidate genes for focussed study. Data from 717 alleles from Sanger Mouse Pipelines (C57BL/6N, LabDiet #5021), was analysed by a Mixed-Model approach. 58 lines (8%) had hits for one or more bone mineral measurement: bone mineral density (BMD – 42 hits), volumetric BMD (vBMD – 19 hits) and bone mineral content accounting for body weight (BMC+BW – 37 hits). Hits were distributed: 1 hit – 26 alleles, 2 hits – 26, 3 hits – 6. Using the power of the Origins of Bone and Cartilage Disease platform (http://www.boneandcartilage.com), we further characterised two lines that displayed reduced bone parameters. Mice lacking the phosphate transporter *Slc20a2* have reduced BMD and BMC+BW (BMC+BW effect size: Females -9.77%; Males -9.38%) in DEXA. Using microcomputed tomography and histology, we found that femurs from *Slc20a2*$^{tm1a(EUCOMM)Wtsi}$-/- mice show impaired linear growth, particularly at younger ages. *Slc20a2*-/- femurs display delayed ossification linked to reduced growth plate width, as well as reduced trabecular bone content and reduced mineralization in both trabecular and cortical bone. Consequently, the mechanical properties of *Slc20a2*-/- femurs were altered in a three-point bend test, resulting in weak, brittle but flexible bones. Similarly, mice lacking *Tram2*, a component of the translocon, had significantly reduced BMD, vBMD and BMC+BW (BMC+BW effect size: Females -18.67%; Males -17.93%) in DEXA. Femurs from *Tram2*$^{tm1a(KOMP)Wtsi}$-/- mice were short, with thinner cortical bone, less trabecular bone and were weak yet flexible in the three-point bend test. As well as long-bone phenotypes, both *Slc20a2*-/- and *Tram2*-/- mice had abnormal skull and craniofacial morphologies. In *Tram2*-/- mice, this, combined with disrupted ossicle shape, correlates with a substantially raised hearing threshold without indication of auditory sensorineural impairment. Overall, this data shows the additive value of combining high-throughput phenotyping with specialist platforms to enhance the knowledge generated for specific areas of high medical need.

M313 From mouse to human and back to mouse: sodium channel mutations and epilepsy. *Miriam Meisler*, Rosie Bunton-Stasyshyn, Julie Jones, Jacy Wagnon. Univ Michigan, Ann Arbor, MI.

A transgene insertional mutation in the early 1990s led to the discovery of the mouse *Scn8a* gene, which encodes one of the most abundant sodium channels involved in generation of action potentials in mammalian neurons. A series of loss-of-function and gain-of-function alleles in the mouse revealed many of the *in vivo* roles of SCN8A and other gene family members. In 2012, a *de novo* mutation of *SCN8A* was identified in a child with Epileptic Encephalopathy, a severe disorder that includes seizure onset in the first year of life, developmental delay and intellectual disability. Overall, *de novo* sodium channel mutations account for 5% of cases, and more than 150 indivduals with *SCN8A* mutations have been identified. Functional studies in transfected cells demonstrate that 8/10 mutations cause incomplete channel inactivation or premature activition, both leading to neuronal hyperexcitability. A knock-in mouse model carrying one human mutation is being used to

characterize the functional impact on various classes of neurons and in the heart, as well as responses to standard epileptic drugs and novel compounds. The interplay of human and mouse genetics has been essential to our understanding of the role of sodium channel gene mutations in neurological disorders.

M314 Cas9 RNA-guided nuclease gene editing – rapid disease modeling in mice. *L. M. J. Nutter*[1,2], J. Ellegood[3], M. Gertsenstein[2], A. Flenniken[2,4], R. M. Henkelman[1], C. McKerlie[1,2], J. P. Lerch[1], J. Rossant[1]. 1) The Hospital for Sick Children, Toronto, ON, CA; 2) The Centre for Phenogenomics (TCP), Toronto, ON, CA; 3) Mouse Imaging Centre, Toronto, ON, CA; 4) 4Lunenfeld-Tanenbaum Research Institute, Toronto, ON, CA.

Cas9 RNA-guided nuclease (CRISPR/Cas9) gene editing is revolutionizing genetic modification in many species. The Model Production Core at The Centre for Phenogenomics (TCP) is a national facility serving academia and industry in Canada and internationally. TCP has implemented CRISPR/Cas9 editing in mice to produce knockout mutations for the International Mouse Phenotyping Consortium as well as knockout and point mutant alleles for investigators seeking to model specific human diseases.

We produced models by co-injecting mouse zygotes with target-specific guide RNA(s), Cas9 endonuclease, and when required, a homologous repair template. We have designed and produced multiple lines with disease-associated point mutations for mechanistic and pre-clinical studies as well as knockout mouse lines for both medium- and high-throughput phenotype screens. Our success rate for requested alleles is >80% at >50 different loci in the C57BL/ 6NCrl background. No off-target mutagenesis was detected for 12 different gRNAs in 93 N1 mice for 852 potential off-target loci. However, real-time quantitative PCR for repair template copy number has identified multi-copy insertions in some mice. Thus, while off-target mutagenesis appears to be a very low risk with locus-specific guide RNAs, quality control should include copy number screens for repair templates when they are used.

We set out to compare phenotypes of ES cell-derived and Cas9-derived mutant lines; assess novel mutants for neuroanatomical phenotypes; and produce mouse models of human disease. We produced two *Tox3* knockout mouse lines; one from targeted ES cells and the other using Cas9, C6N-*Tox3*$^{<tm1b(KOMP)Mbp}$*/MbpCrl* and C6N-*Tox3*^{em1Tcp}, respectively. For phenotyping, we intercrossed heterozygotes within each line. Homozygotes for each of these alleles both died before 4 weeks of age and had reduced brain volume. We also used Cas9 to produce several knockout mouse lines in genes associated with Autism Spectrum Disorder as part of a medium-throughput screen to identify neuroanatomical phenotypes. We show that many of these mutants had both *in vivo* behavioural phenotypes as well as brain dysmorphology compared to wild type. Finally, we have produced and delivered several disease-associated point mutants that are currently under study. One of these presents with seizures, a clinical sign observed in the patient population. This mutant is now being used to assess hyperexcitability in cortical neurons as well as in a drug screen.

In conclusion, Cas9 mutagenesis is an efficient way to produce mutant mouse lines at high throughput with specific gene mutations for modeling human disease.

POPULATION, EVOLUTIONARY, AND QUANTITATIVE GENETICS MEETING

Plenary and Platform Session Abstracts

Population, Evolutionary, And Quantitative Genetics Meeting

SCHEDULE AT-A-GLANCE

Wednesday, July 13		
2:00pm-9:30pm	Speaker Ready Room Open	Hall of Cities - Anaheim
7:00pm-9:00pm	Scientific Session: PEQG Keynote 1	Crystal Ballroom
9:00pm-11:00pm	Opening Mixer with Exhibits	Cypress Ballroom
Thursday, July 14		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities -Anaheim
7:45am-10:00am	Genetics and Determinants of Health Joint Plenary Session	Palms Ballroom
8:00am-4:00pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:30am-12:30pm	Scientific Session: Natural Selection and Adaptation	Crystal Ballroom
12:30pm-1:30pm	Mentoring Roundtables #1	North Tower - Harbor Beach
12:30pm-1:30pm	Speaking Up for Genetics and Model Organism Research	Crystal Ballroom H
1:30pm-3:30pm	Poster Presentations 1:30pm-2:30pm: Even-numbered poster 2:30pm-3:30pm: Odd-numbered posters	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center and Job Fair	Cypress Ballroom 1C
4:00pm-6:00pm	Scientific Session: James F. Crow Symposium	Crystal Ballroom
4:00pm-6:00pm	Plenary Session and Workshop for Undergraduate Researchers	North Tower - Sawgrass
7:45pm-9:45pm	Scientific Session: PEQG Keynote 2	Crystal Ballroom
10:00pm-11:30pm	*Science Cafe Event	Palms Ballroom Sabal
Friday, July 15		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-9:30am	Scientific Session: Cryptic Variation and Robustness	Crystal Ballroom
8:00am-4:30pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:00am-12:00pm	Scientific Session: Mutation & Recombination	Crystal Ballroom
12:00pm-1:30pm	*Editor's Panel Discussion and Roundtable	North Tower - Harbor Beach
1:30pm-3:30pm	Poster Presentations 1:30pm-2:10pm: "A" poster authors present 2:10pm-2:50pm: "B" poster authors present 2:50pm-3:30pm: "C" poster authors present	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center	Cypress Ballroom 1C
2:00pm-2:45pm	GeneticsCareers Workshop - Nailing the Job Talk	Cypress Ballroom 1B
4:00pm-6:00pm	Scientific Session: Molecular Evolution	Crystal Ballroom
6:00pm-7:30pm	*Women in Genetics Panel and Networking	North Tower - Harbor Beach
7:30pm-9:30pm	Development and Evolution Joint Plenary Session	Palms Ballroom

** Ticketed Event*

#TAGC16

Saturday, July 16		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-10:00am	Workshops: See topics and descriptions in the Workshop Section	Multiple locations
8:00am-12:00pm	Exhibits Open	Cypress Ballroom
8:00am-900am	Trainee Bootcamp Workshops: Session 1	North Tower
9:00am-10:00am	Trainee Bootcamp Workshops: Session 2	North Tower
10:00am-12:00pm	Poster Presentations 10:00am-11:00am Odd-numbered posters 11:00am-12:00pm Even-numbered posters	Cypress Ballroom *(Posters must be removed by 1pm)*
10:00am-12:00pm	GeneticsCareers Center	Cypress Ballroom 1C
10:30am-11:15am	GeneticsCareers Workshop	Cypress Ballroom 1B
12:15pm-1:45pm	*Mentoring Roundtables #2	North Tower - Harbor Beach
1:45pm-3:45pm	Scientific Session: Population Genetics	Crystal Ballroom
4:00pm-6:00pm	Scientific Session: Complex Trait Evolution	Crystal Ballroom
7:30pm-9:30pm	Scientific Session: PEQG Keynote 3	Crystal Ballroom
Sunday, July 17		
8:00am-10:00am	Scientific Session: Epistasis	Crystal Ballroom
10:30am-12:30pm	Technology and its Application Joint Plenary Session	Palms Ballroom

** Ticketed Event*

P315 Evolution of gene expression: from mutation to polymorphism to divergence. *Patricia J. Wittkopp*. Univ Michigan, Ann Arbor, MI.

Gene expression is controlled by a complex network of molecular interactions. Genetic changes that alter this network contribute to phenotypic differences within and between species. To better understand how mutation and selection affect the evolution of gene expression, we have investigated properties of new mutations that create regulatory variation in *Saccharomyces cerevisiae* and compared their effects to those of regulatory variants segregating in the wild. Comparisons between these two datasets are providing are being used to make inferences about how regulatory networks evolve. Genetic variation segregating within a species reflects the combined activities of mutation, selection, and genetic drift. In the absence of selection, polymorphisms are expected to be a random subset of new mutations; thus, comparing the effects of polymorphisms and new mutations provides a test for selection. When evidence of selection exists, such comparisons can identify properties of mutations that are most likely to persist in natural populations. We have been investigating how mutation and selection contribute to variation in cis- and trans-regulatory sequences controlling gene expression by empirically determining the effects of new mutations and polymorphisms in *Saccharomyces cerevisiae.*.

P316 The hidden complexity of Mendelian inheritance in natural populations. *J. Schacherer*[1], J. Hou[1], A. Sigwalt[1], D. Pflieger[1], J. Peter[1], T. Fournier[1], M. Dunham[2]. 1) University of Strasbourg, Strasbourg, FR; 2) University of Washington, Seattle, US.

More than a century after the rediscovery of Mendel's law, we still understand relatively little about the spectrum of the genetic complexity of traits. This lack of knowledge constitutes a gap in our basic understanding of how genotype determines phenotype. To obtain a comprehensive picture of the genetic complexity spectrum, we performed a species-wide survey of the inheritance patterns of a large number of traits using the *Saccharomyces cerevisiae* yeast model system. We performed systematic crosses between the laboratory strain (Σ1278b) and 41 diverse natural isolates covering a high genetic divergence. We quantitatively measured the fitness variation in the offspring for each cross across a large panel of stress conditions. By analyzing the distribution and the segregation of the more than 1,100 cross/trait combinations, we obtained the first estimation of the number of cases showing a Mendelian inheritance pattern within a natural population. Our results showed that 8.9% (98 out of 1,105 cases) are monogenic traits with Mendelian inheritance. Using mapping strategies combined with next-generation sequencing, we then precisely identified the genomic loci involved in the observed Mendelian traits. Interestingly, the identified Mendelian loci are not randomly distributed but rather genetically linked, leading to co-segregating phenotypes. In addition, we traced the phenotypic effect and genetic inheritance of one monogenic allele we characterized across multiple genetic backgrounds. Interestingly, increased genetic complexity was observed in 30% of the cases, with significant deviations from the Mendelian expectation. Our results clearly show that monogenic mutations might have different phenotypic outcomes with different inheritance patterns ranging from a Mendelian to a complex inheritance, including cases with intermediate levels of complexity. Overall, these data and analysis provide, for the first time, a comprehensive picture of natural genetic variants contributing to the onset of Mendelian traits, and illustrate the extent to which different genetic background could impact the inheritance pattern of these traits in a continuous way in terms of complexity. In fact, when taking into account the natural population diversity, the hidden complexity of traits could be substantial, perplexing the phenotypic predictability even for simple Mendelian traits.

P317 Parallel Gene Expression Differences between Low and High Latitude Populations of two *Drosophila* species. *Li Zhao*, David J. Begun. Department of Evolution and Ecology, UC Davis, Davis, CA.

Gene expression variation within species is relatively common, however, the role of natural selection in the maintenance of this variation is poorly understood. Here we study geographic differences in gene expression in recently established low and high latitude populations of two closely related species of *Drosophila, Drosophila melanogaster* and *D. simulans*, to determine whether the two species show similar patterns of population differentiation, consistent with a role for spatially varying selection in maintaining gene expression variation. We observed a significant excess of genes exhibiting differential expression in both species, consistent with parallel adaptation to heterogeneous environments. Moreover, the majority of genes showing parallel expression differentiation showed the same direction of differential expression in the two species and the magnitudes of expression differences between high and low latitude populations were correlated across species, further bolstering the conclusion that parallelism for expression phenotypes results from spatially varying selection. Comparison of inter-population sequence differentiation and expression differentiation suggests that cis-acting variants play a role in geographic expression differentiation.

P318 *Trans* regulatory architecture of genetic transcriptome variation from 1,000 yeast individuals. *Frank W. Albert*[1,2], Joshua S. Bloom[1], Jake Siegel[1], Laura Day[1], Leonid Kruglyak[1,3]. 1) University of California, Los Angeles, CA; 2) University of Minnesota, Minneapolis, MN; 3) Howard Hughes Medical Institute, Los Angeles, CA.

* FWA and JSB contributed equally

Regulatory variation is an important source of genetic variation for many traits and can be identified as "expression quantitative trait loci" (eQTL). To date, all eQTL studies have been hampered by low statistical power due to limited sample sizes. As a consequence, the full extent and nature of regulatory variation remains unknown.

We have addressed this limitation in the yeast *Saccharomyces cerevisiae*. We used mRNA sequencing to profile gene expression in more than 1,000 recombinant individuals generated from a cross between two yeast isolates. The statistical power of this dataset is high enough to map thousands of previously "missing" eQTL that together account for ~80% of the heritability of gene expression. Thus, our data provide a nearly exhaustive view of how genetic variation influences the transcriptome.

We identified 34,318 eQTL for 6,210 transcripts. A typical transcript is influenced by a median of 6 and up to 20 eQTL, several fold more than

previously seen. While 43% of all genes had a local eQTL that is located close to the gene, most eQTL are located elsewhere in the genome and influence gene expression in *trans*. The aggregate effect of the *trans* eQTL was larger than that of the local eQTL, illustrating the importance of *trans* acting variation.

Rather than appearing randomly across the genome, the newly discovered *trans* eQTL were highly structured, such that the vast majority fell into one of 111 hotspot regions that each affect the expression of many genes. Some of these hotspots have extraordinarily wide-reaching effects and influence thousands of transcripts across all cellular processes, while others specifically influence certain pathways. By combining information from all genes that map to a given hotspot, we can fine-map the causal hotspot location with high precision, in 26 cases to single-gene resolution of less than 1.5 kb. This permits – for the first time – a systematic and unbiased analysis of the types of genes that act as *trans* eQTL. *Trans*-acting variation generates structure in the yeast transcriptome such that groups of genes are affected by multiple eQTL in a combinatorial fashion. Finally, despite our high statistical power, many local eQTL did not act as *trans* eQTL for other genes. This might indicate that the expression changes these eQTL cause at their local genes do not further affect cellular physiology, at least not in ways that are reflected in the transcriptome. This raises important questions about the characteristics of genes where local regulatory variation does have cellular and phenotypic consequences.

P319 The genomic basis of environmental adaptation in house mice. *M. Phifer-Rixey*[1], K. Bi[1], K. G. Ferris[1], M. J. Sheehan[2], D. Lin[1], S. M. Keeble[3], J. M. Good[3], M. W. Nachman[1]. 1) UC Berkeley, Berkeley, CA; 2) Cornell University, Ithaca, NY; 3) University of Montana, Missoula, Missoula, MT.

Understanding the genetic basis of adaptation is a fundamental goal of evolutionary biology. Many of the first examples connecting adaptive phenotypes to specific genetic variants implicated single loci of large effect (e.g., *Mc1r* in rock pocket mice, *Eda* in sticklebacks, FRIGIDA in *Arabidopsis thaliana*). Nevertheless, the majority of adaptive phenotypes are likely to be complex and contingent on interactions between many genes and the environment. Progress in identifying the genetic basis of complex traits has been more limited. The incredibly successful colonization of the Americas by house mice offers a unique opportunity to study environmental adaptation, complex traits, and population genetics in an invasive species that is also the premier mammalian medical model for humans. Despite arriving in the Americas relatively recently with European settlers, the house mouse (*Mus musculus domesticus*) has expanded into a wide variety of climates and habitats. We sampled five populations of house mice in eastern North America distributed along a latitudinal gradient and established laboratory colonies of mice from the ends of this transect. Using genome and exome scans, gene expression studies, and phenotypic analyses in the field and in the lab, we found strong evidence of environmental adaptation. We identified several phenotypes tied to fitness that differ among the most extreme populations, and those differences persist in the lab over several generations indicating that they have a genetic basis. We also identified specific genes as candidates for environmental adaptation. Importantly, most candidate SNPs are non-coding or synonymous, indicating that regulatory evolution has been key to the success of house mice in varied climates.

P320 Parallel selective sweeps of selfish *Segregation Distorter* complexes in African and European *Drosophila melanogaster* populations. *A. M. Larracuente*, C. Brand, D. C. Presgraves. University of Rochester, Rochester, NY.

Segregation distorters are selfish genetic elements that unfairly achieve biased transmission through the germline. Found across a wide variety of taxa including fungi, plants, insects and mammals, segregation distorters can rapidly increase in frequency in natural populations and trigger the evolution of suppressors across the genome. One of the best-studied male segregation distorters is the autosomal *Segregation Distorter* (*SD*) gene complex of *Drosophila melanogaster*. Males heterozygous for *SD* and a wild type chromosome transmit *SD* to over >95% of their progeny, whereas heterozygous females transmit *SD* fairly to 50% of their progeny. *SD* is found at frequencies of ~1-5% in natural populations across the globe. Cosmopolitan *SD* chromosomes involve a main driving locus on chromosome *2L—Sd-RanGAP—*and several upward enhancers that strengthen drive on both *2L* and *2R*. *SD* chromosomes often tighten linkage among enhancers and *Sd-RanGAP* via the recruitment of chromosomal inversions that suppress recombination. Suppressed recombination prevents the distorter from recombining onto a sensitive target background and generating self-distorting “suicide” genotypes. African and European *SD* chromosomes appear molecularly different—they do not share inversions and we find that suppressors of European *SD* chromosomes do not suppress African *SD* chromosomes. While inversions provide short-term benefits to *SD*, the reduced recombination entails long-term costs due to the associated reduced efficacy of natural selection. To study the consequences of suppressed recombination, we performed whole-chromosome population genomics analyses of *SD* chromosomes sampled from African and European populations. We Illumina-sequenced 10 haploid embryos from Zambia and 10 adults from France. We find a dearth of nucleotide variation on French *SD* chromosomes that begins at *Sd-RanGAP* on chromosome *2L*, spans the centromere and extends 4 Mb into chromosome *2R*. In contrast, we find a more dramatic dearth of nucleotide variation on Zambian *SD* chromosomes that begins at *Sd-RanGAP* on chromosome *2L,* spans the centromere and extends for ~22.5 Mb across chromosome *2R*— a massive sweep signal that suggests very recent and strong selection. We therefore detect a signature of parallel sweeps of independent multi-locus selfish *SD* complexes in Africa and France. Taken together, differences in (*i*) the structure of the selective sweeps, (*ii*) the chromosomal inversions involved, and (*iii*) responses to genetic suppressors of *SD*, imply that Zambian and French *SD* gene complexes have functionally diverged from one another.

P321 Diverse genetic architectures lead to the same cryptic phenotype in a yeast cross. *Ian Ehrenreich*, Matthew Taylor, Joann Phan, Jonathan Lee, Madelyn McCadden. University of Southern California, Los Angeles, CA.

Cryptic genetic variants that do not typically influence traits can interact epistatically with each other and mutations to cause unexpected phenotypes. To improve understanding of the genetic architectures and molecular mechanisms that underlie these interactions, we

comprehensively dissected the genetic bases of 17 independent instances of the same cryptic colony phenotype in a yeast cross. In eight cases, the phenotype resulted from an interaction between a spontaneous mutation and one or more cryptic variants. The total number and specific identities of the detected cryptic variants depended on which gene was mutated. In the nine remaining cases, the phenotype arose without a mutation due to higher-order interactions only involving cryptic variants. These mutation-independent cases fell into two classes, which could be distinguished by the occurrence of recombination within a specific ~1.3 kb genomic interval in the promoter of a required cell surface protein. Our results may be relevant to other species and disease, as most of the mutations and cryptic variants identified in our study reside in components of the evolutionarily conserved and oncogenic Ras signaling pathway.

P322 Genome-wide selection component analysis in a wild pedigreed population of the Florida Scrub-Jay. *Andrew G. Clark*[1], Elissa Cosgrove[1], Huijie Feng[1], Ishaan Jhaveri[1], Ashish Akshat[1], Reed Bowman[2], John W. Fitzpatrick[3], Nancy Chen[3,4]. 1) Cornell Univ, Ithaca, NY; 2) Archbold Biological Station, Venus, FL; 3) Cornell Lab of Ornithology, Ithaca, NY; 4) University of California, Davis, CA.

Analysis of contemporary evolution is the only way to directly test many fundamental questions in evolutionary biology, but we usually lack the combined phenotypic and genomic data over time in natural populations required for such studies. In addition, most current approaches for inferring natural selection do not have the opportunity to incorporate the additional power gained from access to a full pedigree. Here, we study short-term selection using a 25-year genomic, phenotypic, and pedigree dataset in the Florida Scrub-Jay (*Aphelocoma coerulescens*), an iconic species on the U. S. Endangered Species List that has drastically decreased in number during the past half-century. A population of Florida Scrub-Jays at Archbold Biological Station has been studied intensively since 1969, providing a 12-generation pedigree among the most accurate and extensive for any wild vertebrate species. For all locally recruited birds in the population, we have full records of individual lifespans, annual fecundity, and lifetime fitness; similar records exist for immigrant breeders except for exact lifespan. We sequenced and assembled the Florida Scrub-Jay genome and used custom Illumina Beadchips to genotype every individual in our study population over the past two decades (3,838 individuals total) at 15,416 genome-wide SNPs. We used gene dropping to explicitly sample gametes in each generation on the known pedigree and asked whether the observed allele frequency dynamics of each SNP were consistent with a pure drift process. Numerous SNPs departed significantly from the null model and showed frequency dynamics consistent with perturbation by selection. We then tested for selection acting on specific life-cycle stages by tailoring hierarchical selection component analysis to take full advantage of exhaustive population sampling. We identified a number of loci that clearly exhibited male gametic selection, sexual selection, and viability selection. By combining sensitive pedigree-based inferences of net selection with fine-scale dissection of selection components, this study provides a detailed assessment of the role of selection in perturbing allele frequency dynamics in a rapidly declining population. Results suggest a role of selection in maintaining variation even in the face of population decline, and may help guide conservation efforts.

P323 *Drosophila melanogaster*-specific genes rapidly evolved strong fitness effects. *Nicholas VanKuren*, Manyuan Long. University of Chicago, Chicago, IL.

Genome regions that are highly conserved between distantly-related organisms are assumed to have important fitness effects that have been preserved by natural selection over long periods of time. This assumption has led to two others: 1) the evolution of important genome regions is slow and 2) new genome regions do not have significant fitness effects. However, new genetic variants are precisely those that cause individuals to vary and thus drive evolution. A complete understanding of how and how rapidly genomes and fitnesses change thus depends on characterization of the evolutionary forces that act on and the fitness effects of new variants.

We investigated these characteristics of a set of 27 *Drosophila melanogaster*-specific genes. These genes were formed by duplication and fixed after *D. melanogaster* and *D. simulans* diverged (~2 mya). We screened 12 *D. melanogaster*-specific genes for severe fitness and morphological defects using available mutants and RNA interference (RNAi). Specific and constitutive expression knockdown of 4/12 genes is lethal. That is, significantly fewer (1 gene) or no (3 genes) RNAi flies survive to adulthood. Thus, 1/3 genes rapidly acquired a critical fitness effect. Natural selection appears to have dominated this process, as 50% of *D. melanogaster*-specific genes reside in regions with extremely negative Tajima's *D* and/or Fay and Wu's *H* statistics. These findings suggest that *D. melanogaster*-specific genes were subject to recent and strong selection, and are supported by comparisons of polymorphism and divergence in the surrounding regions. While *D. melanogaster*-specific genes exhibit few signatures of rapid sequence evolution, 37% have accumulated insertion/deletions, recruited additional amino acids, or are chimeric relative to their parent copies. In addition, duplicate pairs rapidly diverged in expression patterns across tissues and developmental times.

We thus provide direct and indirect evidence that *D. melanogaster*-specific genes can rapidly evolve significant fitness effects. Our results support a model in which 1) new genes become distinct from their parents concurrent with their formation, 2) selection immediately acts on new genes to drive them to fixation or purge them from the population, and 3) new gene function and fitness effects are refined during and after fixation. These findings suggest that new genes can play important roles in determining organismal fitness shortly after they are formed and that importance does not require long-term conservation.

P324 Examining the effects of natural selection on linked neutral divergence. *T. N. Phung*, C. D. Huber, K. E. Lohmueller. University of California, Los Angeles, Los Angeles, CA.

A major goal in evolutionary biology is to understand the processes that shape patterns of genetic variation across genomes. One process that has received a lot of attention is natural selection. In particular, numerous studies in a variety of species have shown that neutral genetic diversity (intra-species differences) has been reduced at sites linked to those under selection. However, the effect of selection on neutral sequence divergence (inter-species differences) remains ambiguous. While some empirical studies have reported correlations between

divergence and recombination which is interpreted as empirical evidence for natural selection reducing neutral linked divergence, theoretical arguments argued otherwise, especially for species that have diverged long ago. Here we address these outstanding issues by examining how natural selection has affected divergence between both closely and distantly related species. We show that neutral divergence is negatively correlated with functional content and positively correlated with estimates of background selection from primates, which is a measure of how negative selection affects linked neutral sites. These patterns persist even when comparing humans and mice, species that split 75 million years ago. Coalescent models indicate that background selection can generate these patterns, suggesting that natural selection has affected linked divergence between distantly related species. Our theoretical and simulation results show that even when the contribution of ancestral polymorphism to divergence is small, background selection in the ancestral population can still explain a large proportion of the variance in divergence across the genome. Thus, the view that selection does not affect divergence at linked neutral sites needs to be reconsidered. These findings also suggest that the effects of natural selection affecting linked neutral sites cannot be ignored when studying neutral divergence. Our work has important implications for understanding evolution of genomes and interpreting patterns of genetic variation.

P325 Genome-wide signals of adaptation in mammals and the arms race with viruses. *D. Enard*[1], Arya Iranmehr[2], Dmitri Petrov[1]. 1) Stanford University, Stanford, CA; 2) UCSD, San Diego, CA.

In “The Causes of Evolution” published in 1932, Haldane formulated the hypothesis that pathogens are a major driver of hosts' evolution. Adaptation against pathogens has since then been studied mostly in genes specialized in the immune response.

Yet pathogens, and in particular viruses, interact with hundreds to thousands of host proteins that are not specialized in the immune response. Whether adaptation to viruses has typically involved only specialized immune, antiviral proteins or has affected a broad array of proteins is unknown. Here, we analyze patterns of adaptation in thousands of virus-interacting proteins at different evolutionary time scales ranging from the whole mammalian phylogenetic tree to the past 10,000 years of human evolution.

Over long evolutionary time scales, we find that virus-interacting proteins have experienced strongly increased rates of adaptation across the entire mammalian phylogeny, from primates to rodents to bats and elephants. In the human branch, we estimate that 30% of all adaptive protein changes since divergence with chimpanzees were driven by viruses.

During recent human evolution, we find that virus-interacting proteins exhibit a very strong excess of partial and complete sweeps. Importantly, recent human adaptation against viruses is driven not only by immune proteins but instead by proteins from a wide range of host cellular functions.

Our results show that the influence of pathogens extends well beyond the classic cases of specialized immune proteins, and that pathogens are a major driver of adaptation at the whole genome level.

P326 Evolution of gene expression in giant island mice. *Mark Nolte*[1,2], Colin Dewey[3], Bret Payseur[1]. 1) Laboratory of Genetics, University of Wisconsin, Madison, WI; 2) Supported by NHGRI training grant to the Genomic Sciences Training Program 5T32HG002760, Madison, WI; 3) The Department of Biostatistics and Medical Informatics, University of Wisconsin, Madison, WI.

Island colonizing organisms often evolve extreme body sizes. Notable examples of this phenomenon include evolved dwarfism in an extinct insular elephant and hominin, and acquired gigantism in numerous insular rodents. Although the generality of the phenomenon suggests common evolutionary mechanisms, the genetic basis of extreme body size evolution on islands remains poorly understood. A striking case of island gigantism is that observed on Gough Island (GI) where house mice evolved to become the largest wild representatives of their species in just 200 years. To illuminate the genetic basis of metabolic processes contributing to the extreme size of GI mice and to nominate candidate genes involved in gigantism evolution we characterized gene expression evolution in three metabolic organs. A total of 100 RNA-sequencing libraries were constructed for GI mice and a wild-derived strain with size representative of mainland mice (WSB) for five conditions: the liver from embryonic, 2-week, and 4-week old mice, and the gonadal adipose depot and hypothalamus from 4-week old mice. We employed the software package RSEM to align reads and quantify transcripts. Using the R package DESeq2, we detected differential expression between GI and WSB mice at thousands of genes in each condition. The embryonic liver and adipose manifested the most differentially expressed (DE) genes. The adipose exhibited a significant excess of highly expressed genes in large magnitude fold change classes, indicating elevated activity of the pathways constructed by these genes (including metabolically driven cell growth). Monitoring patterns of liver-specific gene expression across three time points enabled grouping of genes with similar temporal expression profiles, shedding light on the evolution of co-regulated genes acting to promote extreme size evolution. In all conditions assayed, we found a significant association between proximal upstream single nucleotide polymorphisms and differential gene expression, suggesting a role for *cis*-regulatory evolution in GI metabolism. We identified 91 DE genes within +/- 3Mb of QTL for body size recently mapped in crosses between GI and WSB mice. A subset of these genes harbor fixed sequence differences between GI and WSB mice in potential upstream regulatory regions. This study further establishes the GI mouse as a model for understanding the genetic basis of insular body size evolution and provides candidate genes for potential functional genetic assays that explore variable and abnormal growth and metabolism in vertebrates.

P327 Does the Y-chromosome facilitate sexual dimorphic evolution or constrain autosomal evolution? *I. C. Kutch*, K. M. Fedorka. University of Central Florida, Orlando, FL.

Non-protein coding regions of the Y-chromosome have been shown to influence the expression of hundreds of autosomal and X-linked genes in multiple species. This Y-linked regulatory variation (YRV) may provide the sex-specific variation in gene expression required for the adaptive evolution of sexually dimorphic traits. This requires that YRV exist within natural populations, influence fitness related traits, and be at least in part comprised of additive genetic variation (i.e. selection must be able to shape it). In this study we investigated the ability for selection to

shape YRV using wild caught *Drosophila melanogaster.*

We collected wild *D. melanogaster* from a single locality to investigate the potential for selection to shape YRV. To determine if YRV exists within a population, we introgressed 30 Y-chromosomes into a single genetic background and assayed for variation in immune gene expression. To determine if YRV has any fitness consequences, we then tested these same Y-chromosome lines for differential responses to a real immune challenge. Lastly, we crossed 4 Y-chromosomes into 4 different genetic backgrounds to determine if intra-population YRV was comprised of additive genetic variation.

Our data suggest that intra-population YRV does exist and that this variation is correlated with survival to a bacterial pathogen. Furthermore, the magnitude of the YRV effect was quite large (~42.6% of genetic variation in our experimental lines). However, to our surprise, this variation was comprised entirely of non-additive genetic variation, suggesting that selection may not be able to act on immune-related YRV and may instead act as a significant constraint to the adaptive evolution of insect immune function. The extent to which this non-additive variation is constraining the adaptive evolution of immune response is currently being tested using experimental evolution on more realistic population level variation. Additional traits are also being investigated as it is possible for YRV to manifest differently for different YRV sensitive traits.

P328 The antibiotic-independent evolution of antibiotic resistance. *R. Hershberg*, W. Field, S. Katz. Technion, Israel Institute of Technology, Haifa, IL.

It has been demonstrated that when bacterial colonies are starved, the frequency of antibiotic resistance tends to increase, even in the absence of any antibiotic exposure. We were able to demonstrate that, contrary to widespread assumption, such starvation-induced increases in antibiotic resistance frequencies were not due to increases in mutagenesis. Rather, it appears that specific resistance mutations to a variety of antibiotics are beneficial to bacterial growth under starvation. We could further demonstrate that one such resistance allele that we identified as adaptive under starvation is alarmingly frequent within natural bacterial populations. This antibiotic resistance allele is particularly frequent within-host environments, where on average ~40% of bacteria carry the allele, independent of levels of antibiotic exposure. Our results suggest that the antibiotic-independent fitness effects of resistance alleles may strongly affect the frequency with which resistance segregates within natural bacterial populations.

P329 Dynamics and feasibility of CRISPR/Cas9-mediated gene drives in natural populations. *P. Messer*, A. Clark, R. Unckless. Cornell University, Ithaca, NY.

The idea of driving an engineered gene to fixation in a population is more than 40 years old. Its potential applications are ambitious and widely varied, including the eradication of disease vectors, the control of pest species, and the preservation of endangered species from extinction. So far, all proposed drive mechanisms have fallen short of these goals. The recently developed CRISPR/Cas9-mediated gene drive (CMGD) now promises a mechanism for quickly driving genetically modified alleles to high frequency in a population regardless of their fitness cost. However, we still lack a comprehensive understanding of the population dynamics of CMGD and are therefore unable to predict its spread in natural populations in the face of potential resistance. Here we develop a comprehensive population-genetic model of CMGD to evaluate the feasibility of this process in natural populations. We specifically study the probability that resistance evolves against a gene drive from alleles that (i) are already present as standing genetic variation when the driver allele is introduced into the population, (ii) arise by de novo mutations while the driver allele is spreading through the population, or (iii) are created by the drive itself when double strand break repair results in mutated target sites that can no longer be recognized by the driver's guide RNA. We use these results to analyze how different drive strategies (breaking a gene versus editing/inserting a gene) are expected to influence the probability of resistance due to different fitness costs of resistance alleles. We finally discuss how our results can be used to identify strategies with reduced potential for resistance evolution in order to facilitate a successful drive, as well as approaches that would promote resistance as a possible mechanism for controlling the spread of a drive.

P330 Legacy of James Crow. *Daniel Hartl*. Harvard University, Cambridge, MA.

no abstract submitted

P331 Estimating Jacquard's general model of relatedness from population genomic data. *Matthew S. Ackerman*[1], Parul Johri[1], Ken Spitze[1], Sen Xu[2], Thomas Doak[1], Kimberly Young[1], Michael Lynch[1]. 1) Indiana University, Bloomington, IN; 2) The University of Texas at Arlington.

Population structure is described by genotypic correlation coefficients between individuals, the most basic of which are Jacquard's nine condensed coefficients. These correlation coefficients form the basis of quantitative genetic analysis, and geneticists perform experimental crosses or pedigree analysis in order to recover them. Previously molecular techniques could only recover four of these coefficients, but we can recover seven coefficients using biallelic loci and a maximum likelihood method. This approach should allow for more robust estimation of the components of genetic variance from population genomic data.

Simulations show that the procedure is nearly unbiased, even at the minimally informative 3x coverage, and that errors in five of the seven coefficients are statistically uncorrelated. The remaining two coefficients have a negative correlation of errors, but their sum provides an unbiased assessment of the overall correlation of heterozygosity between two individuals. These methods are applied to four populations of the freshwater crustacean *Daphnia pulex,* and revealed several interesting characteristics that are not apparent with other techniques. The use of a maximum likelihood method also allows us to assess statistical significance of relationships using a log likelihood ratio test, and we find statistically significant negative estimates of many of these pair-wise relatedness coefficients. Although these coefficients are traditionally regarded as measure of the probability of identity, which cannot be negative, we treat them as measures of conditional association, which can

be negative. These methods are implemented as part of the mapgd package (maximum likelihood analysis of population genomic data) available from https://github.com/LynchLab/MAPGD.

P332 Dynamics of seasonal adaptation in *Drosophila melanogaster*. *Emily L. Behrman*[1], Alan O. Bergland[2,3], Dmitri A. Petrov[2], Paul S. Schmidt[1]. 1) University of Pennsylvania, Philadelphia, PA; 2) Stanford University, Stanford, CA; 3) University of Virginia, Charlottesville, VA.

The rate and tempo of adaptation is a fundamental question in evolutionary biology. Evolution is generally considered to be a gradual process and it is unclear if populations can adapt rapidly to environmental selection pressures. Temporal changes in environmental selection pressures across seasonal time may results in traits associated with fitness to vary as a function of season; this may result in seasonal oscillations of traits associated with high fitness that are favorable for reproduction and population expansion (summer) and those that are not and must be endured (winter). Long-term datasets can be used to investigate the association between environmental variance, fitness-related phenotypes and changing allele frequencies that are driven by natural selection and therefore reflect the adaptive process. This provides a long-term empirical investigation of seasonal life history patterns in natural populations of *Drosophila melanogaster* using isofemale lines collected from a temperate orchard over five consecutive years. There are pronounced and repeated changes in phenotypic performance based on temporal change in the genetic composition of the population from spring to fall; the variance in traits across seasonal time is equivalent to measurements across broad geographic distances over 20º latitude. Changes in both the phenotypic and genetic correlations from spring to fall counters the basic assumption of stable covariance over time and suggests that selection acts rapidly to change the genetic architecture of a population. Whole-genome resequencing of the population across seasonal time reveals thousands of SNPs that cycle as a function of season. Seasonal SNPs are associated with the fitness traits are identified based on genome-wide association and their function is evaluated. Environmental data from the study site is used to identify environmental parameters that correlate with the direction and magnitude of observed seasonal phenotypic and allele variation. The results suggest that selection acts in a rapid fashion with cyclic alternating selection by different environmental parameters between winter and summer phenotypes and genes. The fluctuating-stabilizing selection created by alternating selection pressures across seasons may be integral in the maintenance of phenotypic and genetic variation in natural populations.

P333 The fragile Y hypothesis: The role of Y aneuploidy in the evolution of sex chromosomes and genome architecture. *Heath Blackmon*[1], Jeffery Demuth[2]. 1) University of Minnesota, Saint Paul, MN; 2) University of Texas - Arlington, Arlington, TX.

Y-chromosome losses leading to XO sex chromosome systems are common in many male heterogametic groups (approximately 15% of examined species). However despite a clear understanding of how Y-chromosomes are expected to decay our understanding of why some lineages frequently lose Y-chromosomes while others do not is limited. The fragile Y hypothesis proposes that in species with chiasmatic meiosis the rate of Y-chromosome aneuploidy and the size of the recombining region have a negative correlation. The fragile Y hypothesis provides a number of novel insights not possible under traditional models of sex chromosome evolution. Specifically, as the recombining region of the sex chromosomes shrinks increased rates of Y aneuploidy will impose positive selection for 1) gene movement off the Y; 2) translocations and fusions which expand the recombining region; and 3) alternative meiotic segregation mechanisms (achiasmatic or asynaptic male meiosis). We have built a time-calibrated phylogeny for more than 1,000 beetles and have applied comparative methods to understand the tempo and mode of sex chromosome evolution in beetles with chiasmatic and achiasmatic male meiosis. We show that beetles with chiasmatic meiosis lose Y-chromosomes more frequently and that Y chromosome loss is associated with species that have on average smaller recombining regions. Likewise limited data from mammals is also consistent with Y aneuploidy being an important force in the evolution of sex chromosomes and meiotic mechanisms. This hypothesis and our analyses raise doubts about the long-term stability of the human Y-chromosome despite recent evidence for stable gene content in older nonrecombining regions.

P334 Using network theory to infer and analyze population structure from genetic data. *G. Greenbaum*[1], A. R. Templeton[2,3], S. Bar-David[1]. 1) Ben-Gurion Univ., Midreshet Ben-Gurion, Israel; 2) Washington Univ., St. Louis, MO; 3) Haifa Univ., Haif, Israel.

Clustering individuals to subpopulations based on genetic data has become commonplace in many population genetic studies. Inference of population structure is most often done by applying model-based approaches, for example as implemented in the program STRUCTURE, aided by visualization using distance-based approaches such as multidimensional scaling. While existing distance-based approaches suffer from a lack of statistical rigor, model-based approaches entail often unrealistic assumptions of prior conditions, such as that the subpopulations are at Hardy-Weinberg equilibria. We present a novel distance-based approach for inference of population structure using genetic data by defining population structure using network theory terminology and methodologies. A *network* is constructed from a pairwise genetic-similarity matrix of all sampled individuals. The *community partition*, a partition of the network to dense subgraphs, is equated with population structure, i.e. partition to subpopulations. Furthermore, by applying a threshold for removal of weak connections from the network, we can explore the hierarchical structure of the population. The statistical significance of each hierarchical level of structure can be estimated using permutation tests and evaluation of the partition's *modularity*, a network measure for the quality of community partitions. In order to further characterize population structure, we formulate the *Strength of Association* (SA), the strength in which each individual is associated with its assigned community. We develop the *Strength of Association Distribution* (SAD) analysis, in which the SA distributions are interpreted as isolation and gene flow patterns between the subpopulations. We use both simulated data and real data of 11 human groups, extracted from the HapMap project, to demonstrate the applicability of our method. With the human data, the method detected three statistically significant hierarchical levels, corresponding to African/none-African, African/Indo-European/East-Asian, and fine-scale divisions of the population. SAD analysis showed differences in gene flow patterns between subgroups, for example the African-American and Masai groups showed lower association to the African subpopulation, but evidence of more recent gene flow was observed in the African-American SAD. The approach presented here

provides a novel, computationally efficient, model-free method for inference of population structure that does not entail *a priori* assumptions. The method is implemented in the software NetStruct (available at https://giligreenbaum.wordpress.com/software).

P335 Molecular variation across populations of a widespread North American firefly reveals selection on luciferase but not opsins. *Sarah E. Sander*[1], Kathrin F. Stanger-Hall[2], David W. Hall[2]. 1) Cornell University, Ithaca, NY; 2) University of Georgia, Athens, GA.

Why are animal signals so diverse? This question has received intense scrutiny, and both natural and sexual selection have been implicated as important drivers of signal diversity. Under the framework of sensory drive natural selection is expected to favor signals and receptors that maximize signal detection. A corollary of this is that the genes underlying both signal production and reception should show evidence of selection as they evolve to maximize detection. Fireflies are an excellent system in which to study signal and receptor evolution because the primary genes underlying each are known. Fireflies vary in their signal color, but color is not important for female choice, implying that natural selection for detection is the primary driver of color evolution. Sequence variation in luciferase, the enzyme that catalyzes the light reaction, is thought to cause the color differences between species. Likewise, sequence variation in opsins, the protein components of visual pigments, may underlie the variation in visual sensitivity, which has been shown to match signal color, across species. Here we tested the hypothesis that light color is due to selection acting on luciferase and the resulting predictions that (1) sequence variation in luciferase correlates with observed variation in signal color, (2) sequence variation in opsins correlates with observed variation in signal color (and inferred visual sensitivity), and (3) signatures of selection are evident at these two loci. To do this, we sequenced the luciferase and opsin genes of 192 individuals from 12 populations of the North American firefly, *Photinus pyralis*. We also sequenced the *cytochrome oxidase I* (*COI*) locus and genotyped individuals at 716 single nucleotide polymorphisms (SNPs) using triple digest restriction site associated DNA sequencing (3RAD). We tested for selection while accounting for population structure by comparing differentiation (Fst) at SNPs in luciferase and opsins to the distribution of Fst for the genome-wide 3RAD SNPs. We found no variation at the amino acid level in either luciferase or opsins despite the population differences in signal color. However, silent variation in luciferase, but not opsins, shows high levels of differentiation among populations, strongly suggesting that selection is acting at this locus. The absence of protein variation rejects the paradigm that variation in light color is solely due to variation in luciferase sequence in *P. pyralis*. Instead, natural selection appears to target regulatory or other non-coding variants. If natural selection targets light emission color, our work suggests that either non-coding luciferase variation directly alters emission color through an unknown mechanism, or changes at another gene are correlated with changes in non-coding variants in luciferase.

P336 Fitness pleiotropy and the phenotypic basis of adaptation in experimentally evolving yeast. *S. Venkataram*, Y. Li, A. Agarwala, B. Dunn, D. Fisher, D. Petrov, G. Sherlock. Stanford University, Stanford, CA.

Adaptive mutations drive much of the evolutionary diversity observed in nature. Understanding the relationship between genotype, phenotype, and fitness in adaptive mutations is therefore essential to understanding adaptation and evolutionary processes in general. As fitness is dependent on the environment, it is necessary to study the fitness of adaptive mutations across a range of conditions to characterize fitness pleiotropy. We recently developed a DNA barcoding technology to quantify the fitness effects of ~4,800 independently evolved yeast clones. Each clone contains on average a single adaptive mutation and was isolated from an experimentally evolving population under glucose-limited batch culture conditions. We selected several hundred clones possessing adaptive mutations and performed whole-genome sequencing to comprehensively characterize the genetic basis of adaptation-driving mutations in this low-glucose condition and build a genotype-fitness map. In this work, we used this DNA barcode technology and our previously identified adaptation-driving mutations to study both fitness pleiotropy and understand how these mutations affect cell physiology. We re-measured fitness for all 4,800 independent clones under varying growth conditions, where we systematically varied either the amount of time the cells spend in exponential growth or stationary phase within each batch growth cycle. We found strong evidence of fitness pleiotropy across both sets of experiments, as well as instances of antagonistic pleiotropy where many of our adaptive clones became deleterious if they spend too long in stationary phase between batch culture cycles. In addition, fitness pleiotropy was dependent on both the gene and in some cases the mutation type (i.e. missense, nonsense or frameshift) of the specific adaptive mutation. Our sample size and ability to isolate single adaptive events gives us confidence that all of these results are driven by pleiotropy and not by passenger mutations in these strains. Our findings also showed that all of our adaptive mutations modulated multiple phases of the yeast growth cycle, including the exponential and stationary growth phases. We validated our results using detailed physiological studies measuring the growth of the adapted strains in monoculture and growth when competed against the ancestral strain. Surprisingly, our adaptive mutations appeared to generate antagonistic phenotypic effects even within the exponential growth phase, where many adaptive mutations grew more slowly than the wild-type clone early in exponential growth but grew faster than wild-type late in exponential phase. Our results highlight the complex physiological changes that underlie even single adaptive events, and suggest that the genotype-phenotype-fitness map can be modulated by even slight changes in the environment.

P337 Systems genetics for industry: combining QTL mapping, GWAS and RNA sequencing to improve bone strength in laying hens. *D. J. de Koning*[1,2], H. A. McCormack[2], B. Raymond[1], A. M. Johansson[1], R. Preisinger[3], M. Schmutz[3], I. C. Dunn[2]. 1) Swedish University of Agricultural Sciences, Uppsala, Sweden; 2) The Roslin Institute and R(D)SVS, University of Edinburgh, UK; 3) Lohmann Tierzucht GmbH, Cuxhaven, Germany.

Bone fractures are a major welfare problem in laying hens. In order to determine the potential to selectively breed for bone strength, divergent selection lines were established in the nineties from a commercial breeding line of White Leghorns. Bishop et al (2000) showed a heritability of 0.40 for a bone index that combined breaking strength from different bones. Subsequently an F2 was created from G9 of the selection lines and a quantitative trait locus (QTL) with a large effect was mapped on Chromosome 1 (Dunn et al., 2007).

In this study, we refined the QTL in the F2 by adding SNP markers. We then tested the SNPs from the refined region on the 2006 generation of

the White Leghorn line from which the F2 was derived after 9 generations of divergent selection. Subsequently, birds from the 2011 generation were genotyped for the QTL haplotype and birds with alternative QTL haplotypes were selected for qPCR of candidate genes and RNA sequencing.

For the SNPs with the most consistent effects, the tibial breaking strength for the alternative haplotypes in the 2006 population was 200.4 vs 218.1 Newtons ($p<0.002$).

The RNA sequencing showed strong differential expression for a single candidate gene in the QTL region with clear allelic imbalance suggesting a *cis*-acting effect within the candidate gene.

Subsequently, a full GWAS was carried out on ~750 birds from the 2006 generation using the Affymetrix high-density SNP chip. After quality control around 225K SNP remained for a mixed model GWAS using GenAbel. Additional QTL were identified on chromosomes 3, 8 and 16. The most significant QTL on chromosome 8 had an additive effect of 20 Newton. At present we are validating these SNP effects in commercial crossbred layers from different companies, with different feeding regimes and in different housing systems. This will be the ultimate test for the utility of these genetic markers to improve bone strength under practical farming conditions.

P338 Effect of Genetic Architecture and Sample Size on the Accuracy of Genomic Prediction of Complex Traits. *Fabio Morgante*[1], Wen Huang[1], Christian Maltecca[2], Trudy Mackay[1]. 1) Program in Genetics, Department of Biological Sciences, and WM Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC; 2) Program in Genetics, and Department of Animal Science, North Carolina State University, Raleigh, NC.

Understanding the genetic architecture of complex traits is a fundamental aim of many branches of genetics. Genome wide association studies (GWAS) have been successful at identifying some loci affecting complex traits. However, those loci account for just a very small proportion of the total genetic variation, the widely known phenomenon called “missing heritability”. As a result, prediction of phenotypes based on the loci uncovered by GWAS has had low accuracy. Methods that regress phenotypes on hundreds of thousands of markers concurrently (whole genome regression, WGR,) may be able to capture a consistent amount of the genetic variation of complex traits and, thereby, increase predictive ability. However, most WGR methods assume strict additivity and traits that are potentially affected by non-additive (epistatic) interactions have not shown any gain in predictive ability. Here, we investigated the effect of sample size and genetic architecture on the accuracy of genomic prediction of complex traits. We used G-BLUP methodology and the unique resource of the *Drosophila* Genetic Reference Panel (DGRP), a collection of 205 fully sequenced inbred lines that have been phenotyped for many quantitative traits, as well as simulated data. The results show that the accuracy of prediction increases as the sample size increases, conditional on the genetic architecture of the trait examined being taken into account in the statistical model used. In particular, strict additive models in presence of an epistatic component being part of the genetic architecture may fail completely, even if we have knowledge about the true variants affecting that trait and no matter the size of the sample. However, when an epistatic model is fitted, the accuracy of prediction rises, even with small sample size. In summary, this study shows the importance of accounting for their genetic architecture to increase the accuracy of genomic prediction of complex traits.

P339 A Powerful Yeast Mapping Panel for Complex Trait Genetics. *Daniel A. Skelly*[1], Selcan Aydin[1], Sriram Vijayraghavan[1], John H. McCusker[1], Eric A. Stone[2], Nicolas E. Buchler[1], Paul M. Magwene[1]. 1) Duke University, Durham, NC; 2) North Carolina State University, Raleigh, NC.

Dissecting the genetic architecture of quantitative traits is a formidable challenge in modern biology. Genetic resource populations are a powerful tool for understanding the contributions of alleles segregating in natural populations to variation in quantitative traits. We present the “Yeast Diversity Mapping Panel”, a genetically diverse panel of nearly 800 F2 segregants from crosses between wild, laboratory, and domesticated strains of *Saccharomyces cerevisiae*. This genetic resource population has been carefully designed to capture the bulk of genetic diversity present in budding yeast, maximize allelic richness across the panel, and incorporate ancestry from parental strains that show substantial variability across >250 phenotypic axes. We describe a novel method for computationally deconvolving haplotype blocks from pooled, unbarcoded libraries of genetically diverse segregants, and demonstrate the use of this technique to obtain full genome sequence information for our mapping panel. The resulting high-resolution genotypes can be integrated with patterns of allelic effects to narrow QTL intervals, and will facilitate identification of candidate causal alleles for functional follow-up. We demonstrate the utility of this mapping panel by characterizing the genetic architecture of a quantitatively varying phenotype, growth response to osmotic stress. Overall, this panel constitutes a powerful resource for interrogating the genetic basis of complex trait variation, and will facilitate studies of dynamically variable phenotypes, epistasis, and gene-environment interaction.

P340 Using haplotype-based models for genomic predictions in crossbred animals and multiple breeds. *J. E. Decker*[1,2], M. L. Wilson[1], R. D. Schnabel[1,2], R. Weaber[3], J. F. Taylor[1]. 1) Division of Animal Sciences, University of Missouri, Columbia, MO; 2) Informatics Institute, University of Missouri, Columbia, MO; 3) Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS.

One of the major shortcomings of genomic prediction is the low prediction accuracy across populations. We explore the use of haplotypes, rather than SNP genotypes, as effects in genomic predictions trained to be accurate in multiple populations. We analyzed 651 Angus and 1,095 Hereford purebreds along with 695 Charolais, 283 Limousin, 301 Maine-Anjou, and 516 Simmental sired (with predominantly Angus dams) samples with phenotypes and BovineSNP50 genotypes from the Carcass Merit Project (CMP). We used 3,993 Angus, 101 Charolais, 1,225 Hereford, 2,366 Limousin, 11 Maine-Anjou, and 1,913 Simmental purebred animals in addition to the CMP animals to phase the genotype data and impute missing genotypes using BEAGLE v3. Using GEMMA, we fit traits in a Bayesian Sparse Linear Mixed Model (BSLMM). Core SNPs with

the largest effects were identified from the SNP-based BSLMM analysis. Using the four flanking SNPs to each identified core SNP, we constructed non-overlapping five SNP blocks that were used to form haplotypes. Using GEMMA, we fit this new haplotype matrix as random effects in a BSLMM prediction model. We used two methods to validate these predictions. First, we used a three-fold cross-validation within breeds where clusters were chosen using genomic relationships to maximize relatedness within a cluster and minimize relatedness between clusters. Second, we omitted one breed and trained on the remaining five to assess the model's ability to predict genetic merit in breeds not represented in the training data. Using 500 to 1,000 QTL regions (core SNPs) maximized the correlation between phenotype and predicted breeding value. Results indicate that feature selection maybe more important than the use of haplotypes. When we trained a genomic prediction model with 38,686 SNPs in all breeds, the correlations from three-fold cross validation averaged 0.17 for Hereford. When we trained a genomic prediction model using 22,427 haplotypes representing 1,000 QTLs, the average correlation for Hereford was 0.41. When we trained using the 5,000 SNPs corresponding to the 1,000 QTL regions, the average cross-validation correlation for Hereford was 0.52. When we trained in five breeds omitting Hereford and then validated in Hereford, we observed a correlation of 0.33 for the haplotype model and a correlation of 0.54 for the 5,000 SNP model. Using only the top 5,000 haplotype effects, we achieved correlations of 0.65 for both the three-fold cross validation and the validation when Hereford was excluded from the training set. We have recently genotyped 1,240 additional CMP animals from 5 breeds (Brangus, Gelbvieh, Red Angus, Salers, and Shorthorn) to be used for additional validation. We continue to evaluate models to identify the optimal use of haplotypes in genomic prediction.

P341 Dissection of complex traits in sorghum for the sustainable production of fuels and chemicals. *W. Vermerris*[1], A. Abril[1], T. J. Felderhoff[1], P. S. Rao[1], S. Shukla[1], C. H. Kang[2], S. E. Sattler[3], S. Kadam[4], S. Bardhan[4], F. Fritschi[4]. 1) University of Florida, Gainesville, FL; 2) Washington State University, Pullman, WA; 3) USDA-ARS, Lincoln, NE; 4) University of Missouri, Columbia, MO.

Large-scale cultivation of bioenergy crops on low-productivity land and with minimal inputs of fertilizer and chemicals can contribute to the sustainable production of fuels and chemicals as alternatives to petroleum-derived products. This requires, however, fundamentally different crop improvement strategies than the ones that have been implemented to maximize the yield of commodity crops since the Green Revolution.

Sorghum (*Sorghum bicolor* (L.) Moench), a diploid grass that originated in Africa, is a promising bioenergy crop due to its great yield potential with low input requirements. It is also tolerant to a wide range of growing conditions, due to its great genetic diversity. The availability of the sorghum genome sequence has facilitated genetics and genomics studies.

Genetic improvement of sorghum as a feedstock for renewable fuels and chemicals will benefit greatly from the elucidation of a number of quantitative traits: **1) Disease resistance:** Successful expansion of the crop on low-productivity land, especially in the southeastern United States, depends on resistance against the most prevalent diseases, including anthracnose, a fungal disease that can result in yield losses of up to 70%. Several sources of anthracnose resistance have been identified and we recently fine-mapped two novel, major anthracnose QTL and are currently validating a small number of candidate genes using virus-induced gene silencing. **2) Flooding tolerance:** Production of bioenergy crops on land that is prone to seasonal flooding minimizes competition with food production, but requires plants able to withstand water logging. We have identified a number of sorghum genotypes that can tolerate prolonged flooding by forming aerial roots that float on the water. High-throughput expression profiling of these roots revealed extensive changes to plant metabolism. **3) Cell wall composition:** The efficiency of converting biomass to fermentable sugars is a function of cell wall composition, which can be modified by exploiting natural genetic variation, chemically induced mutants, and transgenic approaches. We have shown up to 30% improvement in the yield of fermentable sugars from biomass by using mutants with altered cell wall composition, cloned the underlying genes, and explained the impact of the mutations via structural analyses, including X-ray crystallography. Detailed understanding of the catalytic mechanisms of these enzymes is now forming the basis for protein engineering studies in which metabolic pathways can be reconfigured to meet specific applications.

This presentation will highlight the main findings of this multidisciplinary approach to dissect these complex traits.

P342 Rediscovering the Diallel: How inbred and F1 data can be used to define, model and estimate heritability of both ordinary and treatment-response traits. *W. Valdar*[1], A. Lenarcic[2], J. Crowley[1], Y. Kim[1], P. Maurizio[1]. 1) UNC Chapel Hill, NC; 2) Securities and Exchange Commission, NY.

The inbred diallel is one of the oldest designs in model organism genetics. Describing the complete (or incomplete) set of F1s produced from a given panel of parental lines, it can be used to cleanly partition different types of heritable effects, from additive, epistatic, parent-of-origin, and sex-specific versions thereof. Since pilot data often corresponds to an incomplete diallel, diallel analysis of such data can powerfully guide selection of follow-up studies. Yet, thanks to a turbulant history marked by racorous disagreements, awkward formulations, and outdated statistical ideas, diallel data is often not analyzed as such -- indeed, diallel analysis is too often treated as an arcane puzzle to be avoided. In our view, this wastes a tremendous opportunity. Motivated by a series of extensive pilot studies on the founders of the Collaborative Cross, we recently revisited diallel analysis from a modern statistical perspective, reformulating the traditional model and approach to leverage what we see as some of the diallel's greatest strengths -- the ability to project into a new design space in order to prioritize follow-up experiments that use genetic combinations of the same parental lines. We have since extended our approach to define and estimate heritable effects on response to an applied treatment, leading to a definition and modeling of GxE that would be almost impossible in human studies. I will first introduce basic concepts of the diallel cross and its decomposition via Bayesian hierarchical modeling; then I will describe how these are extended to model genetic effects on treatment response, illustrating this extension with recent work on understanding the pharmacogenetics of haloperidol treatment, and the immunogenetics of response to flu infection.

P343 A New Trait Mapping Method for *Drosophila* Reveals Oligogenic Adaptation from Standing Genetic Variation. *John Pool*, Justin Lack, Héloïse Bastide, Quentin Sprengelmeyer, Jeremy Lange, Amir Yassin, Dylan Braun, Matthew Monette. University of Wisconsin - Madison, Madison, WI.

Improved genetic mapping approaches are key to a range of biological studies, including investigations of the genetic basis of adaptive trait evolution. Here, we develop a next generation approach to bulk segregant analysis (BSA) in *Drosophila*, using a simulation-based inference framework to deal with overlapping quantitative trait locus (QTL) peaks. Our simulations suggest that BSA yields consistently stronger QTL signals than introgression mapping. We apply this method to traits that differ adaptively between natural *D. melanogaster* populations. These traits include body and wing size in a highland Ethiopian population, and the parallel evolution of melanic pigmentation and ethanol tolerance in three populations each. QTLs of moderate to large effect are readily detectable for each trait, but these often vary among strains from the same population. Examining genetic variation within QTL peaks, we find evidence that local adaptation occurred via selection on standing genetic variation, producing "soft sweep" patterns such as isolated variants with strong population frequency differences. These results begin to crystalize a general model for the genetic architecture of adaptive trait evolution in *Drosophila*. The BSA mapping method introduced here may prove valuable in many studies in *Drosophila* and in other experimentally tractable organisms.

P344 The genomic architecture of interactions between natural polymorphisms and environments in yeast growth. *X. Wei*, J. Zhang. University of Michigan, Ann Arbor, MI.

Gene-environment interaction (G×E) refers to the phenomenon that the same mutation has different phenotypic effects in different environments, and is believed to be common in all organisms. Previous studies in humans and model organisms identified a number of genes or quantitative trait loci (QTLs) exhibiting G×E. However, little is known about the general properties of G×E and its underlying QTLs. Here we use the genotype data of 1005 segregants from a cross between two *Saccharomyces cerevisiae* strains and the growth rates of these segregants in 47 different environments to identify QTLs underlying *g*rowth rate variation in each environment (gQTLs) and QTLs that have different effects in each pair of *e*nvironments (g×eQTLs). We found that 31% of gQTLs are g×eQTLs and on average 78% of g×eQTLs belong to gQTLs, supporting the current practice of identifying genes/QTLs showing different effects in different environments from genes/QTLs with effects in individual environments. Interestingly, most g×eQTLs identified from gQTLs have concordant rather than antagonistic effects in different environments, but as the effect size of a mutation in one environment increases, the probability that it is antagonistic in another environment becomes greater. Antagonistic g×eQTLs are overrepresented in environments rarely encountered by yeast in nature. We found gQTLs to be enriched in nonsynonymous sites and nonsense sites, while g×eQTLs are enriched in introns. Gene Ontology (GO) analysis identified >10 times the number of enriched GO terms for g×eQTLs than gQTLs. Simulations based on the yeast data showed that ignoring G×E causes substantial missing heritability. Together, our findings reveal the genomic architecture of interactions between natural polymorphisms and environments in yeast growth and demonstrate the importance of considering G×E in explaining fitness variation and missing heritability.

P345 How to make drug resistance evolution "difficult": a lesson on epistasis and robustness in malaria parasites. *T. Chookajorn*[1], K. Kumpornsin[1], T. Kochakarn[1], Y. Yuthavong[2], N. White[1,3], D. Fidock[4], O. Miotto[1,3,5]. 1) Mahidol Univ., Bangkok, Thailand; 2) NSTDA, Bangkok, Thailand; 3) Oxford Univ., Oxford, UK; 4) Columbia Univ., New York, NY; 5) Wellcome Trust Sanger Institute, Hinxton, UK.

Drug resistance evolution is an intricate process, requiring a series of epistatic interactions to maintain balance between selective advantage and fitness loss from drug-resistant mutations. The key questions are why treatment with certain antibiotics does not lead to full resistance, and why some drugs can be quickly overcome by drug-resistant mutations. Malaria drug resistance has become an excellent model to study these question since the parasites have been under a plethora of drug regimens for more than a century. Here we used antifolate and artemisinin resistance as models to show that the difference comes from the gain of robustness during the evolutionary process, which allows mutations to be accumulated without fitness loss.

In antifolate resistance, we demonstrated how robustness evolved under selective pressure from an antimalarial drug inhibiting the folate synthesis pathway. A series of four nonsynonymous amino acid substitutions at the targeted enzyme, dihydrofolate reductase (DHFR), render the parasites highly resistant to the antifolate drug pyrimethamine. The stepwise gain of these four *dhfr* mutations results in tradeoffs between pyrimethamine resistance and parasite fitness. The epistatic interactions between *dhfr* mutations and amplification of the gene encoding the first upstream enzyme in the folate pathway, GTP cyclohydrolase I (GCH1) promotes pyrimethamine resistance. *gch1* amplification confers low level pyrimethamine resistance and would thus be selected for by pyrimethamine treatment. Interestingly, the *gch1* amplification can then be co-opted by the parasites because it reduces the cost of acquiring drug-resistant *dhfr* mutations downstream in the same metabolic pathway.

On the other hand, the resistance level of artemisinin, the drug of choice for severe malaria treatment, is relatively low with only clinical presentation of delayed parasite clearance by a few hours. Convergent evolution, driven by multiple trajectories to overcome artemisinin-induced stress, was observed, but it was precluded to become full blown resistance by high fitness cost as shown by unstable resistant phenotypes. Artemisinin-resistant parasites suffered from fitness loss and produced fewer progenies. Under amino acid starvation, the resistant parasites failed to undergo maturation unlike their sensitive counterparts as a result of reduced hemoglobin consumption. The reduction leads to loss in artemisinin sensitivity, but the defect causes significant fitness trade-off. Without the gain of a new robustness system, long-term drug selection experiment failed to drive artemisinin resistance to surpass therapeutic doses, but the selection toward antifolate resistance was quickly and repeatedly accomplished under increased robustness by extra GCH1.

P346 Investigating cryptic genetic variation through position effect variegation in a panel of *Drosophila melanogaster* inbred lines. *J. Y. Kao*[1], D. W. McNelis[1], B. E. Goulet[2], J. Shen[3], M. L. Siegal[1]. 1) New York University, New York, NY USA; 2) Harvard University, Cambridge, MA; 3) Stony Brook University, Stony Brook, NY.

Organisms are constantly adapting and evolving in response to internal and external stimuli. These changes can arise by either new mutations or by genetic variation that was already present in the system. The latter source of standing genetic variation, which does not affect the usual range of phenotypes, but has a potential to modify a phenotype in the event of an environmental or genetic perturbation, is referred to as cryptic genetic variation (CGV). Studying cryptic genetic variation in present times is especially important. Due to the environmental and cultural changes in recent human history, the uncovering of CGV could be a potential explanation for the rising incidences of "diseases of modernity" (i.e. asthma, diabetes, depression, etc.). We believe that cryptic variants are quite prevalent in the genome and do play a significant part in determining the consequences of genetic perturbations in an organism. To demonstrate the release of CGV, we use the fruit fly, *Drosophila melanogaster* and perturb the gene, *His2Av*, which encodes for an alternate histone. Work done in nematode worms and yeast reveal that chromatin regulation may be a factor in suppressing cryptic variation. Additionally, *His2Av* is the ortholog of yeast *HTZ1*, which contributes to robustness of cell morphology against microenvironmental variation and has also been shown to have extensive epistasis with genetic variants present in a panel of yeast mutation-accumulation lines. In our study, we have created a new loss-of-function *His2Av* mutation tagged with GFP and introgressed this mutation via backcrossing into the genetic backgrounds of the Drosophila Genetic Reference Panel (DGRP), which is a panel of naturally-derived inbred *D. melanogaster* lines. We then crossed each of our introgressed lines to the w[m4] line to reveal dominant effects of the *His2Av* knockout in different DGRP genetic backgrounds. The w[m4] line has an inversion on the X chromosome that places the white gene responsible for the red pigmentation in fly eyes next to pericentric heterochromatin thereby creating mottled eyes. We are developing a new image-based position effect variegation assay to characterize the extent of variegation in eye pigmentation. By quantifying the amount of variation in perturbed phenotypic responses within and between DGRP lines as well as with and without the *His2Av* mutation, we can understand the prevalence and behavior of cryptic genetic variation.

P347 The cost of noise in biochemical reactions and the evolutionary limits of cellular robustness. *J. Van Dyken*. University of Miami, Coral Gables, FL.

Finite cell size and the probabilistic nature of biochemical reactions combine to generate substantial cellular "noise", i.e., random fluctuations in molecular abundance. An open question is whether noise affects cell function, and, if so, how and by how much? Here I demonstrate that noise causes a quantifiable loss in cell fitness by slowing the average rate of biosynthesis. The efficacy of selection to suppress noise, however, decays superlinearly with cell size, leading to a stark taxonomic divide between prokaryotes and eukaryotes in the principle design imperatives of genomic and cellular architecture.

Extending previous work, I present an analytical framework for solving the steady-state statistics of molecular species in arbitrarily connected networks of non-linear chemical reactions in mesoscopic volumes, which I validate with extensive stochastic simulations. In general, substrate noise slows the average rate of non-cooperative bimolecular (i.e., Michaelis-Menten or hyperbolic) reactions, including reactions intimately tied to organismal fitness: nutrient uptake and translation. I find that gene expression is unusually sensitive to the cost of noise because, unlike with metabolic reactions, noise-induced slowdown is not buffered by network-level feedbacks. In addition, I find that transcriptional noise directly reduces fitness by slowing the average translation rate. In general, the loss in fitness caused by noise scales inversely with cell size, while random genetic drift scales positively with cell size. Together, the ability of natural selection to suppress noise decays superlinearly with cell size, leading to a stark, cell-size-mediated taxonomic divide in selection pressures for gene regulatory architecture. Greatly weakened selection efficacy in large cells may have facilitated the evolution of transcriptional complexity in eukaryotes as they evolved free of the selective constraints of noise minimization experienced by most prokaryotes. Furthermore, hyperbolic translation kinetics substantially influence the noise statistics of gene expression, with numerous practical implications. In particular, with hyperbolic translation, increasing mRNA/ribosome binding affinity actually reduces protein noise, even as it increases translational bursting. These results thus illuminate the costs of cellular noise, the targets of noise-amelioration, and the evolutionary limits of robustness.

P348 Selection transforms the genetic landscape of Hsp90-interacting variation. *Kerry A. Geiler-Samerotte*[1], Yuan Zhu[1], David Hall[3], Sandeep Venkataram[1], Yuping Li[1], Anisa Noorasa[1], Gavin Sherlock[1], Dmitri Petrov[1], Mark Siegal[2]. 1) Stanford University, Palo Alto, CA; 2) New York University, NY, NY; 3) University of Georgia, Athens, Georgia.

Introduction: The chaperone Hsp90 decreases variation in quantitative traits by buffering the effects of polymorphisms found in nature. This result prompts a question: does Hsp90 increase an organism's robustness to genetic perturbation? The potential for Hsp90 and several other putative buffers to increase robustness to mutation has had a major impact on disease models, quantitative genetics and evolutionary theory. But perhaps Hsp90-buffered mutations are rare, yet appear prevalent in nature because stabilizing selection cannot act efficiently on such mutations. This may allow buffered mutations to persist, while other mutations that have immediate effects on phenotype are purged.

Methods: We measure how Hsp90 inhibition affects phenotypic variation among 174 yeast strains that experienced reduced selection as well as 96 yeast strains that experienced natural or artificial selection. We measure 29 orthogonal quantitative traits related to cell morphology for two million yeast cells in control or Hsp90-inhibited conditions. Ours is the first study to rigorously quantify how Hsp90 influences variance in quantitative traits by using statistics that partition multiple contributions to variance.

Results: Hsp90 tends to *decrease* quantitative trait variation among populations that have been exposed to selection. In contrast, Hsp90 predominantly *increases* quantitative trait variation between yeast strains possessing spontaneous mutations that accumulated under reduced selection.

Conclusions: Hsp90 does not make phenotypes more robust to mutations' effects. Stabilizing selection preferentially allows buffered alleles

to persist, transforming the pool of Hsp90-interacting genetic variation found in natural populations to leave the false impression of robustness.

Other selective regimes: Adaptive evolution may enrich for different types of Hsp90-interacting genetic variation. We very recently tested how Hsp90 influences fitness of 82 yeast lineages that each contain on average a single adaptive mutation in one of eleven genes involved in glucose metabolism. Preliminary results show that the presence and direction of Hsp90-induced changes in fitness depend upon which gene contains the mutation. Understanding how hub proteins, like Hsp90, influence the relationship between genetic variation and quantitative traits, and how selection's sieve enriches for certain relationships, will contribute unique knowledge to the larger goal of mapping genotype to phenotype.

P349 Genetic and cellular architecture of parentally biased seed size determinants. *J. Fitz Gerald*, J. Haymore, C. Manley. Rhodes College, Memphis, TN.

Parental genomic imprinting (PGI) is the selective expression of only one allele after fertilization depending solely on its parent-of-origin. In Arabidopsis, PGI has been characterized in the seed endosperm linking this phenomenon to the provision of maternal resources. Understanding the developmental programs regulated by PGI may provide clues to optimizing breeding strategies for seed size.
Loss of PGI can result in large seeds when maternal silencing is compromised or small seeds when paternal silencing is compromised. In a typical breeding scheme, it might be expected that PGI could lead to parent-of-origin effects or specific maternal/paternal interactions that would be masked as a component of heterosis. For example, the Landsberg *erecta* (Ler) ascension of Arabidopsis has a small seed size, but Ler pollen sires large seeds on a Cape Verde Islands (Cvi) mother. Dissecting these epigenetic effects from heterosis or simple additive effects requires a robust system to examine genome-wide genetic contributions to specific phenotypic effects. This is distinct from the more limited analysis of mechanisms for imprinted gene expression that have been characterized extensively in select genes.
To ascertain the adaptive role of global imprinted gene programs in wild-type plants we are simultaneously assaying the genetic basis for parent of origin effects Ler, Cvi and other ascensions, while exploring the cellular basis for differences in early development. A parental bias is observed in these lines with Ler fathers promoting a transgressive large seed not seen in reciprocal crosses. Using spectral analysis of autofluorescence in the seed, we isolated endosperm from surrounding maternal structures in non-transgenic natural variants. Reconstructed 3D endosperm models show that Cvi development involves an adaxial-abaxial expansion not present in Ler, which restricts expansion on this axis maternally. Ler fathers, however, promote an anterior-posterior expansion.
Using recombinant inbred lines, we are further tracking the parental origins and loci associated with changes in early seed development. Previously characterized QTL have been linked to paternal or maternal effects. Further, geometric morphometrics have been applied to seed shape to better discern if there are independent genetic pathways responsible for aspect of final seed size. We are currently linking these final seed size outcomes to events in early development to better understand the developmental targets of seed size adaptations and the role of PGI.

P350 An X×Y genetic interaction mediates global crossover frequency in house mice. *Beth Dumont*. North Carolina State University, Raleigh, NC.

The rate of recombination is a complex trait, with quantitative variation between individuals shaped by multiple genetic factors. Previously, I identified a large-effect X-linked QTL underlying recombination rate differences between males of two house mouse subspecies, *Mus musculus musculus* (*Mmm*) and *M. m. castaneus* (*Mmc*). Surprisingly, at this locus, the allele conferring the increase in recombination rate derives from the low recombination rate *Mmc* parent. This observation suggests that the native *Mmc* genome harbors at least one recombination rate suppressor that overrides the effect of this recombination-increasing allele. Here, I present multiple lines of experimental evidence to demonstrate that one of these suppressors is on the Y chromosome. Using data from the Collaborative Cross panel of recombinant house mice, I estimate that this Y-linked recombination rate modifier decreases global crossover frequency by ~1 crossover per meiosis. I further show that animals with X^{Mmc}/Y^{Mmm} and X^{Mmm}/Y^{Mmc} genotypes have reduced recombination frequencies, suggesting an antagonistic genetic interaction driven by intersubspecific combinations of alleles on the sex chromosomes. I nominate a pair of homologous, ampliconic, and interacting genes on the X and Y as compelling candidates underpinning these intriguing patterns. Together, these findings suggest that recombination rates may be shaped, in part, by intragenomic conflict and offer new genetic explanations for the striking sex differences in the crossover landscape.

P351 CRISPR-directed mitotic recombination enables genetic mapping without crosses. *Meru J. Sadhu*[1,2], Joshua S. Bloom[1,2], Laura Day[1,2], Elise Pham[1,2], Leonid Kruglyak[1,2]. 1) University of California, Los Angeles, Los Angeles, CA; 2) Howard Hughes Medical Institute, Los Angeles, CA.

Linkage and association studies have mapped thousands of genomic regions that contribute to phenotypic variation in myriad organisms. Narrowing these regions to the underlying causal genes and variants has proven much more challenging, as the resolution of genetic mapping is limited by the local recombination rate. We developed a method that uses CRISPR to build mapping panels with targeted recombination events. We tested the method by generating a panel with recombination events spaced along a *Saccharomyces cerevisiae* chromosome arm, and then by targeting a high density of recombination events to a region of interest. Using this approach, we fine-mapped manganese sensitivity in lab yeast to a single polymorphism in the transporter Pmr1, demonstrating that targeting recombination events to regions of interest allows us to rapidly and systematically identify causal variants underlying trait differences.

Though much of the most interesting phenotypic differences are between species, rather than within species, our understanding of the genetic causes of interspecies differences is very limited, as it is extremely difficult to apply linkage or association studies to interspecies variation. Our novel method builds mapping panels using directed recombination events that occur in mitosis, so it can be used to compare

species capable of forming sterile hybrid diploids. We have generated a mapping panel in hybrids of *S. cerevisiae* and *S. paradoxus*, sister species that are approximately 10% diverged, and used it to map traits that markedly differ between them.

P352 Genetic analysis of an intermediate phenotype for recombination rate variation. *Richard Wang*[1], Beth Dumont[1,2], Bret Payseur[1]. 1) University of Wisconsin - Madison, Madison, WI; 2) North Carolina State University, Raleigh, NC.

During meiosis, chromosomes participate in a delicate choreography, exchanging arms and genetic material in the process of recombination. Recombination occurs in all sexually reproducing organisms, is essential for the proper segregation of chromosomes during meiosis, and shuffles alleles onto new genetic backgrounds. Heritable variation for recombination rate exists among individuals, most of which remains unexplained. To provide insights into the genetic mechanisms governing recombination rate variation, we studied the synaptonemal complex (SC), a proteinaceous scaffold that mediates the pairing of homologous chromosomes during recombination. Rates of recombination are strongly correlated with the length of the SC, forming a material link between genetic distance and a physical structure in the cell.

We focused on the house mouse, a model system in which recombination rate and SC length can be quantified in single cells with immunofluorescent microscopy. We developed a high-throughput method to measure SC length and applied it to cytological images of meiocytes from an F_2 intercross between strains of *Mus musculus musculus* and *Mus musculus castaneus*. With this novel phenotypic data, we mapped three quantitative trait loci (QTL) responsible for variation in SC length – the first known QTL for naturally occurring variation in this trait. Substantial divergence in recombination rate exists between these two subspecies, and previous work (in the same cross) identified loci responsible for this divergence. The QTL with the greatest effect on both recombination rate and SC length lie on the proximal end of the X chromosome. When we mapped QTL for variation in SC length conditioned on recombination rate, we found the locus on the X was no longer significant. This suggests distinct genetic controls underpin these strongly correlated traits. Our results help elucidate the genetic architecture of recombination rate variation and help explain the role that the synaptonemal complex plays in that variation.

P353 Replication timing generates conserved base-substitution mutation rates in concurrently replicated regions of mismatch repair deficient bacterial genomes. *Vaughn Cooper*[1], Marcus Dillon[2], Michael Lynch[3], Way Sung[4]. 1) University of Pittsburgh, Pittsburgh, PA; 2) University of New Hampshire, Durham, NH; 3) Indiana University, Bloomington, IN; 4) University of North Carolina - Charlotte, Charlotte, NC.

Evidence now exists that spontaneous mutation rates are non-uniform within genomes across the tree of life, but the causes and consequences of spatiotemporal variation in mutation rates remain uncertain because precise estimates of genome-wide mutation rates are exceedingly rare. To assess the extent to which base-substitution mutation rates vary within the genomes of bacteria with multiple chromosomes, we carried out mutation accumulation experiments paired with whole-genome sequencing (MA-WGS) in three multi-chromosome bacteria, *Vibrio fischeri*, *Vibrio cholerae*, and *Burkholderia cenocepacia*, and two additional MA-WGS experiments in mismatch repair deficient strains of *V. fischeri* and *V. cholerae*. We find that in the absence of mismatch repair, base-substitution mutation rates vary in a mirrored wave-like pattern on opposing replichores of the large chromosome of *V. fischeri* and *V. cholerae*, where concurrently replicated regions experience similar base-substitution mutation rates. The base-substitution mutation rates on the small chromosome vary less but also tend to follow the patterns of concurrently replicated regions of the large chromosome in both species. We lack a sufficient quantity of base-substitution mutations in the MA-WGS experiments with intact mismatch repair to display significant within genome variation in base-substitution mutation rates, so it is uncertain whether the rates in these studies reflect those of the corresponding mismatch repair deficient MA-WGS experiments. These results support the notion that base-substitution mutation rates vary genome-wide across concurrently replicated regions over the duration of the cell cycle, perhaps owing to the spatiotemporal replication timing program or the conserved nucleoid structure on concurrently replicated bacterial replichores.

P354 Decomposing intra-genomic heterogeneity in mutation bias in coding sequences. *C. Landerer*, M. A. Gilchrist. University of Tennessee, Knoxville, TN.

Synonymous codon usage differs not only between taxa but also within a single genome. Current studies of codon usage bias (CUB) attribute any heterogeneity to variation in selection bias for translational efficiency proportional to gene expression. However, these approaches ignore heterogeneity in mutation bias which is widely known to occur. Previously, we have shown that one can decouple the forces shaping CUB and estimate mutation bias, gene expression and selection bias using a Bayesian framework. Here we extend our approach to allow mutation and selection bias to vary between gene categories according to the cellular conditions shaping CUB. We applied our approach to the yeast *Lachancea kluyveri* which provides a striking example of heterogeneity in GC-content. Specifically, the left arm of chromosome C (C-left) has a 13% increased GC-content compared to the rest of the genome. When all genes are assigned to a single category, the model performs overall reasonably well predicting gene expression ($\rho = 0.58$) but shows anomalous behavior for genes located at C-left. In contrast, when grouping the C-left genes into a separate category the anomalous behavior is resolved and prediction of gene expression improves substantially ($\rho = 0.68$). Our extension allows to categorize genes by mutation and selection bias on CUB and current work is focused on automatic categorization of genes.

P355 Transposon-induced genome rearrangements in maize: mechanisms and genetic impacts. *T. A. Peterson*[1], J. Zhang[1], D. Wang[1], T. Zuo[1], W. Su[1], S. P. Sharma[1], D. F. Weber[2]. 1) Iowa State Univ, Ames, IA; 2) Illinois State Univ, Normal, IL.

Barbara McClintock showed that Transposable Elements (TEs) not only move within the genome, but they can also induce major changes in genome structure and gene expression. How do these changes occur? We are studying the mechanisms by which DNA transposons duplicate and rearrange sequences, ranging from individual genic components (enhancers and exons), up to segments of 15 Mbp and containing 300

genes or more. The highly active maize *Ac/Ds* transposon system can undergo Alternative Transposition (AT) reactions that involve the termini of different nearby TE copies: 5' and 3' termini in direct orientation can undergo Sister Chromatid Transposition to produce flanking deletions and inverted duplications; while termini in reversed orientation can undergo Reversed-Ends Transposition reactions resulting in inversions, deletions, translocations and duplications (Zhang et al., 2009, 2013). *Ac/Ds* elements preferentially insert into genic regions, which greatly enhances the frequency of exon shuffling events. We show that *Ac/Ds* transposons can, in a single step, create new chimeric genes which are expressed and produce functional products and visible phenotypes. In addition, AT events that occur during DNA replication generate novel Composite Insertions by DNA re-replication (Zhang et al., 2014). These Composite Insertions can carry enhancers that induce ectopic expression of nearby genes. AT reactions are not unique to *Ac/Ds* elements, but occur also in other *hAT* family transposons which are widespread in eukaryotes. Our results demonstrate that AT events can be an effective mechanism for the generation of new genes, and provide new insight into the impacts of transposable elements on genome evolution.

For animations of Alternative Transposition, see http://thomasp.public.iastate.edu/transposition.html.

P356 Evidence for the interspecies transfer of a driving X chromosome. *C. Leonard*[1], Z. Fuller[2], R. Young[1], S. Schaeffer[2], N. Phadnis[1]. 1) University of Utah, Salt Lake City, UT; 2) Pennsylvania State University, State College, PA.

Segregation distorters are selfish genetic elements that gain an evolutionary advantage by fundamentally violating Mendel's Laws of inheritance. Such distorting systems are found in a wide swath of eukaryotes and powerfully bias segregation ratios in their own favor. When sex chromosomes gain distorting elements, massive shifts to the population sex ratio may even drive populations or species to extinction. While the selective advantage for selfish segregation is obvious, the origins of these distorting chromosomes have proven much more enigmatic.

The *Sex-Ratio* (*SR*) chromosomes in *Drosophila pseudoobscura* and its sister species *Drosophila persimilis* are classic cases of distorting X chromosomes. Males that carry these distorting X chromosomes produce almost exclusively X-bearing sperm, disrupting the sex ratio of their progeny. These SR chromosomes exist as stable polymorphisms in wild populations; neither species has acquired suppressors to counter distortion in males. Perhaps most fascinating is the observation that the *D. persimilis* SR (DperSR) chromosome lacks a large inversion acquired on the standard *D. persimilis* X (DperST) chromosome, causing it to be collinear with the standard chromosome of *D. pseudoobscura* (DpseST). This collinearity is nearly or entirely complete, and it has been mapped down to a short array of repeats at either breakpoint. The simplest explanation for this strange collinearity between DperSR and DpseST is that the DperSR chromosome may have been derived through re-inversion at the same breakpoints to restore collinearity with DpseST.

Surprisingly, our results show that this is not the case. A sliding window phylogeny test across the X chromosome shows that DperSR clearly clusters with DpseST, forming a monophyletic group across species. This clustering is specifically found at the chromosomal inversion breakpoints, where recombination between DperSR and DperST is heavily limited. Through further statistical methods designed to detect gene flow between populations, we detect robust signatures of introgression between DpseST and DperSR. Together, our data suggest a recent origin for *D. persimilis* SR chromosome through transfer from *D. pseudoobscura*. While gene flow across species is halted by hybrid incompatibilities, this chromosome may have persisted in its sister species through its ability to distort. Thus, while distorting elements have been implicated in generating hybrid incompatibility, our work indicates that segregation distorters may also promote gene flow across species by conferring a strong selective advantage to an otherwise deleterious genotype.

P357 The mutational structure of metabolism in *Caenorhabditis elegans*. *Charles F. Baer*[1], Armand Leroi[2], Sarah K. Davies[2], Rodrigo Liberal[2], John W. Pinney[2], Austin Burt[2], Jacob G. Bundy[2]. 1) University of Florida, Gainesville, FL; 2) Imperial College, London.

We investigate the mutational structure of the *Caenorhabditis elegans* metabolome by means of a mutation accumulation experiment. We find that pool sizes of 29 metabolites vary greatly in their vulnerability to mutation, both in terms of the rate of accumulation of genetic variance (the mutational variance, VM) and the rate of change of the trait mean (the mutational bias, ΔM). Strikingly, some metabolites are much more vulnerable to mutation than any other trait previously studied in the same way. PCA provides strong evidence that metabolite pools are genetically correlated, although we cannot statistically assess the strength of correlations between individual metabolites. Some metabolites are also significantly correlated with fitness. Given this structure, we hypothesized that the sensitivity of a metabolite to mutation might depend on its position in the metabolic network. To test that hypothesis we reconstructed the *C. elegans* metabolic network from genomic data, but find that its topology does not predict either the rate of accumulation of mutational variance or the direction and/or magnitude of the mutational bias. We suggest that the observed patterns depend on higher level regulatory interactions that are not captured by simple metabolic network properties.

P358 Ongoing duplicate gene resolution shapes diversified metabolic networks: a functional comparative study of two yeast *GAL*actose utilization networks. *Meihua Christina Kuang*[1,2], Paul Hutchins[3], Joshua J. Coon[3], Chris Todd Hittinger[1]. 1) Department of Genetics; 2) Graduate Program in Cellular and Molecular Biology; 3) Department of Chemistry, University of Wisconsin-Madison, Madison, WI.

After being fixed in a population, duplicate genes continuously diverge and diversify in different lineages. Duplicate gene diversification impacts gene network evolution, as well as organismal traits. Here we demonstrate that ongoing diversification of two duplicate gene pairs from the whole genome duplication shaped different network architectures and resulted in different parameters for galactose metabolism. We functionally compared the *GAL*actose utilization networks between *Saccharomyces cerevisiae* (*Scer*) and *Saccharomyces uvarum* (*Suva*) and found that the resolution of duplicate fate in this network is ongoing in this genus. The first pair, descended from an ancestral kinase and co-inducer, *ScerGAL1* and *ScerGAL3*, are almost completely subfunctionalized in *S. cerevisiae*, with *ScerGAL1* encoding a kinase with weak co-

inducing activity and *ScerGAL3* encoding a dedicated co-inducer without kinase activity. However, we found that, in *S. uvarum*, *SuvaGAL1* can largely complement the removal of *SuvaGAL3*, while *SuvaGAL3* encodes an inefficient kinase and a co-inducer. Another duplicate pair, *GAL80* and *GAL80B*, encodes two partially redundant *GAL* gene repressors in *S. uvarum*, but *GAL80B* has been lost in *S. cerevisiae*. In contrast to the well-established rapid growth of a *S. cerevisiae gal80*-null mutant in galactose, the *S. uvarum gal80*-null double mutant (*gal80Δ gal80bΔ*) shows initial rapid growth followed by several hours of temporary growth arrest. Surprisingly, closer examination revealed that the *S. cerevisiae gal80Δ* mutant also shows a temporary growth arrest, but to a much lesser extent. We found that this arrest is likely caused by overly rapid galactose catabolism, which leads to excessive accumulation of reactive oxygen species. Differences between the severity of growth arrest in *S. cerevisiae* and *S. uvarum* can largely be explained by differences at the *GAL1* locus. Due to technical limitations in detecting the relatively mild effect in *S. cerevisiae*, this novel observation was not captured by decades of research. Instead, the evolutionary differences between closely related model species rendered this general phenotype more conspicuous and facilitated its discovery. Further examination of *gal80*-null mutants of both species in a mixture of galactose and fructose or mannose revealed a stronger growth arrest, which indicates a possibly general metabolic conflict with the rapid co-utilization of galactose with fructose and mannose. Our study provides clear evidence that ongoing differential resolution of *GAL* gene duplicates shaped metabolic network diversification and revealed novel principles of galactose metabolism that might shed light on tradeoffs in carbon metabolism more broadly.

P359 Young proteins are less ordered, showing preadaptation for *de novo* gene birth. *Ben Wilson*[1], Scott Foy[1], Rafik Neme[2], Joanna Masel[1]. 1) University of Arizona, Tucson, Arizona USA; 2) Max Planck Institute for Evolutionary Biology, Plon, Germany.

Occasionally, new protein-coding genes arise *de novo* from noncoding DNA and not from duplication and divergence of other genes. *De novo* originating proteins will be restricted to the taxa that persist following the emergence of the new protein-coding gene: older *de novo* protein-coding genes will have homologs in more distantly related taxa whereas younger *de novo* protein-coding genes may be restricted to few closely related taxa. The historical progression of *de novo* protein-coding genes---from noncoding sequence to presence in a single species to presence in multiple taxa---has led to the theory that proteins should fall along a "continuum" where the traits of the youngest proteins should more closely resemble those of noncoding sequence than those of older proteins, as would be expected if protein-coding genes emerged entirely by chance. An alternative “preadaptation” theory predicts that the youngest proteins will have extreme gene-like trait values because selection will remove all toxic noncoding sequences, which never go on to persist as protein-coding genes. These trait values may even be more exaggerated in younger proteins than in older proteins that have had more time to evolve subtle solutions to structural constraints. We examined intrinsic structural disorder (ISD) as a trait with the potential to distinguish between the competing theories. We inferred ISD from the sequences of all mouse proteins and paired intergenic noncoding sequences after assigning evolutionary ages to each mouse protein via homology-based phylostratigraphy. We found that younger proteins have a higher intrinsic structural disorder (ISD) than older proteins, and proteins translated from intergenic noncoding sequences have the lowest ISD, contradicting the continuum theory of *de novo* gene birth and confirming the alternative theory of preadaptation. Our results are robust to homology detection bias (*i.e.* to evolutionary rate as a confounding factor), to non-genes erroneously annotated as species-specific proteins, and to correcting the flawed but common practice of exaggerating statistical significance by treating genes, rather than gene families, as independent data points.

P360 Lineage dynamics in adapting yeast populations. *J. Piper*, M. Desai. Harvard University, Cambridge, MA.

Predictions of evolutionary outcomes based solely on genome sequence data is limited in experimental evolution studies. Gene-level parallelism that results in differing fates across populations and mutational cohorts formed via hitchhiking obscure our ability to assign likelihoods as to which lineages will survive. Furthermore, while initial fitness has been shown to be a strong predictor of final fitness in adapting laboratory populations of microbes, it is unknown how this phenomenon arises from a system's underlying genomic and physiological architecture when significant sequence-level stochasticity is observed. Here we explore the relationship between genotype and fitness by examining genealogies from a previous long-term evolution study in S. cerevisiae, in which whole-populations were whole-genome sequenced at various time-points over 1000 generations of evolution. We selected a spread of populations representative of observed dynamics in genomic sequence (hard sweeps, clonal interference, etc) and parallelism in mutation identity. We then isolated clones from whole population samples and performed whole genome sequencing and fitness measurements. Using the resulting genealogies and their associated fitness data, we examine the various population genetics factors resulting in the lineage dynamics of adaptation in this system.

P361 Exploration of bioactive peptides from random sequences: an experimental approach to *de novo* gene evolution. *R. Neme*, C. Amador, B. Yildirim, E. McConnell, D. Tautz. Max Planck Institute for Evolutionary Biology.

The biological sequence space is the theoretical representation of all possible combinations of residues to form a protein or nucleic acid. Among the many possible combinations we can identify those which already have functions in many modern organisms.

De novo genes arise from transcribed/translated non-genic sequences known as "protogenes". While the number of protogenes appearing over time is large, only a few become truly functional genes. We suspect this to be closely related to the distribution of possible functions along random unexplored sequenced space.

We have devised a high-throughput system to screen millions of random proteins *in vivo* for their evolutionary potential and biological activity. We are able to recreate and control a phase of the evolution of new genes that has been difficult to approach through comparative genomics alone.

Preliminary results show that a large fraction of random sequences could have activities relevant to the fitness of the host. This enables us to directly explore the functional sequence space to understand a great variety of aspects of molecular innovation.

P362 Molecular evolution and population dynamics of herbicide resistance in *Amaranthus palmeri*: rapid proliferation of a highly conserved gene modulated by population structure. *A. Lawton-Rauh*[1], K. E. Beard[1], J. D. Burton[2], R. L. Nichols[4], N. R. Burgos[3]. 1) Clemson University, Clemson, SC, USA; 2) North Carolina State University, Raleigh, NC, USA; 3) University of Arkansas, Fayetteville, AR USA; 4) Cotton Incorporated, Cary, NC USA.

Gene copy number proliferation is one mechanism amongst several involved in the evolution and spread of resistance. Pinning down the relative impact of gene copy number proliferation and generalized stress response pathways remains elusive due to phenotype complexity and modulation of selection pressure over spatio-temporal scales. Furthermore, specific genomic process(es) underlying rapid adaptive evolution during resistance, particularly copy number multiplication-mediated resistance at the population level, has been difficult to capture. To understand the genomic origins of gene copy number proliferation during increased resistance amplitudes and the subsequent population level processes expanding resistant biotypes, we investigated genus Amaranthus and populations of the species with the most rapidly spreading resistance, *Amaranthus palmeri*. The primary mechanism underlying glyphosate resistance in *A. palmeri* is massive duplication and random spread of *EPSPS* gene copy numbers across the genome. *EPSPS* produces 5-enolpyruvylshikimate-3-phosphate synthase, an essential enzyme in the aromatic amino acid biosynthesis pathway. Outside of glyphosate-resistant biotypes of some species, *EPSPS* is present in single copy, is highly conserved, and contains a binding domain that is the target site of glyphosate across genus Amaranthus, in ancestral *A. palmeri*, and other plant families. In this study, we estimated *EPSPS* gene copy numbers, EPSP synthase abundances, glyphosate resistance bioassays, and genetic population structures of *A. palmeri* across the southeastern U.S.A. We then compared the divergence population genetics profiles of effective population sizes, divergence times, and the rates and patterns of gene flow of *EPSPS* versus randomized non-target genes. Our results indicate independent origins of glyphosate resistance in *A. palmeri* and that recently-founded resistant populations have differing levels of variance in massive amplification of the *EPSPS* gene (from ancestral single copy state to upwards of 400 copies). This multi-population study of *A. palmeri* with differing levels of glyphosate resistance and genetic structures indicates gene amplification as a dominant source of resistance in some populations in contrast to other, more generalized stress response processes in other populations. Taken together, there seems to be alternative routes of glyphosate resistance in *A. palmeri* which may, in part, be shaped or modulated by population structure.

P363 Secreted Proteins evade the Expression – Rate Anticorrelation. *Felix Feyertag*, Patricia M. Berninsone, David Alvarez-Ponce. Univ. of Nevada, Reno, NV.

The evolutionary rate of proteins can vary by orders of magnitude, both between and within species. A primary factor influencing protein evolutionary rate is protein expression level. A strong negative correlation between expression level and evolutionary rate exists in all studied organisms (the so-called E−R anticorrelation). The translational robustness hypothesis proposes that the main underlying cause of the E−R anticorrelation is the deleterious effect of proteins misfolding due to mistranslation. According to this hypothesis, highly abundant proteins are under strong selective pressure to fold correctly despite translation errors. The misinteraction avoidance hypothesis proposes that highly expressed proteins are under stronger selective pressures in order to avoid unspecific protein-protein interactions. In this study we investigate the E−R anticorrelation in secreted proteins, and find that, in contrast to intracellular proteins, the anticorrelation is weaker and/or non-existent in secreted proteins. We calculated the evolutionary rate of all human proteins from comparison with their mouse orthologs, and divided them into secreted (N = 2279) and non-secreted (N = 13567). In accordance with previous studies, we observed a significant E−R anticorrelation for non-secreted proteins (ρ = -0.259, $p = 4.56\times10^{-181}$); however, for secreted proteins this anticorrelation was not observed (ρ = 0.038, p = 0.084). We confirmed this apparent contradiction to the E−R anticorrelation amongst the class of secreted proteins by repeating our analyses using protein abundance data for 6 individual human tissues, and mRNA abundance data for 32 human tissues. In all cases, the E−R anticorrelation was weaker among secreted proteins. Furthermore, we made similar observations in other species, finding that the E−R anticorrelation is weaker in secreted proteins than in non-secreted proteins in *C. elegans*, *D. melanogaster*, *S. cerevisiae*, *A. thaliana* and *E. coli*. We will discuss how the secretory pathway may reduce the selective pressures of misfolding and misinteraction, thus providing possible explanations as to why the E−R anticorrelation is observed in non-secreted proteins but not in secreted proteins.

P364 The fitness landscape of a tRNA gene. *C. Li*[1], W. Qian[1,2], C. Maclean[1], J. Zhang[1]. 1) University of Michigan, Ann Arbor, MI; 2) Chinese Academy of Sciences, Beijing, China.

Fitness landscapes describe the genotype-fitness relationship and represent major determinants of evolutionary trajectories. However, the vast genotype space, coupled with the difficulty of measuring fitness, has hindered the empirical determination of fitness landscapes. Combining precise gene replacement and next-generation sequencing, we quantify Darwinian fitness under a high-temperature challenge for over 65,000 yeast strains each carrying a unique variant of the single-copy tRNA gene at its native genomic location. Approximately 1% of single point mutations in the gene are beneficial, while 42% are deleterious. Almost half of all mutation pairs exhibit significant epistasis, which has a strong negative bias except when the mutations occur at Watson-Crick paired sites. Fitness is correlated with the predicted fraction of correctly folded tRNA molecules, revealing a biophysical basis of the fitness landscape.

P365 The Critical Functions Encoded by Synonymous Sites. *H. Machado*[1], D. Lawrie[2], D. Petrov[1]. 1) Stanford University, Stanford, CA; 2) University of Southern California, Los Angeles, CA.

Although synonymous sites have historically been considered neutral, and are still used as a neutral reference for many analyses, several processes are known to exert a selective pressure on synonymous sites. Despite evidence for selection on synonymous sites across species and for several different processes, the extent of selection on synonymous sites and the relative contributions of the selective processes are not well understood. Using genome sequence data from two *Drosophila melanogaster* populations, we perform a SFS-based maximum likelihood

estimation of purifying selection on fourfold degenerate synonymous sites using short introns as a neutral control. We estimate that ~15% of fourfold sites are under strong purifying selection. We find that the selection can largely be explained by codon bias and splicing-related factors. If preferred codons and alternatively spliced genes are excluded from the analysis, we find no evidence for selection, and recover the short intron level of polymorphism. These results clearly identify the major processes contributing to purifying selection on synonymous sites, and have implications for creating a neutral reference for other species.

P366 Estimation of Population Phylogeny in an IM framework, with Applications to African Human Hunter Gatherer Populations. *J. Hey*[1], A. Sethuraman[1], J. Lachance[2], S. Fan[2], S. Tishkoff[2], Y. Wang[3,4]. 1) Temple University, Philadelphia, PA; 2) University of Pennsylvania, Philadelphia, PA; 3) Rutgers University, Piscataway, NJ; 4) Ancestry.com, Provo, UT.

A new approach for estimation of population phylogeny together with a demographic model of population sizes and migration rates is presented. By including population phylogeny in a general Isolation-with-Migration (IM) model we address a long-standing problem in population genomics. The method utilizes a Markov chain Monte Carlo simulation over both genealogies and population phylogenies and provides a maximum posterior probability estimate of the population phylogeny by integrating over demographic history. By including an unsampled ghost population as an outgroup in the phylogeny, the method accounts for many kinds of interactions between sampled and unsampled populations. We applied the new method to genomic data from Human hunter gatherer populations from Africa. .

P367 The genetic diversity of a population experiencing selection. *Ivana Cvijovic*, Benjamin Good, Michael Desai. Harvard University, Cambridge, MA.

Empirical studies have uncovered significant evidence for selection in natural populations, but a rigorous characterization of these selection pressures has been difficult. The main reason for this gap is the lack of theoretical predictions for the distortion in genealogies caused by selection, except in a few restricted scenarios: when all mutations are either weakly selected, or have a single effect size. In reality, mutational effects often span several orders of magnitude, but it is impossible to account for this in current theoretical models. In these circumstances, selection leads to correlations between the mutational and genealogical processes that cannot be accounted for by analogy with a population with a stable geographic structure. We describe a new coalescent framework that naturally accounts for these correlations by grouping individuals with the same nonsynonymous mutations into genotypes and considering the dynamics of these genotypes first. We show how our approach naturally extends to purifying selection due to a broad range of fitness effects and predicts patterns of neutral variation. This scheme is significantly more accurate than existing theoretical methods based on reductions in the effective population size and significantly more efficient than the alternative of whole-chromosome simulations. In particular, we show that, in certain scenarios, the genetic diversity of a population steadily declines as the size of the population increases, in contrast to predictions from the background selection limit. We give quantitative predictions for the distortions in the genealogies and patterns of genetic diversity depend on the size of the population, mutation rate and the full distribution of selective effects. Our results have important practical implications for the interpretation of natural sequence variability and the characterization of selective pressures in natural populations.

P368 Estimation of effective number of stem cells in Dugesia worms using temporal variance of allele frequencies. *H. Asgharian*, J. Dunham, T. Kitapci, S. Nuzhdin, P. Marjoram. University of Southern California, Los Angeles, CA.

We applied population genetic theory and statistical analysis to study the mechanism of body regeneration in Planarian flatworms. Several species of Planarians have regained attention in recent years owing to their extraordinary capacity for reconstructing whole bodies from small tissue fragments - promising to be extremely informative towards the efforts in regenerative medicine. It is estimated that stem cells comprise about 30% of their body but details of the regeneration process are largely unknown. For example, it is not clear if all stem cells or only a fraction of them close to the wound site participate actively in each round of regeneration; or whether different stem cells contribute almost equally to the growing body. Due to unavailability of transgenes for these species and lack of a high quality reference genome, many routine molecular and cell biology techniques cannot be applied to this system yet. We modeled each cell as a separate individual and the body of a worm as a population of cells. We tried to estimate the effective number of stem cells based on the temporal variance of allele frequencies across 16 generations sampled every other generations. Preliminary results proved that sequence data contain sufficient information to make the desired estimates, and generated an initial estimate of effective stem cell numbers equal to ~1.58X the number of cell generations between first and last samples (lower bound in our dataset: 22.1, more reasonable estimate is in the order of several hundred). This is a much lower number than the estimated total number of stem cells in the body indicating activity of a small fraction of them at each regeneration cycle. This project yields results to improve our understanding of body regeneration through stem cells, and emphasize the utility of out-of-the-box approaches to research when common methods fail.

P369 Estimating ages of singletons and other rare alleles. *A. Platt*, J. Hey. Temple University, Philadelphia, PA.

The ages of different variants segregating in the human population is a topic of considerable current interest. In a typical population genomic sample, however, a full half of the identified variants are present in only a single copy. Existing methods that estimate ages based on allele frequencies can only assign all of these variants to a single, yougest, age class, and methods based on the decay of linkage disequilbrium or shared haplotypes surrounding rare alleles are inapplicable. This leaves this largest class of variants almost completely uncharacterized.

There exists, however, real information in a population genomic sample that will allow us to estimate the ages of individual singleton alleles. In an infinite sites model where each allele has a unique origin, the mutation that created an allele found in a singleton must post-date the most recent common ancestor shared between the individual carrying the singleton allele and any other individual in the sample. We

propose an estimate of the time since this common ancestor as a function of the maximum length of haplotype shared between the individual containing the singleton allele and any other individual in the sample. Conditional on the age of this ancestor, the probability distribution of the age of the allele is uniform over the open range (0, age of ancestor) and has an expected age half that of the common ancestor.

This estimator applies not just to singletons, but any rare allele. For alleles present in multiple copies we use the singleton model to generate a composite likelihood estimate of the age of the most recent common ancestor shared between all of the individuals carrying the allele and any of the other individuals in the sample. This estimate will be more a more precise than in the singleton case due to combining the estimates from multiple observations. Furthermore, previous methods for estimating the age of the most recent common ancestor of the individuals who share the allele refine the estimate of the age of the allele itself to a uniform density over the interval (age of ancestor among carriers, age of ancestor between carriers and others).

P370 Genetic Interrelationships between Zika Virus, Dengue Virus, Chikungunya Virus and Yellow Fever Virus Strains. *Olaitan Awe*[1], Angela Makolo[1], Segun Fatumo[1,2]. 1) University of Ibadan, Ibadan, NG; 2) Wellcome Trust Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge, CB10 1SA, UK.

Zika virus is an arbovirus in the family Flaviviridae, genus Flavivirus and this virus generally causes a mild febrile illness with maculo-papular rash and paralysis-causing Guillain-Barré syndrome in infected humans. Its main vectors are Aedes mosquitoes, the same type of mosquito that spreads dengue, chikungunya and yellow fever virus. In 2015, Zika virus disease outbreak was reported in South America, with an association of Zika virus infection with microcephaly-babies born with abnormally small heads. This link is based on the surge in microcephaly cases following this outbreak. Nevertheless, little is known about the genomics of Zika virus or its interrelationships with other arboviruses, and treatment is currently based on dealing with the symptoms, but an extensive phylogeny is a step towards a better understanding of the natural history of Zika virus. In this study, we did comparative genomics of certain arboviruses, so as to investigate their genetic interrelationships by obtaining complete genome sequences of Zika, Dengue, Chikungunya and Yellow Fever virus species from Genbank and revealed Zika's phylogenetic position. Results from our analysis revealed that the degree of relatedness in recent Zika virus strains is higher than past Zika virus strains isolated before, and this informs possible genetic changes. We also have a view of the outbreak that started last year in Brazil. We see genetic changes that separate the Brazilian strain of Zika virus from the past strains isolated before. Though, we do not know if there are genetic variations or mutations that have happened within the course of the Zika outbreak event, or mutations within individuals, unless confirmed experimentally. If the virus incurs a mutation, this could also facilitate transmission by another vector. These are important points to put in perspective, developing tools, drugs or having understanding of Zika virus pathogenesis, because these tools are mostly built on the genome sequence of the virus. These Zika strains carry derived mutations which put them in a group with Chikungunya and Yellow Fever Virus, though Chikungunya and Yellow Fever Viruses are more closely related to each other than to Zika. Though, it is hard to speculate on the implications of our results without a few more samples, insights from this study will ultimately help to build the genomic infrastructure needed to study and combat Zika virus. Results from this study could facilitate effective vaccine production pipelines in future. We expect this study to provide better understanding of the genomic knowledge of Zika virus.

P371 Beneficial mutations improve fitness in a *Caenorhabditis elegans* line evolved under conditions of extreme genetic drift. *S. Christy*, S. Estes. Portland State University, Portland, OR.

Most newly arising mutations are deleterious for organismal fitness, yet can readily propagate within populations under a broad range of conditions. Evolutionary genetic processes able to counteract deleterious mutation accumulation include: a) generally beneficial mutations that improve organismal fitness irrespective of genetic background, b) compensatory mutations that specifically mitigate the effects of previously-acquired deleterious mutations through epistasis, and c) reversion mutations back to wildtype. The potential for spontaneous mutations to mitigate the effects of deleterious mutations alters our expectations for the population-level impact of deleterious mutation. However, the capacity of spontaneous mutations to restore ancestral phenotype and the molecular genetic patterns of compensation are still poorly understood in eukaryotic systems. We performed a mutation accumulation (MA) experiment—where mutations are allowed to accumulate in replicate lineages evolving in population sizes of one (i.e., extreme genetic drift)—using a mitochondrial-deficient mutant strain of *Caenorhabditis elegans* nematode, *gas-1*. This strain has lower levels of fitness compared to wildtype. Mutation accumulation is expected to result in further fitness decline due to accumulated deleterious mutations. However, the lines experienced partial phenotypic recovery back to wildtype levels. Next-generation sequencing and bioinformatic analyses revealed several candidate single nucleotide polymorphisms (SNPs) responsible for this recovery. The purpose of this study was to determine the capacity of SNPs to restore fitness in *gas-1*. We introgressed the SNPs onto wildtype and *gas-1* backgrounds, then performed life-history assays to compare the introgressed strains with wildtype and *gas-1* strains. We discovered that the introgressed strains experience a shift to earlier reproduction compared to wildtype and *gas-1* strains and conclude that the SNPs are generally beneficial for fitness. This finding indicates that beneficial mutations are able to accumulate under extremely unfavorable population genetic conditions.

P372 Homoploid hybrid speciation in the wild: yeasts do it too. *G. Charron*[1], J.-B. Leducq[2], L. Nielly-Thibault[1], C. Eberlein[1], C. R. Landry[1]. 1) Université Laval, Québec City, Canada; 2) Université de Montréal, Montréal, Canada.

Hybridization is a powerful mechanism to generate biodiversity, for instance through species formation. However, well-supported cases of homoploid hybrid speciation limited to plants and animals, which indicate that either unicellular eukaryotes have been overlooked or that this mechanism is restricted to multicellular organisms. Experiments in the laboratory have shown that new yeast species can be formed by hybridization, suggesting that this mechanism could be, in principle, observed in nature. Using population genomics, experimental crosses and

fitness assays, we show that homoploid hybrid speciation took place recently in natural populations of *Saccharomyces paradoxus* inhabiting the North American temperate forests. We find (1) reproductive isolation between hybrid and parental species, (2) evidence of hybridization in the genome, and (3) a link between hybridization and reproductive isolation. In addition, we found that the hybrid lineage displays specific growth phenotypes that may reflect a new ecological niche. Our results show that hybridization may play a key role in species formation in fungi. Because there is very little opportunity for pre-zygotic isolation among yeast species, this mechanism of species formation could be common in the wild and contribute to fungal biodiversity. Our system will prove useful for the study of the different roles of hybridization, chromosomal rearrangements and hybrid adaptation in speciation.

P373 The correlation across populations of mutation effects on fitness. *Ryan Gutenkunst*, Alec Coffman, Aaron Ragsdale, PingHsun Hsieh. University of Arizona, Tucson, AZ.

Divergent selection, in which the same allele has different effects on fitness in different populations, drives environmental speciation. Much is known about patterns of genetic variation near loci with given divergent selection coefficients, but little is known about overall genomic patterns of divergent selection. To fill this gap, we developed a framework for inferring the joint distribution of fitness effects (DFE) between pairs of populations, based on a diffusion approximation to the joint allele frequency spectrum. We applied this framework to African and American populations of Drosophila melanogaster, first estimating demographic history and then the joint DFE. As expected, we found that genome-wide nonsynonymous mutation fitness effects were highly correlated between these two populations. Considering functional subsets of genes, however, revealed striking differences. For example, for muscle development genes the joint DFE has a correlation of roughly zero, whereas for neuronal development genes it is almost one. Divergent selection in these populations is thus operating much more strongly on the musculature than the brain, suggesting that adaptation is primarily physiological rather than behavioral. Future work will apply this general framework to other populations and species, thus quantifying how divergent selection varies with environmental and molecular context.

P374 An Ion-channel Gene Causes Natural Courtship Song Variation in *Drosophila*. *Yun Ding*[1], Augusto Berrocal[1,2], Tomoko Morita[1], Kit Longden[1], David Stern[1]. 1) Janelia Research Campus, HHMI, Ashburn, VA; 2) UC Berkeley, Berkeley, CA.

Animal species display enormous variation for innate behaviors, but little is known about how genomes evolve to generate this diversity. The features of *Drosophila* courtship song are easy to quantify and vary widely within and between species, making song an excellent system for genetic studies. We developed a mapping strategy that combined traditional mapping protocol with high-resolution recombinant mapping using engineered visible markers via CRISPR/Cas9 genome editing. This approach allowed us to map the sine song frequency difference between two wild isolates, *Drosophila simulans 5* and *D. mauritiana 29*, to a 966 bp region within the *slowpoke* locus, which encodes a calcium-activated potassium channel. We confirmed that *slowpoke* is the causal locus using a reciprocal hemizygosity test, and further narrowed down the casual mutation to an intronic insertion of retrotransposon that occurred within *D. simulans* populations. Difference in exon usage was observed around the retrotranspon insertion site. Like many ion-channel genes, *slowpoke* is expressed widely in the nervous system and influences many behaviors. The *slowpoke* mutants sing very little song with disrupted features. However, the naturally evolved *slowpoke* alleles cause significant variation in only one component of courtship song, showing that a highly pleiotropic ion channel gene can evolve to generate a specific new behavior pattern through changes in gene regulation. Currently, we are trying to confirm the retrotranspon insertion as the causal mutation by targeted deletion, and identify the molecular mechanism of how the retrotranspon insertion has fine-tuned the *slowpoke* function. .

P375 Allelic variation of an EXOCYST subunit switches between distinct root system architectures. *W. Busch*, T. Ogura, C. Goeschl, D. Filiault, R. Slovak, S. B. Satbhai, M. Mirea. Gregor Mendel Institute of Molecular Plant Biology, Vienna, AT.

Root system architecture (RSA), the distribution of roots in soil, plays a major role in plant survival. RSA is shaped by processes largely governed by the phytohormone auxin. However, auxin has a central role in numerous other processes and it is unclear which molecular mechanisms allow for the regulation of RSA independently of the numerous other processes that are impacted by auxin. Taking advantage of the remarkable natural genetic variation of *Arabidopsis thaliana*, chemical perturbation of the auxin pathway, and genome wide association mapping, we identify a subunit of a highly conserved molecular complex, the EXOCYST, as a modulator of the auxin pathway. Importantly, the auxin modulatory function of this gene is highly specific for root traits and enables specific regulation of RSA without notably affecting above ground plant traits. At the molecular level, it is acting on the protein distribution of a specific auxin efflux carrier. At the organismal level, allelic variation and genetic perturbation of the EXOCYST subunit lead to alteration of the orientation of root growth and root branching, resulting in a switch from a shallow to a deep root system. Finally, the distribution of alleles of this EXOCYST subunit in natural Arabidopsis populations is highly correlated with precipitation seasonality, suggesting an adaptive role in areas with variable rainfall patterns. We corroborate this idea by showing that overexpression and allelic variation of this EXOCYST subunit affect drought resistance in controlled conditions.

P376 Large scale splicing QTL analysis of cancer genomes. *Kjong-Van Lehmann*[1], Andre Kahles[1], Cyriac Kandoth[1], William Lee[1], Nikolaus Schultz[1], Oliver Stegle[2], Gunnar Rätsch[1]. 1) Memorial Sloan-Kettering Cancer Center; 1275 York avenue; New York City, NY 10065; 2) European Bioinformatics Institute; Hinxton; Cambridge; CB10 1SD; United Kingdom.

The large scale efforts of molecular characterizing thousands of different tumors of The Cancer Genome Atlas (TCGA) network, have enabled new opportunities to undertake quantitative trait analysis at unprecedented sample sizes.To facilitate the joint analysis across various TCGA projects, we have re-aligned and re-analyzed RNA and whole sequencing data of ~4.000 individuals comprising 11 cancer types. RNA-seq data has been processed with SplAdder in order to quantify gene expression and splicing changes, reflecting cancer-specific and tissue-specific

splicing variability. We observe a threefold increase in splicing events compared to GENCODE annotation and estimate an increase of ~20% of splicing complexity in tumor samples. In order to account not only for population structure, which is the most common confounding factor in QTL analysis, but also somatic mutations, recurrence patterns and sample heterogeneity, we employ a mixed model allowing us to model tumor specific genotypic and phenotypic heterogeneity. The large sample size in TCGA allows us to not only find local splicing QTL's but also to detect large effect long range changes, affecting the function of splicing factors. We observe splicing mutations in U2AF1 and SF3B1 caused by somatic alterations. We identify various somatic and germline mutations inducing splicing alterations in many genes and insight into their effects may contribute towards a better understanding of cancer development, progression and treatment.

P377 QTL mapping for hitchhiking behavior in *C. elegans* reveals evolutionary trade-off between dispersal and reproduction. *Daehan Lee*[1], Jun Kim[1], Heeseung Yang[1], Heekyeong Kim[2], Young-ki Paik[2], Leonid Kruglyak[4], Erik Andersen[3], Junho Lee[1]. 1) Seoul National University, Seoul, Korea; 2) Yonsei University, Seoul, Korea; 3) Northwestern University, Evanston, IL; 4) UCLA, Los Angeles, CA.

Caenorhabditis elegans is a species with boom-and-bust life style in fluctuating wild environment. Dauer larvae, which can survive in harsh conditions without food for months, disperse to a new habitat with ample food to resume reproductive growth, either through cruising or hitchhiking. Dauer larvae of *C. elegans* and its close relatives have been discovered in the wild associated with other animals such as isopods or snails. Nictation, a dauer-specific standing and waving behavior, is a hitchhiking behavior which facilitates dauers to attach to these carriers. Here, we show natural variation in nictation behavior among wild isolates of *C. elegans* exhibit a high degree of heritability. By quantitative trait locus (QTL) mapping, we discovered and confirmed a QTL, which we named the *nict-1* locus, involved in nictation variation utilizing molecularly distinguished polymorphisms in two wild isolates. We investigated genetic feature of the *nict-1* locus and analyzed its potential effect on behavioral variation. To assess fitness effect of the *nict-1* QTL, we performed a transmission competition assay using isopods. Our results demonstrate a significant effect of the *nict-1* QTL on the transmission to a new habitat through hitchhiking, as well as an unexpected trade-off effect on reproduction. In summary, we discovered a QTL that exerts a trade-off effect between dispersal and reproduction, two crucial traits for survivor and evolution of the species.

P378 Genetic Analysis of Maize Lines Tolerance to Drought and Soil-Nitrogen Stresses. *Bashir Bello*. Fountain University, Osogbo, Osun State, Nigeria.

The frequency of drought coupled with low-nitrogen (N) soil and increasing cost of N fertilizer that limit maize productivity in West and Central Africa, necessitate breeding cultivars tolerance to both stresses. This study assesses (i) the relationship between maize inbreds performance and their hybrids under optimal, drought and low-N conditions and (ii) the mode of inheritance of nitrogen and drought tolerance stresses in maize. Ten extra-early maize inbred lines were crossed in a half-diallel mating design. The resultant hybrids were evaluated in three environments of managed drought-stress, low-N and optimum-N. Drought tolerant inbreds had high grain yield consistently across all the environments. Several hybrids with at least one tolerant parent generated tolerant hybrids, while crosses between susceptible inbreds produced susceptible hybrids. Additive genetic effects influenced grain yield, ears per plant, anthesis silking interval and leaf senescence under drought, while non-additive gene action conditioned grain yield under low-N. Varietal performance under low-N and drought stresses should lay emphasis on high grain yielding hybrids from parents with suitable general combining ability mean squares for grain yield, ears per plant, leaf senescence and anthesis silking interval under drought stress. Non-additive gene action governing grain yield under low-N and significant general combining ability estimate obtained for grain yield under drought stress signified that development of hybrid is desirable by exploring non-additive genetic control under low-N and drought stresses.

P379 Systems genetics in Maize: A multilevel analysis of Maize response to Ozone. *Lauren M. McIntyre*[1], ALison Morse[1], Alison Gerken[1], Felicia New[1], Linda Young[1], Craig Yendrek[2], Gorka Erice[2], Jessica Wedow[2], Lorena Rios Acosta[2], Crystal Sorghini[2], Chris Montes[2], Ilse Barios-Peres[2], Charles Burroughs[2], Ben Thompson[2], Matt Kendzior[2], John Regan[2], Taylor Pederson[2], Patrick J. Brown[2], Andrew D. B. Leakey[2], Elizabeth A. Ainsworth[2]. 1) University of Florida, Gainesville, FL; 2) University of Illinois at Urbana-Champaign.

In an integrative approach, we use genetic variation as the basis of a systems genetics approach to model phenotypic response to ozone (O_3) exposure in maize. Data from all levels of the system including targeted biochemical assays, measurements of photosynthetic capacity, metabolomics, and RNA-seq are combined to develop a comprehensive picture of Maize response to ozone. Tropospheric ozone is an air pollutant that costs ~$14-26 billion in global crop losses and is projected to worsen in the future. We grew the same genetic material under ambient (40 ppb) and elevated O_3 concentrations (~100 ppb) at the Free Air Concentration Enrichment (FACE) site in Illinois. Little is understood about the physiological processes impaired by elevated O_3 in maize or other C4 species, or whether genetic variation for ozone sensitivity can be exploited in development of future crops. Genetic variation was clearly observed in O_3 sensitivity in the varying responses of ~160 inbred lines, including the nested association mapping population founder lines. From these, 50 lines, including the NAM founders, were retested in 2014 and genetic variation was confirmed. In 2015 10 inbreds and 8 hybrids were extensively tested and these data are the basis of our systems genetics analysis.

P380 Deep sequencing of whole transcriptomes across the Drosophila Genetic Reference Panel. *L. J. Everett*, M. A. Carbone, S. Zhou, G. H. Arya, G. St. Armour, L. Turlapati, R. F. Lyman, T. F. C. Mackay. North Carolina State University, Raleigh, NC.

Genetic variation influences organismal phenotypes in part by altering the transcriptional regulatory architecture that controls mRNA expression levels. However, there remain fundamental questions about the relationship between genetic, transcriptomic, and organismal phenotypic variation, in part because there are few data sets encompassing transcript and phenotype measurements from the same

genotypes. The Drosophila Genetic Reference Panel (DGRP) is a collection of 205 inbred *Drosophila melanogaster* lines harboring natural genetic variation derived from wild-caught flies. Genetic variation was previously mapped across these lines, and multiple research groups have used this collection to map the genetic architecture of various quantitative traits, including alcohol sensitivity, aggression, feeding behavior, and growth control. We have now performed total RNA-seq on adult whole flies across this collection to map genetic variation in the entire transcriptome, including thousands of novel transcripts. We have developed novel analysis pipelines to unify RNA-seq data derived from distinct genomes, and have identified heritable expression patterns in the majority of known genes, as well as transposons and novel transcripts. Additionally, we have found that total RNA-seq libraries from whole flies include signatures of Drosophila microbiomes, and reveal heritable patterns of microbial content across the DGRP. Ultimately this data set will allow the mapping of trait-associated transcripts, eQTLs, and expression correlation networks with unprecedented power and resolution.

P381 Steps toward reproducible research. *Karl W. Broman*. University of Wisconsin-Madison, Madison, WI.

A minimal standard for data analysis and other scientific computations is that they be reproducible: that the code and data are assembled in a way so that another group can re-create all of the results (e.g., the figures in a paper). Adopting a workflow that will make your results reproducible will ultimately make your life easier; if a problem or question arises somewhere down the line, it will be much easier to correct or explain. But organizing analyses so that they are reproducible is not easy. It requires diligence and a considerable investment of time: to learn new computational tools, and to organize and document analyses as you go. Nevertheless, partially reproducible is better than not at all reproducible. Just try to make your next paper or project better organized than the last. There are many paths toward reproducible research, and you shouldn't try to change all aspects of your current practices all at once. Identify one weakness, adopt an improved approach, refine that a bit, and then move on to the next thing. I'll offer some suggestions for the initial steps to take towards making your work reproducible.

P383 Domestic pigeon's checkered past: a link between color patterning, introgression, and hereditary blindness. *A. I. Vickrey*, Z. Kronenberg, R. Bruders, E. J. Osborne, M. Yandell, M. Shapiro. University of Utah, Salt Lake City, Utah.

Animals have evolved a vast array of colors and color patterns in response to natural, sexual, and artificial selection. Domestic rock pigeons (*Columba livia*) are a stunning example of this diversity because different individuals within the same species vary tremendously in color and color pattern. Classical genetics suggest that four alternative alleles (T-check, Checker, Bar, Barless in decreasing order of dominance and melanism) at a single locus determine wing color pattern. Although the Bar pattern is thought to be the ancestral phenotype, birds with the Checker and T-check patterns are more numerous in urban environments, possibly due to enhanced fitness. In this study we investigate the genetic basis of wing color pattern variation. We performed whole-genome comparisons between Bar and Checker birds to identify a candidate region containing just a few genes that was highly differentiated between the two phenotypes. Surprisingly, cross-species sequence comparisons suggest that a haplotype in this region that is shared by all Checker birds was introgressed into the rock pigeon from a different species, providing a striking example of cross-species transmission of a potentially adaptive phenotype. One gene in the candidate region shows expression differences among Bar, Checker, and T-check alleles in regenerating feathers indicating a cis-regulatory change at this locus. Lastly, we find that Barless birds, which have an increased incidence of vision defects, are homozygous for a nonsense mutation in a gene that is associated with blindness in humans. Remarkably, the same mutation we find in pigeons is also observed in two human families with hereditary blindness. This study highlights unexpected molecular links between color pattern, adaptive introgression, and vision defects in a classical model organism.

P384 Whole genome sequencing studies of speciation and selection in the Lake Malawi cichlid radiation. *R. Durbin*[1], M. Malinsky[1,2], H. Svardal[1], A. Tyers[3], M. Genner[4], E. Miska[1,2], G. Turner[3]. 1) Wellcome Trust Sanger Institute, Hinxton, UK; 2) Gurdon Institute, Cambridge Univ., UK; 3) Bangor Univ., UK; 4) Bristol Univ., UK.

The thousands of haplochromine cichlid fish species found in the African rift valley great lakes and surrounding rivers constitute perhaps the most dramatic vertebrate evolutionary radiation. Lake Malawi contains over 500 morphologically and ecologically diverse species that have separated within the last million years or so. In this presentation we will relate recently published results on the sympatric separation of two *A. calliptera* ecomorphs in Lake Massoko, a small crater just north of Lake Malawi (Malinsky et al, Science, 2015), to results from unpublished whole genome sequences of over 150 fish from over 85 species in the main Lake Malawi.

In the Lake Malawi sequences we observe a large amount of shared variation segregating across species, with pairwise Fst varying from 5% to 65% (diversity within species 0.05-0.1%, divergence between species 0.1-0.3%). The average phylogeny shows numerous differences to the standard taxonomy, with multiple instances of repeated phenotypic specialization across the phylogeny. As in Massoko, we see evidence of selection in the visual system, and we are testing loci selected in Lake Massoko for signs of independent repeated selection within Lake Malawi. Because of the shared variation the gene phylogeny varies across the genome. In part this is expected due to incomplete lineage sorting, but using D statistics we also see evidence for gene flow between separate branches of the species phylogeny, and are looking for evidence of selection acting on this gene flow.

We recently collected 1500 more samples from ~250 species to enable more detailed studies into speciation and selection. Finally, we are making all our sequences openly available, to enable others already working on the evolution of Malawi cichlids to also study the genetic sources of phenotypic diversity in this rich system, and are also collaborating with the Genome10K initiative to establish reference genome sequences more broadly to cover vertebrate diversity.

P385 How much do chromosomal inversions prevent gene conversion and interspecies gene flow? *Katharine Korunes*. Duke University, Durham, NC.

Many distinct species continually hybridize, and the persistence of these species is attributed in part to recombination barriers such as chromosomal inversions. Chromosomal inversions are proposed to act as barriers to recombination in inversion heterozygotes, but the strength of these barriers remains poorly understood. While single crossover products are prevented within inversions, non-crossover gene conversion can occur in and around chromosomal inversions, potentially even at high rates, rendering inversions less effective at preventing gene flow. There are extremely limited empirical estimates of non-crossover gene conversion rates with respect to chromosomal inversions, so despite the prevailing thinking, inversions may be quite ineffective at keeping hybridizing species distinct. To test the efficacy of chromosomal inversions at maintaining LD in hybrids, I am utilizing the naturally-hybridizing species pair *Drosophila pseudoobscura* and *D. persimilis.* Using whole-genome sequence data from experimental crosses, I detect gene conversion rates within inverted regions of species hybrids that are at least as high as rates based on within-species LD data. With these data, we can assess rates of gene flux and better understand the conditions under which chromosomal inversions reduce interspecies gene flow. Given such high rates of gene conversion in hybrids but high sequence differentiation between species, other features of this system must be maintaining the distinction of these species besides the recombination barrier provided by chromosomal inversions.

P386 Convergent evolution of regulatory regions in flightless birds. *Tim Sackton*, Phil Grayson, Alison Cloutier, Michele Clamp, Scott Edwards. Harvard Univ, Cambridge, MA.

A fundamental question in evolutionary biology is the extent to which convergent changes in phenotype are driven by convergent changes at the sequence level. Recent phylogenetic work strongly supports multiple independent losses of flight in the ratites (ostriches, rheas, kiwis, cassowaries, and emu), providing a compelling system to understand the degree of genomic convergence associated with flight loss. We have sequenced 10 new high-quality palaeognath genomes (including 7 flightless ratites and 3 volant tinamous), and aligned these with 32 existing sequenced genomes of reptiles and birds to identify 284,000 conserved non-exonic elements (CNEEs) greater than 50 bp in length. Of these, 15,000 are specifically accelerated and/or lost in at least one ratite lineage. We show that a disproportionate number of these elements are accelerated in multiple independent ratite lineages, suggesting that convergent changes in putative regulatory sequences may be associated with independent losses of flight. Genes nearest to convergently accelerated CNEEs are enriched for developmental functions, and include several candidate genes with roles in limb development. Limb development is particularly relevant to loss of flight as forelimbs (wings) are reduced in size or lost in all ratites. Ongoing experimental work aims to determine whether genes close to convergently accelerated CNEEs are differentially expressed during the development of volant (chicken) and flightless (emu) species, and ultimately whether the sequence changes occurring in convergently accelerated CNEEs are associated with detectable changes in regulatory function. Overall, our results suggest that convergent evolution of non-coding regulatory sequence is an important driver of convergent phenotypic evolution in flightless ratites.

P387 Genomic imprinting and speciation in mammals. *J. Good*, T. Brekke. University of Montana, Missoula, MT.

Extreme hybrid growth is common in mammals, indicating that disruption of early development may play an important role in mammalian speciation. Hybrid growth effects often follow a parent-of-origin dependent pattern where reciprocal hybrids differ in size and are either larger or smaller than the parental species. Disruption of genomic imprinting, the parent-specific epigenetic silencing (imprinting) of one allele, has been linked to growth effects underlying diverse human diseases and has been hypothesized to be the predominant cause of abnormal hybrid growth. This hypothesis predicts that loss of imprinting in hybrid placentas should result in parent-of-origin dosage imbalances between paternally expressed growth factors and maternally expressed repressors. We have combined transcriptomic and quantitative genetic experiments to dissect the regulatory underpinnings of extreme parent-of-origin hybrid overgrowth between two species of dwarf hamsters. First, we tested for disrupted placental gene expression in overgrown hybrid placentas. We observed extensive transgressive expression of growth-related genes and bi-allelic expression of several paternally imprinted genes generally associated with growth repression. Surprisingly, the apparent disruption of paternal imprinting was also associated with severely reduced expression levels. These patterns are contrary to the predictions of the loss of imprinting model and suggest that misexpression of dosage sensitive genes in hybrids is caused by other mechanisms in this system. Next, we performed a backcross and identified the X chromosome as the major maternal factor explaining hybrid placental overgrowth. Expression analyses on large and normal backcross placentas revealed substantial autosomal misexpression but normal imprinted expression of the X chromosome. These results indicate that X-linked genetic incompatibilities are not caused by chromosome-wide de-regulation but may be involved in epistatic interactions that lead to the disruption of autosomal imprinting. Collectively, our results support a central role for both disrupted epigenetic processes and the X chromosome in the evolution of extreme hybrid growth in mammals.

P388 Cryptic genetic variation and the evolution of complex traits. *A. B. Paaby*. Georgia Institute of Technology, Atlanta, GA.

Conditionally functional mutations are an important class of natural genetic variation, yet little is known about their prevalence in natural populations or how they mediate adaptive trajectories. In this talk I describe a vast reserve of cryptic genetic variation, alleles that are normally silent but which affect phenotype when the function of other genes is perturbed, in the gene networks of C. elegans embryogenesis. I find evidence that cryptic-effect loci are ubiquitous and segregate at intermediate frequencies in the wild. The cryptic alleles demonstrate low developmental pleiotropy, in that specific, rather than general, perturbations are required to reveal them. My findings underscore the importance of genetic background in characterizing gene function and provide a model for the expression of conditionally functional effects that may be fundamental in mechanisms of trait evolution.

P389 Dissecting the large X-effect in Drosophila speciation: high resolution mapping and the identification of hybrid male sterility genes. *C. Meiklejohn*[1], E. Landeen[2], K. Gordon[3], S. Biel[3], D. Stern[4], D. Presgraves[2]. 1) University of Nebraska-Lincoln, Lincoln, NE; 2) University of Rochester, Rochester NY; 3) Indiana University, Bloomington, IN; 4) HHMI Janelia Research Campus, Ashburn, VA.

In animals with heteromorphic sex chromosomes, the first interspecific genetic incompatibilities to accumulate cause sterility in the heterogametic (XY) sex and are disproportionately X-linked. Why the X chromosome has unique roles in the rapid evolution of gametogenesis and speciation is unclear. To determine the evolutionary and molecular genetic basis of the "large X-effect" in speciation, we have generated an ultra high-resolution genetic map of X-linked hybrid male sterility (HMS) factors between two sister species, *Drosophila mauritiana* and *D. simulans,* by introgressing doubly-marked segments of the *D. mauritiana* X chromosome into a *D. simulans* genome. This map comprises over 500 overlapping introgressed segments ranging in size from 2 Mb to 100 kb that were genotyped at thousands of markers by light-coverage resequencing. Our genetic mapping data, coupled with data on introgression between these species in nature, provide insights into the consequences of incompatibilities for gene flow. Furthermore, a recent selective sweep in *D. mauritiana* plausibly associated with a meiotic drive element appears to have prevented the accumulation of linked HMS factors in this region of the X chromosome. We have further refined three regions to a dozen candidate HMS genes, and in one of these regions, we have identified and transgenically validated a new HMS gene. Identification of multiple X-linked HMS genes will allow broad generalizations on the functions of these genes within species, the etiology of HMS, the evolutionary forces involved in interspecific divergence, and the overall molecular basis of the large X-effect.

P390 The naturally variable ELF3 polyglutamine is the hub of an epistatic network in *Arabidopsis thaliana*. *Maximilian Press*, Christine Queitsch. University of Washington, Seattle, WA.

Short tandem repeats are hypervariable genetic elements occurring frequently in coding regions. Their fast mutation rate has been recognized as a potent source of genetic variation contributing to adaptive evolution and the heritability of human disease. We recently proposed that such repeats are likely to mediate epistasis, by supplying compensatory mutations in certain genetic backgrounds. We have previously described one such case, in the *A. thaliana* gene *ELF3*, of a short tandem repeat that encodes a highly variable polyglutamine. In a twofold approach, we 1) dissected the genetic architecture of this incompatibility between two *A. thaliana* ecotypes, and 2) used a yeast two-hybrid strategy to identify proteins whose physical interactions with ELF3 were modulated by polyglutamine status. Using these two orthogonal approaches, we identify specific genetic and physical mechanisms by which the *ELF3* repeat may mediate the observed genetic incompatibilities. Our work elucidates a variety of mechanisms by which repeat variation, which is generally underascertained in population-scale sequencing, can control phenotypic variation. Furthermore, our work suggests that such highly variable loci contribute disproportionately to the epistatic component of heritability. We also show preliminary data from new sequencing technologies identifying more such highly variable repeats.

P391 Functional compensation and dependency between duplicated genes in protein interaction networks. *C. R. Landry*, G. Diss, I. Gagnon-Arsenault, H. Vignaud, C. Berger, A. M. Dion-Coté. Université Laval, Québec, Canada.

According to evolutionary models of gene duplication, paralogous genes are functionally identical after duplication and slowly diverge by partitioning ancestral functions and/or acquiring new ones until becoming functionally independent. Paralogs may influence each other's evolutionary fate during this period. First, the functional redundancy of the two duplicates may affect the fitness consequences of mutations in one member of a pair, for instance by masking deleterious mutations through functional compensation. Second, the function of the two duplicates may be functionally linked by mechanisms such as cross-regulation or physical interaction between gene products. The two paralogs could thus evolve as a single functional unit. Here we study this interdependency in the yeast protein interaction network by examining whether duplicated genes affect each other's protein-protein interactions when deleted. We tested 56 pairs of duplicated genes in the yeast *Saccharomyces cerevisiae* and found that for a large fraction of them, deleting one duplicate leads to gains of interaction by the other, following patterns that are consistent with a mechanism of functional compensation. We also found an unexpectedly large proportion of pairs that exhibit functional dependency, i.e. partial or complete loss of protein-protein interactions of the remaining paralog after the deletion of its sister copy. Our results provide a molecular mechanism for functional compensation between paralogs, show that functional dependency is widespread among duplicate pairs and suggest that paralogs often do not diverge independently but constrain each other's evolutionary fate.

P392 High-throughput measurements of the evolutionary consequences of epistasis. *J. I. Rojas Echenique*[1], A. N. Nguyen Ba[1], S. Kryazhimskiy[2], M. M. Desai[1]. 1) Harvard University, Cambridge, MA; 2) UCSD, La Jolla, CA.

Epistatic interactions underlie foundational problems in evolutionary biology: the role of history and chance in determining the outcomes of evolution, the relative difficulties of evolving different complex adaptations, and the evolution of sex and recombination. To characterize the general patterns of epistasis produced by adaptation, we evolved 20 replicate populations of 36 different yeast gene deletion mutants and measured the epistasis between the initial gene deletions and the mutations acquired in the course of adaptation. We found that rates of adaptation were genotype dependent and sought to explain this result in terms of the measured epistatic effects.

P393 Beyond candidate genes: Mapping monogenic trait modifiers using informative recombinant progeny in yeast. *A. Sirr*, A. Scott, G. Cromie, E. Jeffery, C. Ludlow, T. Morgan, A. Dudley. Pacific Northwest Diabetes Research Inst, Seattle, WA.

Clinically relevant features of monogenic diseases, including severity of symptoms and age of onset, can vary widely in response to environmental differences as well as to the presence of genetic modifiers affecting the trait's penetrance and expressivity. While a better understanding of modifier loci could lead to treatments for Mendelian diseases, the rarity of individuals harboring both a disease causing allele

and a modifying genotype hinders their study in human populations. Our interest in studying the effect of modifiers on monogenic traits has led us to investigate a dimorphic trait of *Saccharomyces cerevisiae* when grown on a colorimetric medium used in clinical identification of fungal pathogens. While the majority of natural isolates of *S. cerevisiae* develop a distinct purple color on this medium, a small number remain white, demonstrating that this trait is dimorphic in the population. To characterize the nature of this variation further, we preformed QTL analysis on a large number of recombinant progeny produced by a cross between white and purple parental strains. Our results identified a major QTL peak over a region that includes the tandemly arrayed paralogs *PHO3* and *PHO5*, suggesting the colorimetric assay is an indicator of acid phosphatase activity. Sanger sequencing of these loci in 28 white strains in our natural variant collection revealed most strains harbored a gene fusion of the two phosphatase genes that deleted the constitutively expressed *PHO3* promoter region. Gradations in color among the progeny of the cross suggested additional genes, unlinked to the *PHO3- PHO5* locus, could modify the acid phosphatase activity. We used time-lapse photography to quantitatively measure the growth and development of purple color of each individual colony over time. By conditioning on the major QTL allele, we identified additional secondary QTL peaks that differed between the two groups and observed a QTL peak with a LOD score that declined over the time course of the experiment. In order to identify modifiers of acid phosphatase activity, we are currently applying a novel high throughput method to isolate informative recombinant progeny for targeted genotyping.

P394 Characterizing patterns of epistasis in yeast experimental evolution. *Gregory I. Lang*[1], Sean W. Buskirk[1], Ryan Emily Peace[2]. 1) Department of Biological Sciences, Lehigh University, Bethlehem, PA 18015; 2) Bioengineering Program, Lehigh University, Bethlehem, PA 18015.

Understanding how genes interact is a central challenge in biology. Non-additive genetic (epistatic) interactions are crucial in constraining evolutionary trajectories and underlie many complex traits. Yet high-throughput methods for uncovering epistatic interactions are lacking. The current “gold standard” approach for characterizing epistasis is to engineer all possible combinations of evolved mutations and to measure the fitness of each genotype. Though powerful, this strategy is not practical for populations that contain more than a few mutations. To overcome this limitation we developed a new high-throughput method for assaying fitness and epistasis in genetic crosses and applied this method to twelve populations from a previously published yeast evolution experiment.

By crossing each evolved clone with its ancestor, we generated large pools of recombinant progeny containing random combinations of evolved mutations. For each of the twelve crosses we determined the distribution of fitness among 192 randomly selected recombinant individuals. Next, we determined the background-averaged fitness effect for all 111 mutations across our twelve evolved populations by propagating the recombinant pools and quantifying the change in frequency of each mutation over time. We developed a theoretical framework that combines these two data sets to reveal the patterns of epistasis among the mutations in each population.

Our analysis shows that most interactions between evolved mutations follow the model of “global diminishing returns” epistasis. This method also succeeded in identifying several new examples of idiosyncratic epistasis. We are beginning to explore the biological basis underlying these interactions. This is the first step towards understanding how epistatic interactions arise in the context of evolution and how epistasis influences the dynamics of adaptation.

P395 Genetic Interactions Suppress Extreme Bone and Weight Phenotypes in a Mouse Intercross. *Gregory W. Carter*, Anna L. Tyler, Leah Rae Donahue, Gary A. Churchill. The Jackson Laboratory, Bar Harbor, ME.

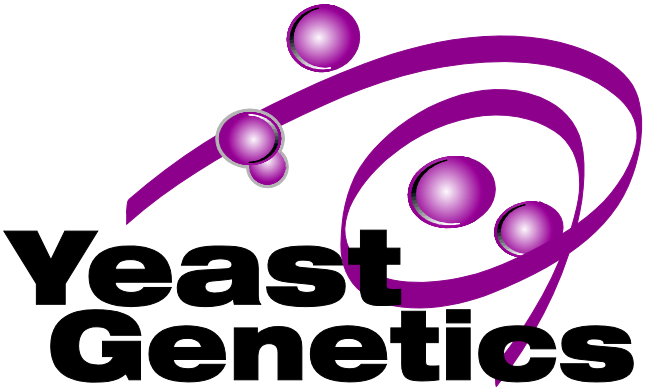
Bone density and other body composition phenotypes are complex traits with many contributing genetic factors, both shared and distinct. Genetic interactions are often suspected to contribute to the genetic architecture of such phenotypes, but understanding their role and extent is commonly limited by sample size. To reduce this limitation, we created a large F2 intercross of over 2000 B6.lit/lit x C3H.lit/lit mice to investigate interactions influencing femoral density, femoral circumference, body fat, and body weight. We used a combined analysis of pleiotropy and epistasis to infer how multiple quantitative trait loci (QTL), sex, and circulating growth factor IGF1 interact to influence all traits simultaneously. A large connected network with dozens of directed genetic interactions among multiple distinct QTL was obtained and analyzed, from which we obtained insights into the genetic architecture of this intercross populations. Recurrent patterns of QTL interactions with sex and circulating IGF1 suggested regulatory and compensatory roles for candidate genes. The QTL network was dominated by genetic interactions that reduced the occurrence of extreme phenotypes when the two interacting loci shared a common parental genotype. Rather than widespread genetic buffering, in which extreme phenotypes arise from strong synergistic interactions between QTL pairs, we found a genetic architecture characterized by (1) weak interactions that adjusted additive variance, and (2) genetic redundancy. This first class of phenotype-stabilizing interactions has effects that are close to additive and are thus difficult to detect except in very large sample sizes. We interpret these findings as a genetic mechanism of homeostasis for each inbred parental strain that is disrupted by allelic assortment via intercrossing. Our results suggest that while epistasis is often weak and unlikely to account for a large proportion of heritable variance, even small-effect genetic interactions can facilitate hypotheses of underlying biology in well-powered studies.

P396 Can epistasis or GxE be predictable? Lessons from mitonuclear interactions in Drosophila. *D. M. Rand*, J. A. Mossman, L. A. Biancani, C.-T. Zhu. Brown Univ, Providence, RI.

Epistasis and genotype by environment interactions (GxE) are likely sources of the missing heritability for complex traits that have emerged from genome wide association studies (GWAS). In an effort to understand the role of gene by gene (GxG) and GxE interactions in the mapping of genotypes in to phenotypes, we have built a panel of 72 mitonuclear genotypes constructed from all pairwise combinations of 6 mtDNAs of D. melanogaster and D. simulans and 12 of the Drosophila Genetic Reference Panel (DGRP) strains. One prediction is that mitonuclear epistasis will scale with mtDNA sequence divergence. Another is that the importance of epistasis will vary across environments. We have quantified

development time and other fitness traits on different isocaloric diets of yeast and sugar to assess the individual and joint contributions of mitochondrial, nuclear and dietary environment to these phenotypes. While certain DGRP lines support the mtDNA divergence prediction, the majority of nuclear backgrounds show no significant effect of mtDNA divergence on development time. However, a number of DGRP backgrounds show striking reversals of phenotype across different mtDNAs. The role of mitonuclear epistasis is most pronounced in the low protein diet with the slowest development. Notably, those DGRP backgrounds that demonstrate the most dramatic epistatic interactions with mtDNAs are the most responsive to dietary modification of development time. These observations raise the hypothesis that GxG and GxE interactions are not distinct phenomena but are related. This notion becomes intuitive if either epistatic partner lies in a pathway where an abiotic environment alters the genetic or cellular environment that governs the expression either gene. This joint mitochondrial and nuclear genomic approach manipulates a number of metabolic processes that can add predictability to GxG and GxE effects underlying the putative missing heritability for complex traits.

YEAST
GENETICS MEETING



Plenary and Platform Session Abstracts

Yeast Genetics Meeting

SCHEDULE AT-A-GLANCE

Wednesday, July 13		
2:00pm-9:30pm	Speaker Ready Room Open	Hall of Cities - Anaheim
7:00pm-9:00pm	Scientific Session: The Dynamic Genome Winge-Lindegren Address presented by Rodney Rothstein	Crystal Ballroom G2
9:00pm-11:00pm	Opening Mixer with Exhibits	Cypress Ballroom
Thursday, July 14		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities -Anaheim
7:45am-10:00am	Genetics and Determinants of Health Joint Plenary Session	Palms Ballroom
8:00am-4:00pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:30am-12:30pm	Scientific Session: Post-Transcriptional Gene Regulation	Crystal Ballroom G2
12:30pm-1:30pm	Mentoring Roundtables #1	North Tower - Harbor Beach
12:30pm-1:30pm	Speaking Up for Genetics and Model Organism Research	Crystal Ballroom H
1:30pm-3:30pm	Poster Presentations 1:30pm-2:30pm: Even-numbered poster 2:30pm-3:30pm: Odd-numbered posters	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center and Job Fair	Cypress Ballroom 1C
4:00pm-6:00pm	Scientific Session: Epigenetics and Transcriptional Regulation	Crystal Ballroom G2
4:00pm-6:00pm	Plenary Session and Workshop for Undergraduate Researchers	North Tower - Sawgrass
7:45pm-9:45pm	Scientific Session: Tackling Human Disease Using Yeast Ira Herskowitz Award presented to Lars Steinmetz	Crystal Ballroom G2
10:00pm-11:30pm	*Science Cafe Event	Palms Ballroom Sabal
Friday, July 15		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-9:30am	Scientific Session: Division and Development	Crystal Ballroom G2
8:00am-4:30pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:00am-12:00pm	Scientific Session: Stress Sensing and Damage Control Yeast Genetics Meeting Lifetime Achievement Award presented to James Broach	Crystal Ballroom G2
12:00pm-1:30pm	*Editor's Panel Discussion and Roundtable	North Tower - Harbor Beach
1:30pm-3:30pm	Poster Presentations 1:30pm-2:10pm: "A" poster authors present 2:10pm-2:50pm: "B" poster authors present 2:50pm-3:30pm: "C" poster authors present	Cypress Ballroom

** Ticketed Event*

#TAGC16

Friday, July 15 (continued)		
1:30pm-3:30pm	GeneticsCareers Center	Cypress Ballroom 1C
2:00pm-2:45pm	GeneticsCareers Workshop - Nailing the Job Talk	Cypress Ballroom 1B
4:00pm-6:00pm	Scientific Session: Evolution in and out of the Lab	Crystal Ballroom G2
6:00pm-7:30pm	*Women in Genetics Panel and Networking	North Tower - Harbor Beach
6:30pm-7:30pm	YGM Program Committee Meeting	TBD
7:30pm-9:30pm	Development and Evolution Joint Plenary Session	Palms Ballroom
Saturday, July 16		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-10:00am	Workshops: See topics and descriptions under the Workshop Section	Multiple locations
8:00am-12:00pm	Exhibits Open	Cypress Ballroom
8:00am-9:00am	Trainee Bootcamp Workshops: Session 1	North Tower
9:00am-10:00am	Trainee Bootcamp Workshops: Session 2	North Tower
10:00am-12:00pm	Poster Presentations 10:00am-11:00am Odd-numbered posters 11:00am-12:00pm Even-numbered posters	Cypress Ballroom *(Posters must be removed by 1pm)*
10:00am-12:00pm	GeneticsCareers Center	Cypress Ballroom 1C
10:30am-11:15am	GeneticsCareers Workshop	Cypress Ballroom 1B
12:15pm-1:45pm	*Mentoring Roundtables #2	North Tower - Harbor Beach
1:45pm-3:45pm	Scientific Session: Revisiting Classical Genetics with New Technology	Crystal Ballroom G2
4:00pm-6:00pm	Concurrent Workshops: Beyond cerevisiae: Exploiting yeast diversity in nature to understand genome evolution in diverse environments Getting Even More Out of SGD	Crystal Ballroom C-D Crystal Ballroom G2
7:30pm-9:30pm	Scientific Session: Structural and Cellular Organization Lee Hartwell Lecture presented by Susan Gasser	Crystal Ballroom G2
Sunday, July 17		
8:00am-10:00am	Scientific Session: The Fat and Sweet Sides of Life	Crystal Ballroom G2
10:30am-12:30pm	Technology and its Application Joint Plenary Session	Palms Ballroom

** Ticketed Event*

Y463 Winge-Lindegren Address. *Rodney Rothstein*. Columbia Univ Med Ctr, New York, NY.
no abstract submitted

Y464 Using Experimental Evolution To Engineer A Low Flocculation Yeast Strain. *Elyse A. Hope*, Clara J. Amorosi, Aaron W. Miller, Kolena Dang, Caiti Smukowski Heil, Maitreya J. Dunham, 2015 CSHL Yeast Genetics and Genomics Course. University of Washington, Seattle, WA.

Yeast flocculation is a community-building cell aggregation trait that is a key contributor to brewing but a nuisance in other industrial processes, the laboratory, and clinical settings. This dichotomy demonstrates the importance of understanding and manipulating the genetic determinants of cell aggregation to achieve phenotypically desirable outcomes on both ends of the spectrum. Experimental evolution in the laboratory provides a unique testing ground for understanding the breadth of genetic mechanisms *Saccharomyces cerevisiae* uses to achieve cell aggregation. The popular lab strain S288C does not aggregate due to a nonsense mutation in the gene encoding transcription factor Flo8, which regulates flocculation. Despite this mutation, these strains evolve the ability to aggregate during 35% of our continuous culture evolution experiments. These evolved strains present an opportunity to examine the adaptive routes that lead to cell aggregation and whether one or many routes are favored.

We have analyzed 23 evolved clones using a combination of whole genome sequencing, quantitative phenotyping, and Bulk Segregant Analysis (BSA), a pooling and sequencing approach. All of the clones exhibit a significantly stronger aggregation phenotype than the ancestor, and none of the strains reverted the point mutation in *FLO8*. We recovered two loss of function mutations in *ACE2*, which has been previously shown in the literature to cause mother-daughter separation defects. BSA revealed causal mutations in *TUP1*, a known regulator of flocculation gene *FLO1*, and *ROX3*, which has not been previously connected to flocculation in the literature. Most interestingly, we discovered a common mutation among 13 of the evolved clones: a Ty1 transposable element insertion in the promoter region of the flocculation gene *FLO1*. Discovering this common adaptive mechanism indicated that rather than many equally favored adaptive routes, changing the regulation of *FLO1* is the preferred route for evolving flocculation. We engineered a *flo1* knockout strain and evolved it comparatively against a wild-type lab strain, with 32 replicates of each. We determined that this single gene deletion reduced flocculation occurrences from 25% to 3% over 250 generations. Deleting *FLO1* had no effect on the evolution of unrelated phenotypes (separation defects and wall sticking), demonstrating the efficacy of this evolve-sequence-design approach for engineering targeted phenotypic outcomes.

Y465 Ploidy tug-of-war: evolutionary and genetic environments influence the rate of ploidy drive in a human fungal pathogen. *M. A. Hickman*[1], A. C. Gerstein[2], H. Kim[2], J. Berman[2,3]. 1) Emory Univ, Atlanta, GA; 2) Univ of Minnesota, Minneapolis, MN; 3) Tel Aviv Univ, Tel Aviv, Isreal.

Ploidy contributes to the evolutionary dynamics and to the cellular physiology of organisms. Significant variation for ploidy is seen throughout the tree of life yet the factors that determine why one ploidy level is selected over another remain poorly understood. Asexual fungal microbes are a unique system to study ploidy dynamics as they are incredibly tolerant to karyotypic variation in both the number of chromosome sets as well as single (or few) chromosomes deviations from euploidy. Such variation can be rapidly selected for or against depending on the environment, yet evolution experiments have revealed a propensity to return to the historical baseline ploidy level under a variety of environmental conditions, a phenomenon that we term here as 'ploidy drive'. To assess the interplay between environmental pressure and strain genotype on ploidy drive, we evolved ten strains of the human fungal pathogen *Candida albicans* of varying initial ploidy under four environmental conditions. We found selection for increased genome sizes under nitrogen limitation and in a complete medium yet selection for smaller genome sizes was observed under phosphorus limitation and in minimal medium. Furthermore, genetic background had a significant role in ploidy drive dynamics, as some genotypes were markedly less stable than others. Combined, this work demonstrates a role for selection at both the environmental and genotypic level in the rate of ploidy drive.

Y466 Mechanism of non-genetic heterogeneity in yeast growth rate and stress resistance. *Shuang Li*, Mark Siegal. New York University, New York, NY.

Despite its genetic homogeneity, a clonal population can display marked heterogeneity in various aspects, such as growth rate and stress resistance. Such heterogeneity may have important clinical implications in tumor biology and microbial pathogenesis. As a model eukaryote and opportunistic pathogen, the budding yeast *Saccharomyces cerevisiae* provides an excellent model for studying non-genetic heterogeneity. Isogenic yeast cells, cultivated under benign conditions, divide at different rates. Slow-growing cells disproportionately survive acute heat shock and have high expression of a stress-protective factor, TSL1. Knocking out TSL1 significantly reduces survival of acute heat shock but has negligible effect on growth. The molecular mechanisms that generate non-genetic variation in yeast-cell growth rate and stress resistance have not been characterized. We identified the paralogous transcription factors MSN2 and MSN4, and the upstream protein kinase A (PKA) pathway, as candidate regulators of this heterogeneity. MSN2 and MSN4 regulate the general stress response. PKA regulates their phosphorylation status, which determines their nuclear import and export rates. Although there have been extensive studies on the function and subcellular localization dynamics of MSN2 and MSN4 under stress, the significance of MSN2 and MSN4 under benign conditions has rarely been explored. We use high-throughput time-lapse microscopy to measure growth and gene expression in microcolonies founded by individual cells. The negative correlation between single-cell growth rate and TSL1 expression level is abolished in msn2 mutants but not msn4 mutants, which suggests only MSN2 is necessary in regulating heterogeneity in TSL1 expression and stress resistance. The population growth-rate distributions of *msn2*, *msn4* and *msn2 msn4* double mutants all show similar, significant reduction of slow growers compared with wildtype. These results suggest that MSN2 and MSN4 are each necessary in regulating slow growers. The proportion of slow growers also decreases significantly in mutants lacking subunits of the PKA complex, TPK1 or TPK2, and the decrease is even stronger than in *msn2* mutants. Therefore, PKA regulates

slow growers, but not solely through MSN2 and MSN4. Monitoring of MSN2 intracellular localization reveals that heterogeneity likely derives in part from its nuclear dwell time: MSN2 enters the nucleus more frequently and stays there longer in slow-growing cells, presumably leading to differential expression of target genes and thereby different growth and stress-resistance states.

Y467 The concerted function of the Shu complex and the Rad51 paralogs in Rad51 presynaptic assembly. *K. A. Bernstein*[1], T. McClendon[1], M. Sullivan[1], W. Gaines[2], S. K. Godin[1], P. Sung[2], J. Yanowitz[1], K. A. Bernstein[1]. 1) Univ Pittsburgh School of Medicine, Pittsburgh, PA; 2) Yale University School of Medicine, New Haven, CT.

Double-strand breaks (DSBs) are one of the most cytotoxic forms of DNA damage. Defects in DSB repair are associated with cancer predisposition, as well as, several heritable diseases including Fanconi anemia. Our work has centered around Rad51 paralog function during DSB repair. Importantly, mutations in the human Rad51 paralogs are found in tumors and also lead to Fanconi anemia. Due to the embryonic lethality of the mouse knockout models and the low abundance and insolubility of the human proteins, there is very limited understanding as to how the Rad51 paralogs function to maintain genomic integrity. To circumvent these obstacles, we are studying the role of a unique Rad51 paralog-containing complex, called the Shu complex, in the budding yeast *S. cerevisiae* and the nematode *C. elegans*. Although the Shu complex was discovered 10 years ago and is critical for DSB repair, its molecular function has remained elusive. We discovered that the yeast Shu complex is highly conserved from archaea through humans. Furthermore, we have identified direct physical interacting partners of the yeast and worm Shu complex and to show that these interactions are necessary and sufficient for the biological efficacy of the Shu complex during DSB repair. We define a molecular role for the yeast and worm Shu complexes in Rad51 presynaptic filament assembly, and thus for the first time gained insight into its mechanistic function. Our work has important implications for how the Rad51 paralogs and the human Shu complex function to prevent tumorigenesis and Fanconi anemia.

Y468 Genome-wide detection of genomic fluctuations in *Saccharomyces cerevisiae*. *K. Palacios Flores*, M. Boege, A. Castillo, J. Garcia, L. Gomez, C. Uribe, G. Davila. Laboratorio Internacional de Investigacion sobre el Genoma Humano, UNAM, Queretaro, Queretaro, MX.

The aim of our study is to define, at the nucleotide level, the most dynamic sites in the *S. cerevisiae* genome. To this end, we designed an experimental strategy that could naturally result in population structure remodelling of a *S. cerevisiae* primary culture. A liquid culture of the S288c strain was prepared and used to plate single cells, which were then allowed to form two-day colonies. Whole genome sequencing was performed for the primary culture and for 91 colonies. To detect genome dynamics in an unbiased manner, we devised a bioinformatics approach that culminates in the formulation of a general signature of variation for sequenced genomes. This signature can be used to scan genomes in the search for different types of variation including SNVs, microindels, deletions, insertions, and amplifications. Herein, we focused on the detection of amplification sites along the genome. Amplifications represent a particularly attractive study case because they provide a physical platform for further dynamic behaviour. We found 187 amplifiable sites located all along the genome, with no obvious preference for any chromosome or chromosomal region. Different sites vary widely in the proportion of cell populations that fall above a 2 fold amplification threshold. The latter suggests the existence of a characteristic dynamic spectrum for each site. For those cases where most of the cell populations pass the amplification threshold, the primary culture does so as well. This suggests that the amplification level of the single cell founders of these populations could have an important role in driving the amplification level of the growing colonies. By comparing the amplification level of each site across cell populations, it became evident that the population structure of the primary culture was remodelled both towards higher and lower levels of amplification, generating a gradation of possibilities. This suggests the action of a stochastic and reversible dynamic process at these sites. Finally, upon computing the dynamic range of amplification levels for each site, we observed up to 4 fold differences. This is considerable when taking into account that derived cell populations were grown under identical conditions and under no obvious selective pressure. We propose the concept of genomic fluctuation: the result of a highly dynamic and reversible process capable of generating genomic differentiation between populations derived from a common and recent origin within a single species. We expect this concept will contribute to the general understanding of the genome as a dynamic entity.

Y469 A cradle-to-grave analysis of *cis*-regulatory variation in yeast. *J. M. Andrie*, G. E. Merrihew, J. Madeoy, D. S. Gordon, D. Akey, E. E. Eichler, D. R. Morris, J. Wakefield, M. MacCoss, J. M. Akey. University of Washington, Seattle, WA.

Cis-regulatory variation is an important source of phenotypic variation within populations and a major target of adaptive divergence between species. However, the molecular processes that are influenced by *cis*-regulatory variation remain poorly understood. To this end, we crossed two genetically diverse wild-derived strains of *Saccharomyces cerevisiae* and studied allele-specific differences in eight molecular phenotypes, including chromatin structure, rates of RNA transcription, decay and translation, RNA secondary structure, binding of proteins to RNA, steady-state protein abundance, and protein decay rates. Furthermore, we performed high-coverage sequencing of both the genome (using PacBio) and the transcriptome, and *de novo* assembled each parental strain to mitigate read mapping biases and ensure accurate estimates of allele-specific phenotypes. We show that *cis*-regulatory variation has pervasive effects on high-dimensional molecular phenotypes, and pleiotropy and buffering are predominant features in the architectural landscape of *cis*-regulatory mutations. Our comprehensive data also provides novel mechanistic insights into *cis*-regulatory variation. We anticipate that our data will be of considerable general interest, and a powerful resource to test hypotheses about the evolution of *cis*-regulatory variation.

Y470 Msn2 regulates cellular response and growth to stress through modulation of its localization and DNA binding. *V. Bharatula*[1], R. Chereji[2], J. Broach[1]. 1) Penn State College of Medicine, Hershey, PA; 2) National Institute of Child Health and Development, Bethesda, MD.

Yeast perceive and respond to changes in their external environment by rapidly modulating its transcriptional program. A core set of genes are upregulated and repressed under several stresses examined. These genes are under the transcriptional control of the stress responsive factor Msn2. Single cell microscopy studies revealed that Msn2 re-localizes from the cytoplasm to the nucleus when cells are stressed. This change in localization is dynamic, which is observed by the presence of short-lived random bursts of Msn2 nuclear occupancy. We observed that Msn2 localization was uniquely modulated for different stresses. We investigated the consequence of this dynamic behavior of Msn2 on transcription of genes using microarrays. Ectopic expression of transient and prolonged nuclear Msn2 forms led to differential gene expression kinetics. Some genes were readily induced *i.e* fast responding and others required a constant nuclear presence of Msn2. This suggests that by modulating transcription factor dynamics yeast can selectively regulate gene expression depending on the stressor. Whole genome chromatin immunoprecipitation and nucleosome positioning studies post nutrient downshift and oxidative stress corroborated our findings and we observed Msn2 binding to promoters of common stress regulated genes as well as genes pertaining to the stressor. Isogenic cells also responded to stress distinctly, with some cells showing prolonged nuclear accumulation of Msn2 and others a more transient phenotype. Overexpression of constitutively nuclear phospho-mutant Msn2 causes cells to undergo cell-cycle arrest, suggesting a direct role for Msn2 in cell growth. Our studies elucidate the complex and inter-dependent mechanisms underlying gene expression changes and the process of "bet hedging" which occurs during stress.

Y471 Stress-dependent transcriptome changes serve to reallocate translational capacity during stress acclimation. *Yi-Hsuan (Elisha) Ho*[1], Evgenia Shishkova[2], Joshua Coon[2], Audrey Gasch[1]. 1) Laboratory of Genetics,University of Wisconsin- Madison, Madison, WI; 2) Department of Chemistry, University of Wisconsin- Madison, Madison, WI.

Proper response to environmental stress is pivotal for cells to survive and adapt to constantly changing environments. Activation of stress defense systems is often coupled with arrest of growth and cell cycle progression. The transcriptomic response of *Saccharomyces cerevisiae* to stress includes a common stress transcriptomic change called the environment stress response (ESR). The ESR includes induced expression of ~ 300 genes involved in stress defense and reduced expression of ~ 600 genes required for active growth, namely genes encoding ribosomal proteins and other translation machinery. There has been a debate if activation of the ESR, and in particular reduced expression of growth-promoting ESR genes, is an active response to stress or is merely an indirect byproduct of reduced growth and cell-cycle progression. Since stress responses are generally correlated with growth and division arrest, it is hard to deconvolute responses triggered directly by stress versus indirectly by growth reduction. Here, we decouple the response to external stress and growth control by following transcriptomic and proteomic changes of arrested cells upon stresses. Our results show that activation of the ESR, including repression of growth-promoting genes, is not associated with cell-cycle phase and is independent of growth arrest. Instead, arrested cells exposed to stress actively induce the ESR, even though they are no longer making significant biomass – this indicates that reduced expression of growth-promoting genes is not necessarily related to growth control. We are using mathematical modeling of transcriptome and proteome changes, along with polysome profiling to measured global translation rates, to understand the function of transcriptome changes under different conditions. Our results suggest that differences in transcript abundance during steady-state growth serve to set the levels of protein production required for a given growth rate, whereas changes in transcript levels during active stress help to reallocate translational machinery during acclimation.

Y472 Genetic factors controlling accelerated mRNA degradation during a nitrogen upshift. *Darach Miller*, David Gresham. New York University, New York, NY.

The release of yeast cells from nitrogen limitation triggers remodeling of cellular physiology to enable a faster rate of cell growth. While the population growth rate increase is not apparent until approximately 2 hours after, the nitrogen upshift triggers extensive transcriptional reprogramming on the timescale of minutes. Conserved signalling pathways, including TORC1 and Ras/PKA, are known to regulate steady-state growth, but the role of these pathways during the first few minutes of a nutrient upshift is unclear. Using the transcriptome to define the cellular state, we show that this upshift doesn't simply entail a shift from a slow growth steady-state to a fast growth steady-state, but rather proceeds through a transient alternative state. Using time-series modeling of transcriptome dynamics, we show evidence for a pulse of ribosomal component mRNA overproduction and accelerated degradation of high-affinity nitrogen transporter mRNAs, including *GAP1* and *DIP5*. To identify factors regulating this process, we used a branched DNA single molecule RNA FISH (smFISH) assay with flow cytometry to quantify transcript abundance in single cells during the upshift. Combining this assay with FACS and barcode sequencing (Barseq), we screened the pooled homozygous deletion collection for mutants defective in the accelerated degradation of GAP1 mRNA upon a nitrogen upshift. Our approach provides an effective means of screening for mutants defective in transcriptional dynamics that occur on the timescale of minutes.

Y473 P bodies regulate the rewiring of a transcription network by controlling the expression of the *YOX1* repressor during DNA replication stress. *R. Loll-Krippleber*, GW. Brown. Department of Biochemistry, University of Toronto, Toronto, Ontario, CA.

P bodies are RNA-protein granules that form in the cytoplasm of eukaryotic cells in response to various stresses and are thought to serve as sites of degradation and/or storage of mRNAs. We recently discovered that P bodies form in yeast in response to replication stress induced by HU (hydroxyurea), an anti-cancer drug that inhibits dNTP synthesis and slows DNA replication. P body components are required for cell survival of replication stress as mutants lacking key P body components Lsm1, Pat1 and Dhh1 are strongly sensitive to HU. Here, we aimed to identify mRNAs that are processed by P bodies during replication stress. First, we performed a transcriptome study on *lsm1*Δ cells upon acute HU exposure to identify mRNAs that are stabilized in the absence of a functional P body-dependent mRNA degradation pathway. Second, we used

an SGA-based suppressor screen to identify genes whose expression is toxic in the absence of Lsm1 and Pat1 during replication stress. We found that the transcriptome in *lsm1*Δ is altered both during normal growth and during replication stress, with more than 800 mRNAs being stabilized in *lsm1*Δ compared to wild type. Interestingly, we found that inactivation of the coding sequence of 6 of those 800 mRNAs was able to suppress HU sensitivity of *lsm1*Δ and *pat1*Δ strains suggesting that these genes encode mRNAs that need to be degraded in a P body dependent manner upon HU exposure. Among these we identified *YOX1*, a gene encoding a transcription repressor critical for the regulation of cell cycle and DNA replication genes. The suppressor effect of *yox1*Δ in *lsm1*Δ and *pat1*Δ cells is specific to HU and not to other drugs that induce DNA replication stress or to other environmental stresses. Consistent with P bodies regulating *YOX1* mRNA abundance, we found that *YOX1* mRNA localizes to P bodies and accumulates at P bodies in the absence of the mRNA exonuclease Xrn1. To gain insight into the role of Yox1 during replication stress, we identified 156 genes that are down-regulated upon *YOX1* overexpression. Among this set of targets, we notably found genes important for ribonucleotides biosynthesis (RNR genes), DNA unwinding during replication (MCM genes), cell cycle regulation and cytokinesis. Work is underway to identify which targets play a critical role during replication stress. Together, our data suggest a model where *YOX1* mRNA abundance is post-transcriptionally regulated by P bodies in order to reduce the level of the Yox1 transcription repressor and therefore prevent repression of genes necessary for survival of DNA replication stress.

Y474 Gene control by prion-like conformations of intrinsically disordered proteins. *D. F. Jarosz*, Richard She, Anupam Chakravarty, Curtis Layton, Johan Andreasson, William Greenleaf, James Byers. Stanford University, Stanford, CA.

Punctuated bursts of protein expression are common in biological systems. Yet whether these exert a lasting influence on future generations is largely unknown. We tested this by transiently overexpressing virtually all open reading frames in *Saccharomyces cerevisiae*. Strikingly, expression of nearly fifty intrinsically disordered proteins (IDPs) created heritable new traits that persisted after overproduction was stopped. The proteins were strongly enriched in RNA binding proteins (RBPs) and transcription factors (TFs). The inheritance patterns of these traits resembled protein-based genetic elements, known as prions. RBPs control the fate of nearly every transcript in a cell, but no existing approach for studying these post-transcriptional gene regulators combines transcriptome-wide throughput and biophysical precision. We used commonly available hardware to generate a uniform and highly redundant array of RNA transcripts, spanning an entire genome, on an Illumina MiSeq chip. We harnessed this transcribed genome array (TGA) to identify hundreds of new targets of Vts1/Smaug, which coordinates the degradation of maternal RNAs during development and emerged as a robust hit in our screen. This approach provided nucleotide resolution and direct measurements of affinity and dissociation in a single experiment, supplanting prior knowledge of determinants driving Vts1 substrate recognition. Acquisition of the [*VTS1*$^{+}$] prion enhanced the decay rates of target mRNAs and expanded the protein's substrate repertoire. The capacity to self-template was conserved in *Drosophila* and human Smaug proteins. Our data force a re-examination of the mechanism of Vts1/Smaug-mediated gene regulation, which was thought to occur via binding to 3'-UTRs from a handful of targeted studies. Instead, we found that binding throughout the transcript resulted in target degradation. Moreover, the idealized equimolar binding landscape of the TGA revealed previously unknown roles for Vts1/Smaug in the birth of new genes from proto-ORFs and in the regulation of stress responses. Our findings transform our understanding of Vts1/Smaug function, and have important implications for the many other IDPs involved in eukaryotic gene control.

Y475 Parallel pathways for export of tRNAs from the nucleus to the cytoplasm. *Anita K. Hopper*[1], Kunal Chatterjee[1], Jingyan` Wu[1,2], Shubhra Majumder[1]. 1) Ohio State Univ., Columbus, OH; 2) Stanford Univ., Palo Alto, CA.

Appropriate biogenesis and subcellular trafficking of tRNA are essential for decoding the genome and for regulating the proteome in response to nutrient availability and stress. Hundreds of gene products function in tRNA biology and mutations in numerous of these gene products cause a variety of human disorders ranging from metabolic diseases, to neuromuscular diseases, and to cancer. Yet, there is a very incomplete understanding of tRNA biology. To address this information gap, we conducted a screen of nearly the entire essential and unessential yeast proteome and identified 162 novel proteins that function in tRNA biology (Wu et al., 2015). Although we identified gene products that function in virtually every step of tRNA biosynthesis and subcellular dynamics, we are particular interested in those proteins that function in tRNA nuclear export because it is essential that tRNAs be efficiently transported to the cytoplasm where protein synthesis occurs, but mutants missing the known tRNA nuclear exporters, *los1Δ*, *msn5Δ*, and *los1Δ msn5Δ* are perfectly viable. Surprisingly, we identified Mex67 (vertebrate TAP), Mtr2 (vertebrate p15), and Crm1 (vertebrate Exportin-1) as potential tRNA nuclear exporters. Previous studies in yeast and vertebrate cells have shown that Mex67 and Mtr2 form heterodimers that function in mRNA nuclear export, whereas Crm1 functions in nuclear export of proteins possessing a leucine-rich NE motif. Three lines of evidence support the role of these proteins in tRNA nuclear export: (1) as determined by Northern analysis, at the nonpermissive temperature, *mex67-5*, *mtr2-x*, and *crm1-1* ts mutants each accumulate unspliced tRNAs and (2) as determined by FISH, each mutant has enhanced tRNA nuclear pools; (3) furthermore, *los1Δ* and *crm1-1* have synthetic growth defects and Mex67 can completely substitute for Los1 when it is over-expressed in *los1Δ* cells. Thus, it appears that there are at least 3 parallel pathways that function to export tRNAs from their site of synthesis in the nucleus to their site of function in the cytoplasm, underscoring genetic redundancy for this essential cellular process. Preliminary data indicate that Mex67/Mtr2 and Crm1 are more error prone in tRNA nuclear export than Los1, raising questions regarding tRNA quality control during the nuclear export process. We are currently conducting *in vivo* biochemical analyses to determine whether tRNAs can be cross-linked to Mex67/Mtr2 and/or Crm1 as they exit the nucleus.

Y476 Yeast telomerase and RNase P/MRP: when two different worlds come together. *N. Laterreur*[1*], B. Lemieux[1], A. Perederina[2], JF. Noël[1], ML. Dubois[1], AS. Krasilnikov[2], RJ. Wellinger[1]. 1) Université de Sherbrooke, Sherbrooke, Québec, CA; 2) Pennsylvania State University, PA, USA.

Telomerase is a specialized ribonucleoprotein that maintains genome integrity by adding telomeric DNA to the ends of eukaryotic chromosomes. For many years now, it has been established that the telomerase complex is minimally composed of an RNA molecule, Tlc1, and a reverse transcriptase called Est2p. In the cell, Tlc1 serves as a scaffold for many other proteins (Est1p, Est3p, yKu, Sm_7) and add telomeric repeats at shortened chromosome ends by reverse transcribing an essential template within the RNA molecule.

Using mass spectrometry analysis and a native system to purify Tlc1-bound proteins, we have been able to identify new partners for *Saccharomyces cerevisiae* telomerase. These proteins are components of the RNase P/ RNase MRP complexes; two related, highly conserved and essential RNPs that are involved in the processing of tRNA, rRNA and certain mRNAs. These ribonucleoproteins both contain a catalytic RNA subunit (Rpr1 for RNase P and Nme1 for RNase MRP) that is bound by a group of proteins.

We report that the Pop1, Pop6 and Pop7 proteins of the RNase P/MRP complexes bind to yeast telomerase RNA and are essential constituents of the telomerase holoenzyme. The trio binds to a specific and highly conserved RNA domain present in Tlc1. This domain, called P3 domain, is also shared by the RNAs of RNase P/MRP. The results also show that Pop1/Pop6/Pop7 function is to maintain the essential components Est1 and Est2 on the RNA in vivo. Consistently, addition of Pop1 allows for telomerase activity reconstitution with wild type telomerase RNA in vitro. Thus, the same chaperoning module has allowed the evolution of functionally and, remarkably, structurally distinct RNPs, telomerase and RNases P/MRP, from unrelated progenitor RNAs.

Note: N. Laterreur & B. Lemieux contributed equally to the work..

Y477 Coordinated regulation of heterochromatin inheritance by Daf1/Dpb4 complex. *Fei Li*[1], Haijin He[1], Yang Li[2], An-Yun Chang[3], Qianhua Dong[1], Feng Gao[2], Zongxuan Chi[1], Min Su[2], Rob Martienssen[3], Yu-hang Chen[2]. 1) Department of Biology, New York University, New York, NY; 2) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; 3) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Histone H3 lysine 9 (H3K9) methylation and histone hypoacetylation are two conserved epigenetic hallmarks of heterochromatin. How H3K9 methylation and histone hypoacetylation are faithfully inherited and how these two processes are coordinately regulated during replication remain poorly understood. We previously showed that the inheritance of H3K9 methylation during DNA replication depends on the catalytic subunit of DNA Polymerase epsilon, Cdc20/Pol2, in fission yeast. Here we show that two histone-fold subunits of Pol epsilon, Daf1/Dpb3 and Dpb4, in fission yeast form a heterodimer that plays a crucial role in the inheritance of histone hypoacetylation. Our findings reveal a previously unrecognized link between histone deacetylation and H3K9 methylation, and suggest a mechanism for how two processes are coordinated during replication. Surprisingly, we found that Dpb4 associates with both positive and negative regulators of heterochromatin silencing. Particularly, we show that SWI/SNF chromatin remodeling complex subunits interact with Daf1-Dpb4 dimer and act an anti-silencing factor essential for heterochromatin disassembly. Upon solving the 1.9 Å crystal structure of the Daf1-Dpb4 complex we observed that Daf1 and Dpb4 form an H2A-H2B-like heterodimer, and gained further insight into the role of this dimer in chromatin regulation. To our knowledge, this represents the first time a crystal structure has been resolved for Dpb3-Dpb4. Together, our findings indicate that the Daf1-Dpb4 complex provides a platform for the recruitment of chromatin modifiers and remodelers during DNA replication, which in turn mediate the disassembly and re-assembly of heterochromatin and ensure the accurate perpetuation of heterochromatin marks.

Y478 The Replication Kinase Cdc7 Marks Histones to Regulate Biosynthesis Genes. *P. A. Grant*[1], E. Sendinc[1], S. A. Hoang[1], X. Xu[1], M. Zakari[2], J. L. Gerton[2], S. Bekiranov[1]. 1) University of Virginia School of Medicine, Charlottesville, VA; 2) Stowers Institute for Medical Research, Kansas City, MO.

Post-translational histone modifications (PTMs) play important roles in regulating various DNA-templated cellular functions including transcription, DNA damage repair, DNA replication and telomeric silencing. Histone H3 Threonine 45 phosphorylation (H3T45ph) is a modification that is enriched during S-phase of the cell cycle in *Saccharomyces cerevisiae* and is carried out by the kinase Cdc7-Dbf4 (DDK) *in vivo* and *in vitro.* To understand the function of this mark we identified the genomic H3T45ph locations by performing chromatin immunoprecipitation followed by high throughput sequencing (ChIP-Seq) of wild-type budding yeast cell chromatin. Remarkably, this PTM is observed not only at origins of DNA replication, but is also uniquely distributed on the promoters of highly transcribed RNA polymerase I, II and III-regulated genes dedicated to protein synthesis and glycolysis. Here we show that H3T45 phosphorylation, mediated by Cdc7-Dbf4, is required for polymerase recruitment and full expression of ribosome protein (RP) and tRNA genes as well as for the regulation of cohesin loading. Collectively, we identify an unexpected gene regulatory function for the DNA-replication kinase Cdc7-Dbf4 and identify a novel histone modification regulating the protein biosynthesis gene machinery.

Y479 An oncometabolite disrupts epigenetic processes and increases gene silencing in *Saccharomyces cerevisiae*. *Ryan Janke*, Jasper Rine. University of California, Berkeley, Berkeley, CA.

Aberrant accumulation of the metabolite D-2-hydroxyglutarate promotes certain types of cancers, but much uncertainty remains regarding which specific cellular processes are impacted by the oncometabolite to promote tumor growth. The structures of α-ketoglutarate and D-2-hydroxyglutarate are sufficiently similar that D-2-hydroxyglutarate can act as a competitive inhibitor of α-ketoglutarate-dependent enzymes both *in vitro* and *in vivo*. Several key epigenetic regulators, including members of the TET family of DNA demethylases and Jumonji histone demethylases, are among the known enzymes inhibited by D-2-hydroxyglutarate. Separating the effects of DNA and histone hypermethylation has been a challenge to the field. *Saccharomyces cerevisiae* naturally lacks DNA methylation machinery and thus provides a unique

opportunity to study exclusively the effect of D-2-hydroxyglutarate accumulation on histone demethylation. Here, we have demonstrated that mutations analogous to those that cause D-2-hydroxyglutarate accumulation in human tumors recapitulated the effect in yeast. We have additionally uncovered a novel pathway for naturally producing and degrading D-2-hydroxyglutarate in yeast. Through a combination of genetic and molecular analyses, we found that D-2-hydroxyglutarate accumulation increased silencing of genes within heterochromatin. This effect was specifically mediated through D-2-hydroxyglutarate-inhibition of histone demethylase enzymes specific for H3K36 methyl marks. Our work has uncovered a mechanism by which the impact of D-2-hydroxyglutarate is leveraged by disrupting a widespread epigenetic regulatory process that leads to altered chromatin states and transcription. Inspired by these results we have expanded our study and uncovered multiple unexpected nodes in metabolism that impact heterochromatin stability, which will be discussed.

Y480 Mechanistic insight into the role of the Paf1 complex in histone modification. *S. Branden Van Oss*[1], Margaret K. Shirra[1], Alain R. Bataille[2], Adam D. Wier[1], Kuangyu Yen[2], Vinesh Vinayachandran[2], Andrew P. VanDemark[1], B. Franklin Pugh[2], Karen M. Arndt[1]. 1) University of PIttsburgh, Pittsburgh, PA; 2) Penn State University, State College, PA.

Post-translational modification of the core histones represents an important level of epigenetic regulation of eukaryotic transcription. One such modification, the monoubiquitylation of histone H2B lysine 123 in yeast (H2B K123ub), is a well conserved mark implicated in activation of transcription, silencing of heterochromatic regions, establishment of other histone modifications, and regulation of development and differentiation in higher eukaryotes. The five-subunit yeast Paf1 complex (Paf1C), an important regulator of all stages of transcription, is required for the establishment of H2B K123ub, largely through the activity of its Rtf1 subunit. We have identified a 66-amino acid region in Rtf1, the Histone Modification Domain (HMD), which is capable of promoting H2B K123ub *in vivo*, even in a strain that lacks all Paf1C subunits. Aided by our crystal structure of the HMD, we constructed a large collection of HMD mutant strains and identified residues important for H2B K123ub, H3 K4 and H3 K79 methylation, and telomeric silencing. Guided by analysis of these mutants, we performed *in vivo* crosslinking experiments using the phenylalanine analog *p*-benzoyl-L-phenylalanine (BPA). Exposure of cells to ultraviolet radiation induced site-specific crosslinks with a short linker distance, allowing us to identify proteins directly interacting with the HMD. Among the collection of proteins that crosslinked to the HMD, we identified the ubiquitin conjugase for H2B K123 ubiquitylation, Rad6. The residues that crosslink to Rad6 lie along one face of an alpha helix within the HMD. Importantly, a mutation that inactivates the HMD eliminates the Rtf1-Rad6 crosslink. To further probe the functions of the Rtf1 HMD and the entire Paf1C, we have used ChIP-exo technology to map the occupancy of Paf1C subunits, as well as the isolated HMD and components of the H2B K123 machinery, with high resolution genome-wide. The results of these studies support the idea that Paf1C occupancy is dictated by its interactions with the RNA polymerase II elongation machinery, but the Rtf1 HMD localizes to histone H2B. Finally, using a minimal *in vitro* ubiquitylation assay, we have shown that the HMD stimulates the specific *in vitro* ubiquitylation activity of Rad6 in a manner that is dependent on the ubiquitin ligase Bre1. Collectively these results provide new mechanistic insight into the critical role of the highly conserved yeast Paf1C in establishing histone modifications during transcription elongation.

Y481 Protein abundance control by non-coding antisense transcription. *F. Huber*[1], D. Bunina[1], I. Gupta[2], A. Khmelinskii[1], M. Meurer[1], P. Theer[1,3], L. Steinmetz[2], M. Knop[1,3]. 1) University of Heidelberg, Heidelberg, DE; 2) European Molecular Biology Laboratory (EMBL), Heidelberg, DE; 3) Deutsches Krebsforschungszentrum, Heidelberg, DE.

High throughput studies have revealed the pervasive nature of transcription in eukaryotic genomes, resulting in the identification of a multitude of non-coding RNAs (ncRNAs). In yeast, a substantial number of ncRNAs overlap protein-coding genes in antisense direction. While numerous reports on antisense-dependent gene regulation have been published, most studies focus on a few selected cases or rely on correlations derived from RNA-based high throughput experiments. Therefore, our understanding of which antisense RNAs exert a biological function and whether functional antisense RNAs share certain features remains incomplete. To address these shortcomings, we developed a genetic strategy to specifically abrogate antisense transcription in selected genomic loci and to directly measure the resulting impact on sense protein levels using GFP as a reporter. To gain a systematic understanding, we applied this technique to 162 yeast genes known to be overlapped by antisense RNAs. Subsequently, we used quantitative high throughput microscopy to score the effects of each tested antisense transcript on sense protein abundance under several growth conditions, thus discriminating between functional and non-functional antisense RNAs. We found that only a minority of the genes (20%) were affected by antisense transcription and that in those cases regulation was generally weak and led to a reduction in protein amounts. Interestingly, antisense regulated genes also showed reduced protein expression noise. When investigating whether certain features distinguish functional from non-functional antisense RNAs we found that the functional antisense transcripts were more likely to extend into the transcript start site of the sense. The effect of antisense increased at high antisense RNA levels but was reduced at high sense RNA levels. While we could not identify any specific sequence motifs, we found that antisense regulated genes had increased H3K4 di- and trimethylation levels. Our data shed light on the general principles that underlie antisense-mediated gene expression regulation and will provide a rich resource for detailed mechanistic studies.

Y482 Promoter scanning during transcription initiation in *Saccharomyces cerevisiae*: Pol II in the "shooting gallery". *Craig Kaplan*. Texas A&M University, College Station, TX.

Transcription initiation is the first step in gene expression. RNA Polymerase II (Pol II) is recruited to promoter DNA by activators, and together with core promoter elements and general transcription factors (GTFs), Pol II initiates transcription. Where Pol II initiates and how efficiently it does so is determined by the biochemical properties of the transcription machinery, and how it interacts with promoter architecture. In *Saccharomyces cerevisiae*, Pol II finds transcription start sites (TSSs) by scanning a promoter in a directional fashion from upstream to downstream. As in higher eukaryotes, many TSSs are used at any individual promoter. We wish to understand the mechanisms by which TSSs

are identified, and how TSS usage and overall efficiency are shaped by promoter architecture (promoter DNA sequence, the spatial relationship between promoter elements, and chromatin structure). To this end, we have used alteration of Pol II and GTF activities to perturb the initiation process. We find that alteration of TFIIH activity (*SSL2*-encoded subunit) affects TSS selection differently than alteration of Pol II (large subunit, encoded by *RPO21*), TFIIB (*SUA7*), or TFIIF (*TFG2*-encoded subunit) activity. Furthermore, we find that promoter scanning appears to operate at all yeast promoters, regardless of promoter class. However, promoter scanning appears to be differentially affected by promoter architecture. We integrate our observations with previous data into a model for Pol II initiation in yeast – the "shooting gallery". In this model, Pol II catalytic activity, and the rate and processivity of the engine for Pol II scanning, TFIIH, determine the distribution of TSSs and their usage levels together with promoter sequence.

Y483 Ira Herskowitz Award. *L. Steinmetz*. Stanford University, Stanford, CA.
no abstract submitted

Y484 Pathway transplantation into yeast as a model for human disease. *Neta Agmon*, Jef D. Boeke. NYULMC Institute for Systems Genetics, New York, NY.

Decades of research have led to the development of a numerous high throughput libraries and technologies for screening in yeast. In combination with the most resent advances in synthetic biology, yeast cells can be manipulated to serve as a "factory" for producing a desired product or as a tool to study cellular pathways. We evaluated whether we could express an entire human metabolic pathway in yeast. We chose the purine biosynthesis pathway as our first working model. It is a highly conserved pathway from yeast to humans. In humans there are >20 disorders associated with the pathway that have a variety of symptoms. Importantly, most of these diseases lack any established treatment. However, in some cases the evidence linking mutations in the gene to disorders is rather thin. We propose to use yeast for expressing human mutant alleles of human diseases in order to study whether these mutations actually impact the ability to biosynthesize adenine or other purines and if so, their effect on the cell's entire metabolic network and for screening for possible treatments. Using a synthetic biology approach, we are swapping the entire purine biosynthesis network of the yeast with the cognate human genes. We have engineered a yeast strain "humanized" for the full *de novo* adenine biosynthesis pathway. We deleted all of the yeast genes involved in the pathway and complemented them using a neochromosome expressing the human reading frames under the transcriptional control of their cognate yeast promoters and terminators. The "humanized" yeast strain shows growth in the absence of adenine, indicating complementation of the yeast pathway by the full set of human proteins. While the strain with the neochromosome is indeed prototrophic, it grows slowly in the absence of adenine. Dissection of the phenotype revealed that the human ortholog of *ADE4*, PPAT, shows only partial complementation. We have used several strategies to understand this phenotype, pointing to a possible fundamental difference between the human and the yeast pathways. We also introduced mutations from patients into the human genes to examine their effect on the "humanized" strain. Surprising results suggest that certain missense mutations presumed to underlie human disease conditions have no detectable impact on adenine biosynthesis in yeast. This could be explained by either a second function for the affected protein or an incorrect interpretation of a mutation. Other mutations recapitulate metabolic aspects of the known human disorder LND. Finally, the purine metabolic network can serve as a proof of principle for our ability to take human diseases and the associated pathway/gene networks and establish new yeast models for human disease.

Y485 Genetic and environmental backgrounds constrain the course of evolutionary rescue by compensatory mutations. *Véronique Hamel*, Marie Filteau, Marie-Christine Pouliot, Isabelle Gagnon-Arsenault, Alexandre K. Dubé, Christian R. Landry. Département de Biologie, PROTEO and Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, Qc, Canada.

Deleterious mutations segregate and often fix in small populations during the course of evolution. These deleterious mutations can be compensated for by secondary mutations known as compensatory mutations. We examined if the trajectory of compensatory evolution to a strongly deleterious mutation is dependent on the environment and the genetic background in which compensatory evolution takes place. We performed an evolutionary rescue experiment in a yeast model for the Wiskott-Aldrich syndrome (mutation in *LAS17*) in two genetic backgrounds and two carbon sources. We found that compensatory mutations tend to be overrepresented in the physical interaction network surrounding Las17p, showing that protein interaction partners are prime targets for compensatory mutations. In addition, we found that multiple aspects of the evolutionary rescue outcome depend on the genotype, the environment and their combination. Specifically, the compensatory mutation rate and type, the molecular rescue mechanism, the genetic target and the associated fitness cost varied across contexts. Overall, our results show that the course of evolution following the fixation of a deleterious allele is highly contingent on the initial conditions in which a deleterious mutation occurs.

Y486 The genomic repercussions of *RAD5* overexpression. *Robert Reid*[1], Eric Bryant[2], Ivana Sunjevaric[1], Rodney Rothstein[1]. 1) Columbia University Medical Center, New York, NY; 2) Columbia University, New York, NY.

The human *HLTF* gene is located on chromosome 3q24 and is frequently amplified in squamous cell lung carcinomas. HLTF is the human ortholog of the *Saccharomyces cerevisiae RAD5* gene, a DNA helicase and ubiquitin ligase that functions in post replication DNA damage repair. Overexpression of *RAD5* results in slow growth and genome instability. We used Selective Ploidy Ablation (SPA), a rapid high throughput plasmid screening protocol, to identify *Saccharomyces cerevisiae* genes whose function becomes essential when *RAD5* is overexpressed. Many DNA replication and DNA double strand break repair genes were identified covering multiple steps in these processes, including replication fork protection, lagging strand DNA synthesis, DNA end resection, homology dependent strand invasion and resolution of Holliday junction

intermediates. Curiously, genes regulating the process of post replication repair (PRR) are unaffected in the screen. Cells encountering DNA damage that results in replication fork stalling enter the PRR pathways via modification of PCNA/Pol30 by ubiquitylation. Using multiple mutant backgrounds, we confirmed that ubiquitylation of PCNA is not necessary for the growth suppression resulting from *RAD5* overexpression. Since Rad5 is a multidomain polypeptide containing helicase, ubiquitin ligase and DNA binding functions, we investigated the contribution of these domains to *RAD5* toxicity. Mutant protein constructs were screened using the high throughput SPA method to transfer each *RAD5* mutant into an array of sensitized mutant strains. Automated quantitative analysis of the growth curves was used to define the effects of individual *RAD5* mutants in strains deficient for specific repair functions. We find that the individual domains of overexpressed *RAD5* have separate contributions to cell toxicity in different genetic backgrounds. For example, we have uncovered instances where deletion of a specific Rad5 domain increases toxicity, suggesting that it normally performs a protective function. As the function of Rad5 is conserved in human cells, the identified SDL interactions may help to develop targeted therapeutic approaches for squamous cell carcinomas of the lung.

Y487 Functional characterization of human gene alleles using inter-species genetic approaches. *Q. Zhong*, S. Chen, I. Haider, M. Islam, R. Santhnakrishnan, S. Ju. Wright State University, Dayton, OH.

Genome-sequencing technologies bring extraordinary opportunities in identifying genetic variations. Interpretation of functional consequences of genetic variations in a give individual, however, remains to be a daunting challenge. This is in part due to our limited understanding of the effect of any single genetic variation on cellular and organismal function. More difficult is to interpret functional impact of multiple genetic changes simultaneously occurring in an individual. We recently discovered that, despite vast evolutionary separation, human and yeast proteins still widely retain the ability to mediate inter-species protein-protein interactions. Surprisingly, such human-yeast inter-species interactions are not limited to proteins conserved between the two species, but frequently correspond to functional links between proteins mediating new species-specific functions and conserved ancestral cellular machinery. Based on these findings, we have developed three experimental platforms to systematically measure effects of human genes on cellular functions and to rapidly classify genetic interactions between human gene alleles using yeast cells as the "mediator" host. Our data demonstrates the feasibility and value of using yeast a a model system to characterize evolutionarily conserved human gene function. Such inter-species genetic approaches may help identify both genetic and environmental factors that modulate complex phenotypes in human disease.

Y488 Genome-Wide Analysis in Yeast to Identify Molecular Targets Promoting Readthrough. *Mert Icyuz*[1*], John L. Hartman, IV[1], Eric J. Sorscher[2]. 1) University of Alabama at Birmingham, Birmingham, AL; 2) Emory University, Atlanta, GA.

Nonsense mutations, single nucleotide base changes that result in an in frame premature termination codon (PTC), make up ~20% of the ~43,000 disease associated mutations residing in gene coding regions and are responsible for ~11% of all inherited human diseases. Restoring functional protein by inducing readthrough of PTCs has been accomplished using aminoglycosides that have been considered as potential therapies. The overall efficacy of readthrough achieved with and the toxicity caused by currently available compounds has not been desirable. The goal of our project is identify genes that promote readthrough and we have employed cystic fibrosis, which is an autosomal recessive disease caused by loss of function of the cystic fibrosis transmembrane conductance regulator (CFTR), as model disease. Previously, we showed that Yor1 (Yeast Oligomycin Resistance-1, a member of the ABC gene family) could serve as a robust model for genome-wide phenomic analysis of CFTR. The approach demonstrated evolutionary conservation of gene-gene interactions between yeast and human for proteins that regulate CFTR-ΔF508 biogenesis; and resulted in discovery of novel targets for correction of CFTR misfolding. The CFTR-G542X mutation is the most common premature termination codon in CFTR, and the second most common CF-causing allele. We have introduced a homologous mutation of CFTR-G542X, G704X, in YOR1, and using growth on oligomycin as readout show that yor1-G704X behaves in a similar fashion to the yor1-null allele. This data indicates very little or no full-length protein is expressed, analogous to CFTR-G542X. In the presence of G418, an aminoglycoside shown to restore partial CFTR function in mammalian cell lines, we observed oligomycin resistance specifically mediated by the Yor1-G704X mutant, establishing that yor1- G704X is suitable as a reporter for genome-wide yeast phenomic screening and identification of molecular targets that allow readthrough of nonsense variants. Among the hits produced by our unbiased genome-wide phenotypic screen with Yor1-G704X are the members of nonsense mediated decay (NMD) pathway, Nam7 & Upf3. Suppression of NMD pathway was previously shown to lead to recovery of CFTR protein function. We identified some members of the 20S proteasome as modifiers of Yor1-G704X function. These candidates will be further tested in cell lines for CFTR protein function with siRNA knockdown. Resulting genes and pathways will serve as a basis for new therapeutic strategies to achieve functional CFTR expression in CF patients carrying premature stop codons.

Y489 Systematic functional analysis of resistance-conferring mutations. *L. H. Wong*[1], S. Sinha[1], J. R. Bergeron[2], G. Giaever[1], P. Flaherty[3,4], C. Nislow[1]. 1) University of British Columbia, Vancouver, BC, Canada; 2) University of Washington, Seattle, WA, USA; 3) Worcester Polytechnic Institute, Worcester, MA, USA; 4) University of Massachusetts, Amherst, MA, USA.

The emergence and prevalence of drug resistance is an inevitable consequence of the biological response to selective pressures like those that accompany drug therapies. We therefore developed an unbiased and systematic screen to identify the functional impact of drug resistant variants in a fast and cost-effective manner. Current methods to understand and predict drug resistance often require significant time, computational power and clinical constraints. These challenges are exacerbated by the lack of statistical methods to accurately call allele frequency variants.

By combining deep sequencing and a Bayesian statistical model we provide a comprehensive survey of drug resistance alleles from complex variant populations. Using dihydrofolate reductase (DFR1), the target of methotrexate chemotherapy as a benchmark we identified and validated functional alleles correlated with methotrexate (MTX) resistance in yeast. This massively parallel strategy allowed us to catalog alleles

with dominant resistance phenotypes independent of pre-existing competitive fitness traits. We confirmed 10 MTX-resistant missense mutations in DFR1/dfr1Δ heterozygotes, 2 of which also confer growth advantages and 3 hypomorphic alleles. In parallel we confirmed 8 functional missense mutations in the haploid dfr1Δ strain. The recovery of these distinct dfr1 MTX resistant-alleles underlines the power of using genetic-based assay in S. cerevisiae to dissect complex functional determinants of drug resistance. Because many of these novel resistant variants map to structurally conserved binding pockets within the Dfr1 active site, this approach should yield insight into the mechanistic differences between antifolate resistant alleles and folate metabolism.

Y490 A trade-off between invasion and sexual reproduction is mediated by the DNA-binding mechanism of a conserved transcription factor. *M. W. Dorrity*[1], J. C. Cuperus[1,2], C. Queitsch[1], S. Fields[1,2]. 1) University of Washington, Seattle, WA; 2) Howard Hughes Medical Institute, Seattle, WA.

Complex traits are challenging to study because there are numerous underlying genetic factors, interactions between genes and the environment, and interactions between the traits themselves. In the yeast *Saccharomyces cerevisiae,* two important complex traits – invasive growth and sexual reproduction – rely on a single transcription factor, Ste12, for proper expression. We used deep mutational scanning to examine the interaction between these complex traits by analyzing single amino acid perturbations in the DNA-binding domain of Ste12. Because alternative modes of DNA binding are implicated in the diverse functions of Ste12, we focused on a critical segment of the protein that is likely to contact DNA. By selecting for the ability of yeast either to invade or to mate, we sought to find sites in the protein needed either for both traits equally or for one at the expense of the other. We identified residues within the DNA-binding domain of Ste12 that could not be mutated without loss of mating, while mutations at these same positions still allowed invasion. In fact, some mutations even increased the ability of cells to invade at the cost of mating, revealing that a trade-off between invasion and sexual reproduction is encoded within a short segment of Ste12's DNA-binding domain. To explore the role of the environment, we identified mutations that increase the dependence of mating on temperature. We found that temperature-dependent variation overlaps with Hsp90-dependent variation, implying that Ste12's DNA-binding function may be stabilized by chaperones. Given the role of Ste12 as the ultimate recipient of diverse signals and the results of our mutational analysis, we suggest that flexibility in DNA-binding may be critical to Ste12 function. These findings increase our understanding of the interactions between complex traits, while also revealing a possible mechanism for the evolutionarily conserved link between invasion and sexual reproduction.

Y491 Meiotic Crossing Over Requires Attenuation of an Intrinsic Degron in the MutS Homolog Msh4. *Neil Hunter*[1], Wei He[1], Prasada Rao[1], Shangming Tang[1], Nikhil Bhagwat[1], Dhananjaya Kulkarni[1], Maria Chang[1], Christie Hall[1], Xiangyu Chen[2], Nancy Hollingsworth[2], Lepashki Singh[3], Petr Cejka[3]. 1) Univ California, Davis, Davis, CA and the Howard Hughes Medical Institute; 2) Stony Brook Univ., New York, NY; 3) University of Zurich, Zurich, Switzerland.

Crossing over is essential for the faithful disjunction of homologous chromosomes during the first meiotic division. Regulatory processes that control crossing over remain poorly characterized. Our recent studies indicate that regulated protein destruction is a key aspect of crossover control. Here, we show that the pro-crossover function of the conserved meiosis-specific recombination factor, MutSγ (a complex of Msh4 and Msh5), requires its stabilization via phosphorylation. Our analysis reveals that Msh4 is an intrinsically unstable protein by virtue of a disordered domain in its N-terminus, which targets it for proteasomal destruction. The degron activity of this domain is attenuated by its phosphorylation, which is essential for the crossover activity of MutSγ. Msh4 phosphorylation requires the initiation of recombination and chromosome synapsis. These and other genetic requirements suggest that Msh4 phosphorylation occurs *in situ* at sites of recombination. Consistent with this idea, we show that Msh4 phosphorylation is mediated by the chromatin-based kinase, DDK (Dbf4-Dependent Kinase, a.k.a. Cdc7-Dbf4). We suggest a model in which the intrinsic instability of a subset of meiotic recombination factors enables crossover control via differential stabilization at crossover versus non-crossover recombination sites.

Y492 The ascus persists after post-germination budding and influences bud-vs-mate decisions in *S. cerevisiae*. *M. A. McMurray*, L. R. Heasley. Univ Colorado Anschutz Medical Campus, Aurora, CO.

Diploid *Saccharomyces cerevisiae* cells undergo meiosis and sporulation during nutritional deprivation. Unlike many other fungi, *S. cerevisiae* spores remain encased in the ascus, a sac-like structure derived from the vegetative cell wall of the pre-meiotic mother cell, unless environmental conditions degrade the ascus and promote spore dispersal. Upon exposure to nutrients, spores germinate (i.e., partially break down the spore wall) and either begin mitotic proliferation immediately, or mate with other cells and then proliferate mitotically. Homothallic spores have the additional option of mating-type switching after the first haploid budding event and then mating with the bud or its progeny. If the original diploid cells are heterozygous at important loci, these three options (mate, bud, or bud/switch/mate) have drastically different consequences for the possible allelic combinations following germination and, thus, for fitness. Although this basic question has been considered from a genetic perspective for many decades, the basic cell biology of germination is surprisingly understudied. It is widely assumed in the literature that the act of budding reflects or induces "global" breakdown of the ascus wall that liberates all constituent spores simultaneously. Strikingly, we find, instead, that budding and mating reflect "local" remodeling of the ascus, such that individual spores can bud or mate "out" of the ascus without liberating their sisters. To address the influence of ascus persistence during germination on the bud-vs-mate decision, we developed three independent assays: (i) the Sex with Sister Selection (SwiSS), which allows colony growth only when a spore mates with its sister; (ii) the Germination Abstinence eNforcer (GermAN), which allows colony growth only when a spore avoids mating; and (iii) appropriate colony color markers to visualize mating between sister spores as half-sectored colonies. These approaches demonstrate that an intact ascus promotes mating as the first post-germination event. Together with our observations of ascus perdurance after budding, these

findings uncover a hitherto unappreciated role for the ascus in the control of genetic stability through the yeast life cycle. As vegetative conditions influence cell wall durability, ascal control of post-germination events may create a regulatory circuit in which environmental conditions prior to sporulation dictate the duration of ascus persistence and, consequently, the extent of intra-ascus mating. In addition to the poorly understood *S. cerevisiae* ecological niches, these mechanisms are likely at play in certain industrial settings, such as vinification.

Y493 Kar4p regulates meiosis at both the transcriptional and translational levels. *M. D. Rose*, M. Remillard, A. Sporer. Princeton University, Princeton, NJ.

Yeast cells undergo several pathways of differentiation, including mating and meiosis. Whereas mating is regulated by exposure to pheromones, meiosis is regulated by integration of multiple signaling pathways that assess the cellular state and nutritional environment. Kar4p is required for both mating and meiosis. Kar4p is a conserved member of the MT-A70 family of mRNA adenine-methyl transferases. In humans, mRNA methylation is carried out by the MT-A complex comprising METTL3, METTL14 and WTAP. In yeast, mRNA methylation is catalyzed by the MIS complex comprising Ime4p, Slz1p and Mum2p. Ime4p is homologous to METTL3 and Mum2p is homologous to WTAP. Although Kar4p is homologous to METTL14, it contains mutations in the active site that would render it inactive. During mating, Kar4p interacts with the transcription factor Ste12p to activate expression of genes required for nuclear fusion. However, Kar4p's role in meiosis was unknown. To analyze Kar4p's separate functions in mating and meiosis, we isolated 28 mating (Mat^-) or meiosis (Mei^-) specific mutations. The two sets of alleles map to non-overlapping surfaces on a predicted protein structure. All 13 Mat^- alleles abolished Kar4p's interaction with Ste12p. *kar4*Δ and the Mei^- alleles blocked entry into meiosis, prior to S-phase and recombination. Overexpression of Ime1p, the major meiotic transcriptional activator, suppressed the Mei^- defect. Transcriptional profiling revealed that ~100 Ime1p-dependent transcripts are strongly decreased and/or delayed in the *kar4*Δ mutant. Ime1p overexpression suppressed the *kar4*Δ early transcription defect. However, Ime1p overexpression was not sufficient to complete sporulation. Approximately half of the mutants were also defective for sporulation (Spo^-), becoming blocked after S-phase and recombination. The Spo^- phenotype was not correlated with Mat^- or Mei^- alleles, implying that the Spo^- alleles serendipitously affect yet another function of Kar4p. Overexpression of *RIM4*, a meiotic translational regulator, efficiently suppressed the Spo^- defect. Consistent with a translational role, Ime2p levels were strongly reduced in the *kar4*Δ Spo^- mutant, although *IME2* mRNA was expressed normally. Rim4p overexpression restored Ime2p expression to normal levels. Overexpression of both *IME1* and *RIM4* in *kar4*Δ cells allowed rapid and efficient sporulation, effectively recreating the transcriptional profile of wild-type. Taken together, these data suggest that Kar4p regulates meiosis and sporulation in two different ways, first by enhancing the activity of Ime1p at a subset of its transcriptional targets and second by enhancing the activity of Rim4p for the translation of Ime2p and other genes.

Y494 A cytokinesis checkpoint. *Eric Weiss*, Jennifer Brace. Northwestern Univ, Evanston, IL.

The steps of cell division happen in precise sequence, even though late processes often don't inherently require successful completion of preceding ones. In the final stage of division, for example, cells are cut into separate entities by strictly ordered morphological rearrangements of cytokinesis and abscission. It's not clear how cells enforce the exact sequence of these events. In budding yeast cell separation actomyosin ring contraction is closely coordinated with synthesis of a septum that's immediately degraded once it's completed. How do cells avoid destroying the septum before it's finished? We find that cytokinetic mutants that significantly delay septum formation are highly sensitive to overproduction of the cell separation chitinase Cts1 and are suppressed by *cts1*, indicating that proper control of the cell separation program is critical when septum formation is defective. A signaling system called the RAM network links *CTS1* transcription to mitotic exit. However, we find that transcription of septum destruction genes turns on just before actomyosin ring contraction, and that cells with defective septum synthesis produce the chitinase protein with normal timing but don't secrete it. The dependency of cell separation enzyme secretion on completion of cytokinesis requires Fir1, a largely intrinsically disordered substrate of the RAM network kinase Cbk1. Deletion of *FIR1* eliminates *Cts1* secretion delay in cytokinesis mutants, which exhibit strong negative genetic interaction with fir1. While the RAM network isn't required for anterograde membrane trafficking during growth, Cbk1 promotes secretion of chitinase. *Fir1* inhibits this Cbk1 function as well as other aspects of post-cytokinesis secretion. *Fir1* is normally quickly degraded during cell separation, disappearing from the cytokinesis site after actomyosin ring contraction, but is stabilized when septum formation is abnormal. Thus, an unknown mechanism links Fir1's inhibition of septum destruction enzyme delivery to status of septum formation. Eukaryotes have evolved checkpoint systems that actively block late processes until preceding ones are complete, enforcing dependencies among otherwise mechanistically independent cell cycle events. We propose that a novel checkpoint protects the integrity of cytokinesis by blocking irreversible late steps until the process's early stages are finished, and that Fir1 is a key part of this checkpoint in budding yeast.

Y495 The CWI Pathway Regulates Cell Wall Degradation During Mating. *A. Hall*, M. Rose. Princeton University, Princeton, NJ.

Cell fusion is a universal process in eukaryotic organisms. We are studying the process of cell fusion using mating of the budding yeast, *S. cerevisiae.* To mate, haploid yeast cells detect each other by pheromone signaling, polarize their growth toward the mating partner, and then fuse to form a diploid zygote. The initial step in yeast fusion is the removal of the cell wall between the mating partners, to allow plasma membrane fusion. Because the improper timing or location of cell wall degradation would lead to cell lysis and death, the initiation of cell fusion is a highly regulated process. The signal(s) that indicate when and where cell wall degradation should occur have not been identified. Previous hypotheses proposed that cells may require a high local concentration of mating pheromone, physical contact, an as yet unidentified protein receptor, or a combination of all three. Several pieces of evidence suggest that the cell wall integrity (CWI) pathway may play a role in this signaling. A hyperactive mutation of *PKC1*, a central downstream component of CWI pathway, causes a cell fusion defect. Moreover, the CWI pathway is activated by pheromone-induced morphogenesis. The five transmembrane protein sensors (Wsc1-3p, Mid2p, and Mtl1p) of the

CWI signaling cascade have been suggested to act as mechanosensors. We hypothesize that cell-cell contact relieves CWI signaling to allow cell wall degradation. Loss of *MID2* causes "mating-induced death" after exposure to pheromone. We find that the mating-induced death is due to localized loss of cell wall and plasma membrane integrity. Mutations in several genes required for cell fusion (*FUS1, FUS2, RVS161, CDC42*), but not nuclear fusion (*KAR5*) suppress *mid2*, suggesting that the mating-induced death is due to unregulated cell fusion. Fus2p functions late in mating to regulate cell wall removal and loss of Fus2p causes a block in cell fusion. Deletion of *WSC1* or *MID2* partially suppresses the cell fusion defect of *fus2*Δ,*fus1*Δ, *rvs161-10* and *cdc42-138*. These data suggest a role for the CWI pathway in regulating cell wall degradation during yeast mating. Interestingly, loss of *WSC1* specifically leads to zygote/diploid cell death. Although *fus2*Δ suppresses the phenotype of *mid2*Δ, it does not suppress the defect of *wsc1*Δ. Taken together these data suggest distinct roles for *WSC1* and *MID2* in cell fusion and diploid cell wall integrity.

Y496 Flipping the Switch": ROS-induced degradation of Med13 by SCF^{Grr1} mediates mitochondrial fragmentation and cell death. *D. Stieg*[1], S. Willis[1], J. Scuorzo[2], M. Song[2], K. Cooper[1]. 1) Dept. of Molecular Biology, Rowan SOM, Stratford, NJ; 2) Dept. of Medicine, Rowan SOM, Stratford, NJ.

In response to stress, the yeast[1] and mammalian[2] cyclin C translocate from the nucleus to the cytoplasm where it associates with the GTPase Drp1/Dnm1 to drive mitochondrial fragmentation and apoptosis. Therefore, the decision to release cyclin C represents a key life or death rubicon. In unstressed cells, the cyclin C-Cdk8 kinase regulates transcription by associating with the Mediator of RNA polymerase II. We previously reported that that the Mediator component Med13 anchors cyclin C in the nucleus[3]. Loss of Med13 function leads to constitutive cytoplasmic localization of cyclin C resulting in fragmented mitochondria, hypersensitivity to stress and mitochondrial dysfunction due to loss of mtDNA. Recently we showed that this molecular switch operates in a two step process. First, efficient cyclin C nuclear release requires its ROS-induced phosphorylation by the MAP kinase Slt2[4] in a carboxyl terminal region of cyclin C that includes a putative Med13 interaction site. Also, exposure of cells to high ROS damage activates the AMP kinase Snf1 that promotes cyclin C release through an indirect mechanism. The second step involves ROS-induced Med13 destruction by the SCF^{Grr1} ubiquitin ligase. Med13 associates with Grr1 in two-hybrid assays and SCF mediated degradation of Med13 requires active cyclin C-Cdk8. Taken together, these results indicate that the cell gauges damage severity by sequential activation of multiple signaling pathways. These results are consistent with a model in which cyclin C phosphorylation permits its disassociation from Med13 and that Med13 destruction allows full cyclin C release and prevents reaccumulation of the cyclin in the nucleus.

[1]Dev. Cell. 2014, 28:161; [2]Mol. Biol. Cell. 2015, 26:1030; [3]Mol. Biol. Cell, 2104 25:2807; [4]Mol. Biol. Cell. 2014, 25:1396.Grant support W.W. Smith Charitable Trust (CO 604) and NIH R15-113196 to K.F.C.

Y497 Protein sequestration after genotoxic stress regulates splicing. *Peter Stirling*[1,3], Annie Tam[1], Christopher Hughes[2], Gregg Morin[2,3], Veena Mathew[1]. 1) Terry Fox Laboratory British Columbia Cancer Agency, Vancouver, BC, CA; 2) Genome Sciences British Columbia Cancer Agency, Vancouver, BC, CA; 3) Department of Medical Genetics University of British Columbia, Vancouver, BC, CA.

The cellular response to stress is coupled to an array of dynamic events. In response to DNA damaging stresses; DNA repair proteins move to sites of damage, the transcription machinery is modified, RNAs are organized into stress granules and P-bodies and proteins can be sequestered in aggregates. These dynamic changes collaborate to repair DNA, and alter the transcriptome and proteome to enable stress recovery. We identified an unexpected relocalization of the splicing factor Hsh155 to both intranuclear (INQ) and cytoplasmic (CytoQ) sites of protein quality control (PQC) upon exposure to the genotoxin methyl methanesulfonate (MMS). Hsh155 localization to PQC sites is promoted by molecular chaperones Hsp42 and Btn2 and is suppressed by the action of the Hsp104 disaggregase and the INQ component Apj1. Hsh155 protein is stable after MMS treatment and, like other aggregates, new translation is required for Hsh155 relocalization. Functionally, Hsh155 localization to PQC sites is correlated with a precipitous drop in splicing efficiency. To extend previous microarray studies, we conducted whole proteome analysis after MMS treatment and observed specific depletion of ribosomal proteins, whose mRNA products together command the largest need for spliceosomal function in yeast. We propose a model in which sequestration of Hsh155 is required to regulate splicing activity when ribosomal protein gene synthesis is arrested by genotoxic stress. These analyses reveal a novel function of PQC structures and highlight protein sequestration as a potential tool in the dynamic cellular control of the transcriptome and proteome under stress.

Y498 The DNA damage checkpoint targets the exoribonuclease, Xrn1, in response to damage. *J. P. Lao*[1], K. M. Ulrich[1], A. A. Vashisht[2], J. R. Johnson[1], N. J. Krogan[1], J. A. Wohlschlegel[2], D. P. Toczyski[1]. 1) University of California, San Francisco, San Francisco, CA; 2) University of California, Los Angeles, Los Angeles, CA.

Cells incur DNA damage from both endogenous cellular metabolism and exogenous sources. The highly conserved DNA damage response (DDR) pathway monitors the genomic integrity of the cell and protects against genotoxic stresses. Genome instability is a common characteristic of cancer cells, and components of the DDR machinery are often mutated in cancer. In *Saccharomyces cerevisiae*, DNA damage activates the sensor kinases, Mec1 and Tel1 (ATR and ATM in human, respectively). The response is further amplified by activation of the effector kinase, Rad53, to regulate a variety of cellular processes, including cell cycle progression, DNA damage repair, chromatin remodeling, and transcription.

To improve our understanding of the global response to DNA damage, we sought to identify targets of Rad53 using a mass spectrometry-based phosphoproteomic screen in a strain deleted for phosphatases known to counteract checkpoint kinases. Of the top 40 hits, we found that 17 display DNA damage-dependent mobility shifts on SDS-PAGE gels and determined that Rad53 phosphorylates 22 out of the 40 hits by *in vitro* kinase assay. Many of the hits are proteins that are involved in RNA metabolism. In particular, we are following up on Xrn1, a highly conserved 5' exoribonuclease, which functions primarily in mRNA decay. Xrn1 is modified upon DNA damage in a checkpoint-dependent

manner and is directly phosphorylated by Rad53 *in vitro*. We used SILAC to identify proteins that interact with Xrn1 in a *RAD53*-dependent manner. Preliminary data suggests that the association of Lsm1 and Lsm3 with Xrn1 is reduced in a *rad53Δ*. In addition, we have found that at least one member of the decapping complex rescues the strong damage sensitivity of an *xrn1*Δ. We are currently conducting an unbiased screen to identify other suppressors of this mutant to determine the pathway relevant to its damage sensitivity. We hypothesize that the DDR regulates mRNA turnover by targeting Xrn1 in response to damage.

Y499 The lysine acetyltransferase NuA4 regulates glucose-deprived stress granule formation through cellular acetyl-CoA levels. *S. Huard*[1,2], M. Rollins[1,2], A. Morettin[3], J. Takuski[1,2], M. Fullerton[2], J. Côté[3], K. Baetz[1,2]. 1) Ottawa Institute of Systems Biology; 2) Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Canada; 3) Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada.

Eukaryotic cells form cytoplasmic RNA-protein aggregates or stress granules (SGs) under a variety of stress conditions and their formation is associated with both neurodegenerative diseases and cancer. For each stress condition distinct stress-activated signaling pathways regulate SG formation, however the molecular details of these pathways remain largely unknown. We have determined that the *Saccharomyces cerevisiae* lysine acetyltransferase complex NuA4 is required for SG formation specifically upon glucose deprivation but no other stresses tested. Similarly the Tip60 complex, the human homolog of the yeast NuA4 complex, is also required for SG formation in human cell lines indicating that NuA4/Tip60 is a conserved signaling pathway regulating SG dynamics. Surprisingly we found that the impact of NuA4 on glucose-deprived SG formation is not through the regulation of core SG protein levels, or inhibition of translation, rather it is through regulation of acetyl-CoA levels. Cells in which the Acetyl-CoA synthetase *ACS1* is deleted, which have decreased acetyl-CoA levels, display increased SG formation upon glucose deprivation. In agreement, cells in which the Acetyl-CoA carboxylase *ACC1* is mutated or in cells exogenous treated with acetate, which have increased acetyl-CoA levels, glucose deprived SG formation is suppressed. Like NuA4, acetyl-CoA levels appear to only contribute to SG formation upon glucose deprivation. Remarkably we determined that mutants of NuA4 have increased acetyl-CoA levels, decreased Acc1 activity and mislocalized Acc1-GFP. Our observations indicate that NuA4 is regulating acetyl-CoA levels through Acc1 and that acetyl-CoA is acting as a signaling rheostat for SG formation upon glucose deprivation.

Y500 The Quick and the Dead: Single-cell Demography at the Yeast Thermal Limit. *P. M. Magwene*, C. S. Maxwell. Duke University, Durham, NC.

What factors limit where a microbial species grows and thrives? Population growth rate is a function of two demographic parameters – rates of birth and death. Analyzing changes in birth and death rates in response to environmental stress can help to identify physiological constraints that limit microbial niches; however measuring them in microbial populations is challenging. In order to overcome this obstacle, we developed a novel cellular mark-release-recapture technique called "TrackScar" that enables single-cell measurement of age-structured birth and death rates in budding yeast. We used this method to study the demographic and physiological factors that limit population growth during heat stress in a genetically diverse panel of *Saccharomyces cerevisiae* strains. We find that the average population growth rate during stress is often a poor predictor of the behavior of individual cells because of heterogeneity in fecundity. Age-structured mortality is one cause of this heterogeneity and is also highly variable across strains. Some genetic backgrounds senesce prematurely during heat stress, while others show the opposite pattern, with elevated rates of early life mortality. Age structuring of birth and death rates points to potential genotype-by-environment effects on processes that regulate asymmetric cell division; consistent with this we find that a mitochondrial inheritance defect explains the early life mortality phenotype of one of the strains we studied. This study demonstrates that characterizing the behavior of individual cells is critical to understanding the physiology of microbes and highlights how the interplay of cellular physiology, genetic variation, and environment influences where microbial populations survive and flourish.

Y501 The RSC complex functions to maintain ploidy in *Saccharomyces cerevisiae*. *T. L. Sing*[1,2], M. P. Hung[1,2], S. Ohnuki[3], J. Ou[1,2], B. J. San Luis[2,4], M. Costanzo[2,4], C. Boone[2,4], Y. Ohya[3], G. W. Brown[1,2]. 1) Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada; 2) Donnelly Centre for Cellular and Biomolecular Research, Toronto, Ontario, Canada; 3) Department of Integrated Biosciences, University of Tokyo, Tokyo, Japan; 4) Department of Molecular Genetics, University of Toronto, Ontario, Canada.

Ploidy is tightly regulated in eukaryotic cells, and is critical for cell function and survival. Cells must coordinate multiple pathways to ensure replicated DNA is segregated both accurately and in a timely fashion to prevent changes in chromosome number. Several cellular processes have been implicated in ploidy maintenance, including spindle pole body (SPB) duplication, mitotic spindle formation and kinetochore attachment.

We have discovered an unanticipated role for 6 of 7 non-essential subunits of the RSC (Remodels the Structure of Chromatin) complex in ploidy maintenance. Using flow cytometry, we demonstrated that deletion of *RSC1, RSC2, RSC30, LDB7, NPL6,* or *HTL1,* but not *RTT102,* causes a rapid transition from haploid to diploid DNA content following germination of haploid mutant spores. Unlike normal diploids that arise from mating, *rscΔ* mutant diploids retained the ability to mate, indicating that diploidization is the result of autopolyploidization rather than mating-type switching. Interestingly, morphological analysis revealed that diploidized *rscΔ* mutants have an elongated bud phenotype, which makes them distinct from wild type diploids.

Microscopic analysis revealed that *rscΔ* mutants do indeed have defects in SPB duplication and maturation that explain the increase-in-ploidy phenotype. Using RNA sequencing, we showed that these defects were not due to obvious changes in transcript levels in the *rscΔ* mutants. Therefore, we performed synthetic genetic array (SGA) analysis, which implicated the RSC complex in regulating proteins important for inserting newly synthesized SPBs into the nuclear envelope. Surprisingly, we were unable to detect physical interactions between the RSC

complex and SPBs by yeast 2-hybrid (Y2H) or bimolecular fluorescence complementation (BiFC). Instead, evidence has lead us to a model where the RSC complex facilitates nuclear pore complex (NPC) stability and/or localization, which is necessary for proper SPB insertion.

Understanding how the RSC complex regulates ploidy will contribute to an integrated view of how multiple pathways converge to maintain the haploid state and propagate it from one generation to the next.

Y502 Yeast Genetics Meeting Lifetime Achievement Award. *J. R. Broach*. Penn State College of Medicine, Hershey, PA.

no abstract submitted

Y503 A comprehensive genotype-fitness map of adaptation-driving mutations in yeast. *Barbara Dunn*[1], Sandeep Venkataram[2], Yuping Li[2], Atish Agarwala[3], Jessica Chang[1], Emily Ebel[2], Kerry Geiler- Samerotte[2], Lucas Herissant[1], Sasha Levy[4], Jamie Blundell[4,5], Daniel Fisher[5], Gavin Sherlock[1], Dmitri Petrov[2]. 1) Dept of Genetics, Stanford Univ, Stanford, CA, USA; 2) Dept of Biology, Stanford Univ, Stanford, CA, USA; 3) Dept of Physics, Stanford Univ, Stanford, CA, USA; 4) Dept of Biochem & Cell Biol, Stony Brook Univ, Stony Brook, NY, USA; 5) Dept of Applied Physics, Stanford Univ, Stanford, CA, USA.

Adaptive evolution is a major driving force behind the phenotypic variation observed in nature, and recent advances in high-throughput sequencing now make possible the deep sampling of adaptive events during the course of a controlled evolution experiment. We developed a DNA barcoding approach to track over time the changes in population frequency of ~500,000 otherwise identical clones of the budding yeast *Saccharomyces cerevisiae*, and have monitored these clonal "fitness trajectories" for up to ~250 generations under a glucose limited regime (Levy, Blundell, et al., Nature, 2015). In this study, we have isolated 4,800 independently evolved clones from the 88 generation timepoint of the barcoded evolution, when most adaptive clones are likely to contain only a single adaptive mutation, and have developed an assay to assign fitness values to each individual clone in a highly parallel manner. This allows us to determine if the clone is adaptive (carries a presumptive adaptive mutation) or non-adaptive (carries either neutral or no mutations). We performed whole genome sequencing on hundreds of individual known adaptive clones, as well as many neutral clones as controls, and have identified many of the adaptive mutations that have independently arisen in these evolutions. There are two major classes of adaptive mutations: (1) self-diploidization, conferring an average fitness benefit of ~3%, and (2) mutations in the nutrient-responsive Ras/PKA and TOR/Sch9 pathways, conferring fitness benefits of ~5% to ~15%, and ~5% to 10%, respectively. Our large sample size and precision of measurement have allowed us to observe that the differential fitness benefits conferred by these mutations are dependent on the affected pathway, the individual gene, and even the type of mutation within a single gene. Additionally, we observe consistent differential fitness advantages for clones carrying mutations in paralogous genes; for example, *IRA1* mutations confer on average a higher fitness advantage than do *IRA2* mutations, while *GPB2* mutations confer higher fitness benefits than *GPB1* mutations. In summary, we have been able to link the specific molecular targets of adaptation to their fitness effects and build a comprehensive genotype-fitness map of the adaptive mutations that drove the initial evolutionary process in this system.

Y504 Extrachromosomal Circular DNA – A Key Player in Creation of Copy Number Variation? *Henrk D. Møller*[1], Tobias Mourier[2], Lance Parsons[3], Birgitte Regenberg[1]. 1) Department of Biology, University of Copenhagen, Copenhagen, Denmark; 2) Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark; 3) Lewis-Sigler Institute for Integrative Genomics, Princeton University, United States.

Numerous human cancer types are caused by copy-number variations (CNVs) of proto-oncogenes. Yet, detecting chromosomal CNVs before they reach establishment in large cell populations is a major challenge. By screening for a potential deletion-byproduct of CNVs, the so-called extrachromosomal circular DNA (eccDNA), we reasoned that we might elucidate some of the early ongoing processes in genomic rearrangements.

To explore the existence of circular DNA in eukaryotes we have developed a highly-sensitive eccDNA purification method, Circle-Seq, that relies on removal of linear DNA and next-generation sequencing of circular DNA. We reveal that CNVs, in the form of eccDNAs, are common in the budding yeast *Saccharomyces cerevisiae.* More than a thousand different eccDNAs larger than 1 kb were recorded in the S288c strain background increasing the known number of eccDNA more than a hundred fold. A number of eccDNAs were found repeatedly in S288c and in the CEN.PK strain background, suggesting conserved hotspots for DNA circularization, e.g. at ribosomal RNA genes, glucose transporter genes *HXT6*, *HXT7,* metallothionein genes *CUP1-1*, *CUP1-2*, Ty-retrotranposons and Y'-telomeric genes. Recording of *HXT6-HXT7* geneotypes indicated selection for [$HXT6/7^{circles}$] in continued yeast cultures under glucose-limitation and [$HXT6/7^{circles}$] also appeared to serve as intermediates of chromosomal amplifications. Sequenced recombination junctions of circular Ty-elements revealed that retrotransposons can form by recombination both within the genome and during the classical retrotranspositional life cycle.

Our results suggest that eccDNAs are frequent genomic rearrangements in *S. cerevisiae* that lead to deletions and transient gene amplifications. Consequently, *S. cerevisiae* is a useful model organism for studying the molecular basis and evolutionary consequences of eccDNA.

Y505 Deciphering common principles governing gene replaceability in yeast. *Aashiq H. Kachroo*[1], Jon M. Laurent[1], Azat Akhmetov[1], Edward M. Marcotte[1,2,3]. 1) Center for Systems and Synthetic Biology, Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712, USA; 2) Center for Computational Biology and Bioinformatics, University of Texas at Austin, Austin, TX 78712, USA; 3) Department of Molecular Biosciences, University of Texas at Austin, Austin, TX 78712, USA.

Owing to common ancestry, organisms share genes across vast evolutionary distances, with many eukaryotic genes shared among humans, yeast, plants, and even bacteria. "Swapping" such orthologous genes between species gives a simple, reasonably high-throughput, method for

testing conserved functions and learning principles of replaceability. Recently, we showed that several hundred genes in yeast can be "humanized", in the process learning some of the rules that govern functional replaceability. Notably, we found that replaceability is determined at the level of gene modules, such that genes in the same pathway or complex are often either largely replaceable or not (Kachroo *et al*. (2015) *Science*, 348:921-925).

However, it is unknown if these trends are truly general in nature and apply equally well to other species. To learn general rules of replaceability, we have now replaced (one gene at a time) a large set of budding yeast (*S. cerevisiae*) genes with their orthologs from bacteria (*E. coli*), and to a lesser extent, from plants (*A. thaliana*). We find that a large proportion (roughly half) of the bacterial or plant genes tested can successfully replace their yeast orthologs and complement an otherwise lethal loss of an essential yeast gene. As with our systematic tests of yeast humanization, we observe genes in the same pathway and complex to be similarly replaceable or not. Strikingly, individual genes of the yeast heme biosynthesis pathway are almost entirely replaceable by their orthologs from *E. coli*, plants, or humans, indicating that this ancient pathway has remained functionally intact and largely unaltered—at least in terms of its critical, enzymatic functionality—since the divergence of the ancestors of modern eubacteria and eukaryotes. Finally, we'll describe our extensions of this work, testing for the effects of mitochondrial localization, and using CRISPR-based integration of the donor genes directly into the yeast chromosomes, to more finely control the human/plant/bacterial-ized genes' expression patterns.

Y506 The 1002 yeast genomes project. *J. Schacherer*[1], J. Peter[1], M. De Chiara[2], D. Pflieger[1], JX. Yue[2], A. Bergstrom[2], A. Sigwalt[1], A. Llored[2], K. Freel[1], S. Engelen[3], A. Lemainque[3], P. Wincker[3], A. Friedrich[1], G. Liti[2]. 1) University of Strasbourg, Strasbourg, FR; 2) Institute of Research on Cancer and Ageing, Nice, FR; 3) Institut de Génomique - Genoscope, Evry, FR.

Genome-wide investigation of the patterns of polymorphism in a large sample of individuals is the first step to assess the relationship between genotype and phenotype within a species. To date, yeast population genomics only focused on a limited number of isolates. In this context, we initiated a project with the goal of describing whole-genome sequence variation in more than 1,000 natural *S. cerevisiae* genomes, avoiding monosporic strains (http://1002genomes.u-strasbg.fr/). Genomes were sequenced using an Illumina HiSeq 100-bp paired-end strategy that yielded a 200-fold coverage on average. Sequenced strains were selected to include as much diversity as possible in terms of global locations (including Australia, Europe, Russia, Vietnam and South Africa), as well as ecological sources (such as dairy products, trees, insects, flowers, fruit and wine). In addition, almost 1,000 were phenotyped on different conditions impacting various physiological and cellular responses, including different carbon sources, membrane and protein stability, signal transduction, sterol biosynthesis, transcription, translation, as well as osmotic and oxidative stress. In total, we analyzed 34,740 measurements for 36 traits.

Due to the broad diversity of isolates selected, this population genomic dataset revealed an accurate picture of the genomic variation (*i.e.* ploidy, aneuploidy, copy number and gene content variation). We found that genomic variations are correlated with the ecological origin of the isolates but also have a direct and general impact on fitness. Concerning the single nucleotide polymorphisms, a total of 58,912,916 high-quality SNPs were detected across the 1,011 genomes, which are distributed over 1,625,809 polymorphic positions. The frequency spectrum of the observed polymorphisms is highly skewed towards an excess of low-frequency alleles. The heterozygosity level also depends on the ecological origin and losses of heterozygosity are considerable in *S. cerevisiae*, with an average of 21 regions covering ~5 Mb per heterozygous genome.

Overall, our study led to a comprehensive view of the multiple genome evolution patterns across subpopulations within the *S. cerevisiae* species. Furthermore, because we performed extensive phenotyping, the high SNPs density allowed us to perform genome-wide association studies. This dataset led to the identification of a large set of functional polymorphisms that underlie phenotypic variation.

Y507 Integrative Analysis of the Variation in the Regulatory Network Among Strains of Yeast. *R. Srivas*[1], B. Dunn[1], T. Kawli[1], L. Jiang[1], E. Li[1], K. Choe[2], J. Gallagher[3], M. Snyder[1]. 1) Stanford University, Stanford, CA; 2) N. Carolina State Univ, Raleigh, NC; 3) West Virginia University, Morgantown, WV.

Regulation of gene expression is a complex affair involving changes in chromatin structure and histone modifications, as well as coordinated action amongst trans-acting transcription factors (TF). To date the extent to which variation in these regulatory mechanisms can influence gene expression, and thus contribute to phenotypic variation, has been limited to examining each regulatory layer in isolation or in the case of TFs, just one or two factors. Here, we present a unified analysis of the regulatory network in five diverse strains of the yeast S. cerevisiae, including genome-wide measurements of the binding locations of 52 TFs (~25% of all TFs), chromatin accessibility and four histone modifications. We observed a broad-range (between 0.1 and 0.75) in the fraction of binding sites different between strains across all TFs, with highly conserved and essential TFs displaying significantly lower binding variability. Variable binding regions and chromatin states were enriched for single-nucleotide variants, and coordinated changes across multiple regulatory layers, in contrast to any single data type, were highly correlated with differences in gene expression. Our data provide a unique resource to examine the impact of sequence-level variation across multiple layers of gene regulation.

Y508 Comparative translatomics reveal a conserved class of noncanonical uORFs in yeast. *Joel McManus*[1], Armaghan Naik[1], Pieter Spealman[1], Gemma May[1], Scott Kuersten[2], Lindsay Freeberg[2], Robert Murphy[1]. 1) Carnegie Mellon University, Pittsburgh, PA; 2) Illumina, Inc., Madison WI.

Upstream Open Reading Frames (uORFs) have emerged as major *cis*-acting elements involved in regulating mRNA turnover and translation (Wethmar, 2014; Ingolia 2014). We and others have found that genetic variation in putative uORFs has contributed to variation in translation in yeast (McManus et al., 2014), mice (Hou et al., 2014), and humans (Cenik et al., 2015). Furthermore, mutations in uORFs are associated with

human disease (Calvo et al., 2009). Functional uORFs can be challenging to identify, as they often reside in alternative regions of mRNA transcript leaders, and have limited sequence conservation. Curiously, recent work has uncovered a large number of uORFs initiating with non-AUG start codons, with functions implicated in development and stress responses (Ingolia et al., 2009; Brar et al., 2011). While the catalog of such noncanonical uORFs continues to increase, little is known about their functions and evolution.

To study the evolution of uORFs, we used targeted RNA-sequencing to map transcript start and end sites in all five *Saccharomyces sensu stricto* yeast species. Comparison of UTR lengths in these species revealed that genes involved in translation and glycolysis have highly conserved transcription start and end sites, many that have remained entirely stable over 15 million years. Many genes contain conserved alternative transcription start and end sites, suggesting transcriptional regulation of UTR sequences. As expected from prior work, "AUG" triplets are depleted from 5' UTRs in each species. Surprisingly, we find that "UUG" is the most enriched triplet in 5' UTRs, suggesting selective pressure to maintain uORFs initiating with this codon. To investigate the importance of UUG enrichment, we searched 5' UTRs for functional uORFs using ribosome profiling data from *S. cerevisiae*, *S. paradoxus*, and *S. uvarum* using a novel machine learning algorithm (uORF-seeker). This analysis identified both conserved and species-specific AUG- and UUG-uORFs in hundreds of orthologous 5' UTRs. Interestingly, conserved UUG uORFs are highly enriched in genes involved in regulating cellular growth during stress. Our results show that UUG- and AUG-uORFs have similar rates of conservation and implicate UUG-uORFs in repressing cellular growth genes in response to stress.

Y509 Mating-type switching in the methylotrophic yeast *Hansenula polymorpha* is regulated by yeast mating and differentiation pathways. *Sara Hanson*, Kevin Byrne, Kenneth Wolfe. Conway Institute and School of Medicine & Medical Science, University College Dublin, Dublin, IE.

Changes in environmental conditions can result in remarkable variations in the expression of phenotypes and the induction of differentiation processes. An important factor distinguishing *Saccharomyces cerevisiae* from many other yeast species is the role of environmental signals in the induction of sexual processes. Natural isolates of *S. cerevisiae* are primarily diploid (diplontic lifestyle). An environmental cue (nitrogen starvation) is required to induce them to sporulate, but the resulting haploids can then go through mating-type switching and mating without any additional environmental input. In contrast, many other yeasts such as the methylotrophic species *Hansenula polymorpha* are primarily haploid (haplontic lifestyle). In these species mating-type switching, mating, and sporulation are coordinately induced by nitrogen limitation. Mating-type switching in *H. polymorpha* occurs through a two-locus chromosomal inversion mechanism that is ancestral to the well-characterized three-locus MAT/HML/HMR mechanism in *S. cerevisiae*. Although nitrogen limitation has been identified as the primary nutritional signal, the molecular pathway from this signal to mating-type switching in *H. polymorpha* is unknown. We surveyed *H. polymorpha* strains and found one strain that is unable to switch mating-types. This strain was crossed with a switching strain and bulk segregant analysis revealed a frameshift in the transcription factor *EFG1*, homolog of *S. cerevisiae PHD1* and *SOK2*, as the causative mutation. Although *EFG1* has a well-characterized role as a key regulator of filamentous growth and the white-opaque phenotypic switch in *Candida albicans*, it was not previously known to have a direct role in mating in any species. Through functional genetics and mRNA-seq analysis, we have found that *EFG1* is part of a transcriptional network that integrates components of the *S. cerevisiae* mating and *C. albicans* differentiation pathways to control mating-type switching and mating responses in *H. polymorpha*.

Y510 The 3D organization of the diploid *Saccharomyces* genome. *Seungsoo Kim*[1], Ivan Liachko[1], Gurkan Yardimci[1], Kate Cook[1], William Noble[1], Maitreya Dunham[1], Jay Shendure[1,2]. 1) University of Washington, Seattle, WA; 2) Howard Hughes Medical Institute.

The genome is packed into the nucleus in a highly organized manner, and this organization has consequences for transcriptional regulation, DNA break repair, and other nuclear processes. In *Saccharomyces* budding yeasts, the genome is positioned in a Rabl orientation throughout the cell cycle, with centromeres attached to the spindle pole, the nucleolus opposite to the spindle pole, and telomeres at the nuclear periphery. Although various studies have suggested additional features of *Saccharomyces* budding yeast nuclear organization, such as clustering of tRNA genes and origins of replication, genome-wide chromatin conformation capture (Hi-C) data from haploids lack evidence of long-range interactions driven by DNA sequence and can largely be explained by simple polymer models simulating a Rabl configuration. Further, although FISH- and recombination-based studies have suggested mitotic pairing of homologous chromosomes in diploid yeast, whether mitotic pairing occurs remains controversial and has not been interrogated in a genome-wide manner. To systematically uncover the 3D organization of the diploid budding yeast genome, we performed Hi-C on interspecific hybrids of *S. cerevisiae*, *S. paradoxus*, and *S. uvarum* during exponential growth and stationary phase. Here, we report that the organization of diploid *Saccharomyces* genomes, unlike those of haploids, substantially deviates from the simple polymer model. First, the homologous chromosomes carrying the rDNA arrays interact preferentially, even at their centromeres. Although preferential pairing of sequences near the rDNA arrays could be predicted by simple polymer models, the pairing of the centromeres is less easily explained. Second and even more surprisingly, we find that specific homologous sequences on chromosome 13 interact with each other, only in stationary phase. A deletion of one copy of this region in *S. cerevisiae* x *S. uvarum* eliminated the interaction, and sequentially smaller deletions allowed us to narrow down the source of the interaction to a ~1 kb intergenic region. Unlike previously reported interactions between genes relocated to the nuclear pore complex upon induction, this novel interchromosomal interaction appears to be repressive, as the region is bound by stress-induced transcriptional repressor Xbp1 and is flanked by genes repressed during stationary phase. Finally, we show that in both hybrids of diverged *S. cerevisiae* strains and *S. cerevisiae* x *S. paradoxus* hybrids, interactions between homologous regions are subtly but clearly enriched even after controlling for their distances from centromeres; we conclude that in diploid budding yeasts, mitotic pairing of homologous chromosomes occurs genome-wide.

Y511 A global yeast genetic network maps cellular function. *M. Costanzo*[1], B. VanderSluis[2], E. Koch[2], A. Baryshnikova[3], C. Pons[2], G. Tan[1], C. Myers[2], B. Andrews[1], C. Boone[1]. 1) Univ Toronto, Toronto, ON, CA; 2) Univ Minnesota, Minneapolis, MN; 3) Princeton Univ, Princeton, NJ.

We generated a global genetic interaction network for *Saccharomyces cerevisiae*, constructing 23.4 million double mutants, identifying ~620,000 negative and ~390,000 positive genetic interactions. Our network maps genetic interactions for essential gene pairs, highlighting essential genes as densely connected hubs. Genetic interaction profiles enabled assembly of a hierarchical model of cell function, including modules corresponding to protein complexes and pathways, biological processes, and cellular compartments. Negative interactions connected functionally related genes, mapped core bioprocesses, and identified multifunctional pleiotropic genes. Positive interactions often mapped general regulatory connections among gene pairs, rather than shared functionality. The global network illustrates how sets of genetic interactions occur within and between genes encoding complexes and pathways and how this topology can be exploited to explore genetic interactions underlying human disease.

Y512 A programmable sensor for protein solubility in yeast uncovers ecological prion-switching factors. *G. A. Newby*[1,2], S. Kiriakov[3], C. Kayatekin[1], M. Khalil[3], S. Lindquist[1,2]. 1) Whitehead Institute, Cambridge, MA; 2) MIT, Cambridge, MA; 3) Boston University, Boston, MA.

Protein aggregation is frequently associated with disease and dysfunction, but is also vital to many aspects of normal biology, from meiosis to memory. Unfortunately, protein aggregates are notoriously difficult to compare and quantify. We have developed a synthetic sensor for protein aggregation in yeast by coupling the solubility of a given protein to a fluorescent or enzymatic reporter. This modular platform is amenable to quantitative, high throughput screening and selection, or can be used to measure individual live cells with microscopy. We applied the tool first to study yeast prions, which are heritable, self-templating protein conformations that confer different phenotypes depending on their conformational state. Screening a pool of random mutations in prion genes, we identified dominant mutations in yeast prions that solubilize and cure the endogenous, wild type prion aggregate. One of these mutations is already harbored by rare, wild strains of yeast. We predict that this strain would be immune to a yeast prion and would cure the prions of its mating partners. We then conducted screens of environmental stresses and a natural product library to uncover drugs and conditions that affect prion switching. We identified a number of drugs and conditions that yeast may encounter in their natural habitat and are active in switching their prion state. Encountering these factors would cause a heritable prion switch that would be 'remembered' by the population for generations to come, significantly altering their phenotype. Our technology is also applicable to the analysis of non-prion aggregates. We have utilized the technique to measure sequestration of endogenous cellular proteins by aggregation-prone human disease proteins *in vivo*. We used this to learn about the sequence elements that influence the interaction of the disease aggregate with endogenous proteins. This technique unlocks previously-inaccessible questions in the investigation of protein aggregation.

Y513 Identifying synthetic cytotoxic genetic interactions with DNA damaging therapeutic agents. *Xuesong Li*, Nigel J. O'Neil, Phil Hieter. The University of British Columbia, Vancouver, B.C., CA.

Many tumors contain mutations that affect the DNA Damage Response (DDR) and genome stability. These mutations differentiate tumour cells from normal surrounding tissue and could be targeted by therapeutics that are based on negative genetic interactions with these mutations. While some genetic interactions result in synthetic lethality, other genetic interactions may increase the sensitivity of tumor cells to DNA damaging therapeutic agents, resulting in a class of conditional synthetic lethality we call Synthetic Cytotoxicity (SC). To identify SC interactions, we are screening yeast DDR mutants for SC interactions with several different DNA damaging agents using both a genome-wide array of non-essential mutants and a curated mini-array of mutants enriched for DDR genes. To date, we have screened mutations affecting the DNA damage checkpoint Tel1 (the yeast ATM ortholog) and the four core components of the non-homologous end joining (NHEJ) machinery: Yku70/Yku80 (DNA end–binding Ku heterodimer) and Dnl4/Lif1 (DNA ligase IV) for SC with four different DNA damaging agents: Bleomycin (radio-mimetic), Camptothecin (TopI inhibitor), Cisplatin (interstrand crosslinker), and MMS (alkylating agent). We characterized and investigated the biology of several strong SC interactions and tested for evolutionary conservation in *C. elegans*. Our SC interaction network of DDR pathways will help interpret the complex networks that maintain genome stability after exposure to DNA damaging therapeutic agents, and will provide a valuable resource for the rational design of personalized therapy based on tumour genotypes.

Y514 High throughput protein-protein interaction sequencing using iSeq. *Z. Liu*[1,2], U. Schlecht[3], JR Blundell[1,2,4], R. Bennett[1,2], RW Davis[3], RP St. Onge[3], SF Levy[1,2]. 1) Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, NY; 2) Department of Biochemistry and Cellular Biology, Stony Brook University, Stony Brook, NY; 3) Stanford Genome Technology Center, Department of Biochemistry, Stanford University, Palo Alto, CA; 4) Department of Applied Physics, Stanford University, Stanford, CA.

In *Saccharomyces cerevisiae*, several large-scale efforts have systematically catalogued the protein-protein interactions in a single environment. However, little is known about how the protein interactome changes across genetic or environmental perturbations. Current technologies, which assay one PPI at a time, are too low throughput to make it practical to study these protein interactome dynamics. Here, we develop a massively parallel protein-protein **i**nteraction **Seq**uencing platform (iSeq) using a novel double barcoding system in conjunction with the murine dihydrofolate reductase-based protein-fragment complementation assay (PCA). Random barcodes are inserted into yeast and are mated to existing PCA strains. Mating of barcoded haploid PCA pools and translocation of barcodes *in vivo* and *en masse* yields diploid PCA strains, each with a double barcode representing a specific PPI. In a pilot study, we generated a pool of 2500 strains that represent 100 PPIs, each tagged with 25 unique double barcodes. Growth of this cell pool and sequencing of double barcodes yields an accurate fitness measurement of each double barcode in the pool, which can be translated to an interaction score for each pairwise protein combination. We find that PPI interaction scores using iSeq are highly reproducible across both double barcodes and growth replicates. Under standard growth

conditions, our system detects most previously-reported PPIs. Additionally, we identify many additional putative PPIs with weaker interaction scores. By growing this pool in four different conditions, we identify several dynamic PPIs that reproducibly change in strength across environments. Verification of new and dynamic PPIs is ongoing. Finally, we demonstrate that the iSeq platform is capable of generating and assaying millions of PPIs in parallel. Current efforts are focused on building larger iSeq yeast pools to perform genome-scale dynamic PPI studies.

Y515 Scalable tools for the quantitative analysis of chemical-genetic interactions from sequencing-based chemical-genetic interaction screens. *S. W. Simpkins*[1], J. Nelson[1], R. Deshpande[1], S. Li[2], J. S. Piotrowski[3], C. M. Boone[4], C. L. Myers[1]. 1) Computer Science and Engineering, Univ. of Minnesota, Minneapolis, MN, USA; 2) Center for Sustainable Resource Science, RIKEN, Saitama, Japan; 3) Yumanity Therapeutics, MA, USA; 4) Donnelly Centre for Cellular and Biomolecular Research, Univ. of Toronto, ON, Canada.

Driven by the vision of high-throughput, unbiased, functional chemical screens and enabled by the functional genomics resources available for *S. cerevisiae*, it is now possible to screen tens of thousands of compounds for chemical-genetic interactions in just a few months' time. To fully take advantage of this substantial increase in throughput, scalable computational tools must be developed to assist in both generating and interpreting chemical-genetic interaction profiles from these data. To this end, we developed two computational tools, BEAN-counter and CG-TARGET, the former to convert raw barcode sequencing data into chemical-genetic interaction profiles and the latter to derive actionable mode-of-action predictions from chemical-genetic interaction profiles.

BEAN-counter provides a complete toolset for processing multiplexed sequencing data from barcoded mutant pools into chemical-genetic interaction profiles. The pipeline implements several quality control and normalization steps to detect and remove technical artifacts or other systematic biases. A Python implementation of this pipeline is available at https://github.com/csbio/BEAN-counter.

CG-TARGET is a pipeline for predicting the molecular targets of compounds from chemical-genetic interaction profiles. It leverages the phenomenon that a compound's chemical-genetic interaction profile will be similar to the genetic interaction profile of its target(s); as such, CG-TARGET uses a reference genetic interaction network to interpret the provided chemical-genetic interaction profiles. Target predictions are first made at the level of individual genes, followed by aggregation of these individual gene scores into process or pathway prediction scores in order to improve statistical confidence. An R implementation of this pipeline is available at https://github.com/csbio/CG-TARGET.

These computational toolsets have been applied to analyze more than 18,000 chemical-genetic interaction screens in *S. cerevisiae*. Validation experiments provide convincing evidence that CG-TARGET has the power to predict a compound's mechanism of action at the resolution of biological processes or pathways from a single chemical-genetic interaction profile. These pipelines have enabled the functional annotation of thousands of compounds and are readily adaptable to other compound collections and mutant libraries.

Y516 One library to make them all: streamlining the creation of yeast libraries via a SWAp-Tag strategy. *U. Weill*[1], I. Yofe[1], M. Meurer[2], S. Chuartzman[1], E. Zalckvar[1], O. Goldman[1], S. Ben-Dor[1], C. Schütze[3], N. Wiedemann[3], M. Knop[2], A. Khmelinskii[2], M. Schuldiner[1]. 1) Weizmann Institute of Science, Rehovot, IL; 2) Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), DKFZ-ZMBH Alliance, Heidelberg, Germany; 3) Institut für Biochemie und Molekularbiologie, ZBMZ Universität, Freiburg, Germany.

The yeast Saccharomyces cerevisiae is ideal for systematic studies relying on collections of modified strains (libraries). Despite the significance of yeast libraries and the immense variety of available tags and regulatory elements, only a few such libraries exist, as their construction is extremely expensive and laborious. To overcome these limitations, we developed a SWAp-Tag (SWAT) method that enables one parental library to be modified easily and efficiently to give rise to an endless variety of libraries of choice. To showcase the versatility of the SWAT approach, we constructed and investigated a library of ~1,800 strains carrying SWAT-GFP modules at the amino termini of endomembrane proteins and then used it to create two new libraries (mCherry and seamless GFP). Our work demonstrates how the SWAT method allows fast and effortless creation of yeast libraries, opening the door to new ways of systematically studying cell biology.

Y517 NGS for "No-pain Genetic Screens": Using transposons and Next-Gen Sequencing to unveil all important yeast loci in one go. *A. Michel*, P. Kimmig, B. Kornmann. ETH Zurich, Zurich, CH.

Yeast genetic screens have been absolutely instrumental in our understanding of cell biology. Yet they remain tedious and oftentimes incomplete. Next generation sequencing on the other hand is fast and exhaustive. I have implemented a transposon-based approach combined with deep sequencing to define the complete set of genes that are essential for growth in a particular condition, in one go. The idea is to saturate the yeast genome with independent transposon insertions. Transposons cannot insert in genes that are essential in a given condition. Deep-sequencing of the transposon-genome junctions of the whole library identifies the locations that tolerate the presence of the transposon and allows to deduce those that cannot, revealing the corresponding set of essential genes. The strength of the method lies on the fact that it interrogates the entire genome at once and is readily amenable to multiple growth conditions for comparison. When used to compare different genetic backgrounds, it reveals genetics interactions. When applied to compare drug-treated to untreated cells, it reveals the set of genes conferring resistance or sensitivity to the drug. In addition to identifying essential genes, this method also generates informative alleles. For instance, transposon insertions can yield truncations of essential genes, allowing to map functional protein domains.

Our method thus allows to screen the yeast genome with an unprecedented throughput and resolution.

Y518 Exploring Functional Genetic Suppression Interactions on a Global Scale. *J. van Leeuwen*[1], C. Pons[2], J. Mellor[1], T. Yamaguchi[1], M. Costanzo[1], C. Myers[2], B. Andrews[1], F. Roth[1], C. Boone[1]. 1) University of Toronto, Toronto, ON, Canada; 2) University of Minnesota, Minneapolis, MN, USA.

Functional genetic suppression interactions occur when the phenotypic defects caused by one mutation are rescued by a mutation in a second gene. To explore the principles of genetic suppression, we collected both literature-curated and unbiased experimental data to map a large-scale suppression network among yeast genes. We found that most suppression pairs involve functionally related genes that are often co-annotated or co-expressed, or that are encoding co-localized proteins or members of the same pathway or complex. Although the suppression network includes interactions identified in other genetic networks, most suppression interactions identify novel functional relationships. For example, we exploited the suppression network to assign a possible function as a novel phospholipid scramblase for the previously uncharacterized gene *ANY1* (*YMR0101W*), a detailed mechanistic prediction that could not be made using other available genetic interaction data. Genetic suppression also involved general regulatory mechanisms associated with protein degradation, mRNA decay, and metabolic rewiring. Whole-genome sequencing of >300 yeast strains, many of which were derived from the yeast deletion mutant collection, revealed additional frequent loss-of-function mutations in a set of specific genes, including *WHI2*, *IRA1*, *IRA2*, and *RIM15,* all of which encode negative regulators of Ras signaling and its role in cell cycle progression. These mutations do not affect exponential cell growth but cause a delay in the onset of stationary phase, which appears to select for their enrichment within a propagating population. Our data allow us to formulate some fundamental mechanisms of functional suppression.

Y519 Lee Hartwell Lecture. From Yeast to Worms and Beyond: Folding Dynamic Chromatin. *Susan Gasser*. Friedrich Miescher Institute for Biomedical Research, Basel, CH.

no abstract submitted

Y520 The yeast polo kinase, Cdc5, inhibits cell growth and affects nuclear morphology during a mitotic arrest. *A. D. Walters*, O. Cohen-Fix. NIDDK/NIH, Bethesda, MD.

Normal cells of a given type possess nuclei of a particular size and shape and maintain a constant nuclear:cell volume ratio. It has been known for many years that abnormalities in nuclear size and morphology are hallmarks of cancer and aging. However, our understanding of the factors that determine nuclear size and shape remains poor. In higher eukaryotes, the nuclear envelope (NE) must expand after it is reformed at the end of mitosis to allow for chromosome decondensation. In yeast, which undergo closed mitosis, the NE must expand to allow chromosome segregation and the formation of two daughter nuclei. The mechanisms by which NE expansion is achieved and controlled are unknown.

Work in our lab has shown that when *S. cerevisiae* is delayed in mitosis the NE continues to expand despite the fact that chromosome segregation is paused, and a nuclear extension, or "flare", is formed adjacent to the site of the nucleolus. We hypothesize that flare formation allows maintenance of the nuclear:cell volume ratio in the face of continued NE expansion during an arrest of cell growth. Through a genetic screen, we have discovered that reduced activity of Cdc5, a key cell cycle regulator, causes a no-flare phenotype, where the nucleus remains round during a mitotic arrest. Our previous experiments have shown that Cdc5 is not affecting nuclear morphology through rDNA condensation or any of its other known roles in mitosis. We have also shown that the nuclei of *cdc5-nf* mutants expand isometrically during a mitotic arrest, indicating that the no-flare phenotype is not a result of reduced NE expansion. In our current work, we found that *cdc5-nf* cells grow significantly more than WT during a mitotic arrest, indicating that Cdc5 plays a role in inhibiting cell growth. We tested whether the increased cell growth in *cdc5-nf* is the cause of the no-flare phenotype, reasoning that in WT a flare forms because cell growth is inhibited while NE expansion continues, whereas in *cdc5-nf*, continued cell growth could allow isometric nuclear expansion without disturbing the nuclear:cell volume ratio. We inhibited cell growth in *cdc5-nf* using cycloheximide or mutations in the Sec pathway. However, inhibition of cell growth in *cdc5-nf* did not restore the flared nuclear phenotype. This result demonstrates that Cdc5 plays a role in the inhibition of cell growth and regulation of nuclear morphology through different pathways during a mitotic arrest, and that Cdc5 is required for specifying the site of the nucleolus for nuclear expansion during a mitotic delay.

Y521 Reconstitution of the microtubule nucleation system of *Candida albicans*. *E. Schiebel*[1], T. Lin[1], A. Neuner[1], D. Flemming[2], R. Arkowitz[3]. 1) Center for Molecular Biology, University of Heidelberg, Heidelberg, DE; 2) Biochemistry Center, University of Heidelberg, Heidelberg, DE; 3) Institute of Biology Valrose, University of Nice, Nice, FR.

Microtubules are hollow cylinders composed out of alpha/beta-tubulin with essential functions in cell architecture, cell movement and chromosome segregation in mitosis and meiosis. Microtubules are dynamic polymers that assemble from the heterodimer alpha/beta-tubulin in a process known as microtubule nucleation. gamma-tubulin, a member of the tubulin family, functions in microtubule nucleation by providing a template for the assembly of alpha/beta-tubulin into microtubules. How does gamma-tubulin provide this template function? gamma-tubulin assembles together with the proteins Spc97/Gcp2 and Spc98/Gcp3 into the small gamma-tubulin complex (gamma-TuSC). gamma-TuSC oligomerizes into a left-handed spiral (the alpha/beta-tubulin template) upon interaction with receptors (gamma-TuR) at the yeast spindle pole body (SPB), the microtubule organizing centre (MTOC). Spc110 is one such gamma-TuR on the nuclear side of the SPB while Spc72 has this function on the cytoplasmic side. Therefore, Spc110 and Spc72 are essential for the organization of nuclear end cytoplasmic microtubules, respectively. Budding yeast *Saccharomyces cerevisiae* has the simplest microtubule nucleation system of all model organisms (gamma-TuSC, Spc72 and Spc110). In *Schizosaccharomyces pombe* and higher eukaryotes, microtubule nucleation requires additional factors named GCP4, GCP5, GCP6, the "mitotic-spindle organizing protein associated with a ring of gamma-tubulin 1" (Mzt1) and Nedd1 (*Drosophila* and human cells). Interestingly, the opportunistic pathogen *Candia albicans* represents an intermediate between *S. cerevisiae* and the more complex microtubule nucleation systems of higher eukaryotes. The *C. albicans* genome encodes gamma-tubulin *CaTUB4*, *CaSPC97/GCP2*, CaSPC98/GCP3, the receptors *CaSPC72* and *CaSPC110* and *MZT1*. Here we report the reconstitution of the microtubule nucleation system of *C.*

albicans with purified components and the analysis of their function *in vivo*. This analysis provides important insights into the function of Mzt1 and how *S. cerevisiae* bypassed the requirement of the normally essential *MZT1* gene.

Y522 Capture-shrinkage of astral microtubules by budding yeast dynein in cells lacking cortical endoplasmic reticulum tethering proteins Scs2 and Scs22. *Wei-Lih Lee*, Safia Omer, John Beckford. University of Massachusetts Amherst, Amherst, MA.

Cytoplasmic dynein at the cell cortex exerts pulling forces on astral microtubules powering spindle movements during asymmetric cell divisions in a variety of organisms. In budding yeast, the only mechanism of dynein-mediated “cortical pulling" known to exist is thought to occur via side-on interactions (pulling along the lattice of microtubules), as exhibited by sliding of astral microtubules along the cortex during spindle movements across the mother-bud neck. Whether dynein mediates end-on capture-shrinkage of microtubules and how dynein is regulated between side-on and end-on interactions with microtubules are not known. Here we show by direct quantitative imaging that dynein uses end-on pulling mechanism for spindle positioning in cells lacking the cortical endoplasmic reticulum (ER) attachment molecules Scs2 and Scs22. We found that *scs2Δ scs22Δ* cells displayed a dramatic loss of cortical Num1 patches (dynein anchorage sites), exhibiting fewer than four Num1-GFP patches compared to >10 in wild-type cells. Quantitative immunoblot analysis of Num1 protein revealed that its level was unaffected by deletions of *scs2* and *scs22*, indicating that the loss of Num1-GFP patches was not due to a defect in protein stability. Interestingly, despite being at a lower density, the remaining Num1-GFP patches in *scs2Δ scs22Δ* cells, seen primarily at the bud tip and the distal end of mother cortex, quantitatively rescued the spindle misorientation and binucleate phenotypes of *num1Δ*, indicating functionality in the dynein-mediated spindle orientation pathway. We found that dynein and dynactin components co-localized with the residual Num1 patches in *scs2Δ scs22Δ* cells. Strikingly, two-color imaging of spindle movements showed that these Num1 patches mediate capture-shrinkage of astral microtubule plus ends, generating pulling forces that rescue misaligned spindles into the mother-bud neck. Disrupting Num1-dynein interaction (using *num1^{LL}* mutant allele), but not deleting Kar9 and Bud6 (the canonical capture-shrinkage pathway), abolished end-on capture-shrinkage of microtubule plus ends, demonstrating that dynein mediates spindle movements across the mother-bud neck via end-on interactions in these cells. These findings explain the long-standing enigma reported for yeast dynein from *in vitro* studies and indicate that cortical ER regulates side-on versus end-on mechanisms of dynein-mediated “cortical pulling”.

Y523 Role of the microtubule cytoskeleton in the regulation of Cdc42 dynamics. *M. Rodriguez Pino*, Illyce Nunez, Fulvia Verde. University of Miami, Miami.

Schizosaccharomyces pombe is a great model system to understand the signaling mechanisms regulating polarized cell growth. Upon the completion of mitosis, *S. pombe* cells grow in a monopolar fashion from their old tips in a process termed Old End Takes Off (OETO). Once the cell reaches a certain length, growth is activated in the second tip through a process called New End Take Off (NETO), resulting in bipolar growth.

Active Cdc42 GTPase, a key regulator of cell polarity, displays oscillatory dynamics that are anti-correlated at the two cell tips in fission yeast. This system globally controls active Cdc42 distribution, modulating cellular dimensions, and controlling the activation of growth at the secondary growing tip (Das, et al., 2012). Mathematical modeling and experimental analysis suggest Cdc42 dynamic oscillations result from the coordinated function of Cdc42 regulators, including Cdc42 GEF proteins, Gef1 and Scd1, Cdc42 GAP protein, Rga4, and Cdc42-dependent Pak1 kinase, in an interplay of positive and negative feedback mechanisms.

Here, we report that the microtubule cytoskeleton is required for the regulation of Cdc42 GTPase activation. Cells treated with the microtubule depolymerizing agent MBC show asymmetrical distribution of active Cdc42 and delay bipolar growth activation (NETO) (Das et al., 2015). Similarly, cells expressing a mutated form of the microtubule tip–associated protein Tea4, which is unable to target the phosphatase Dis1 to the growing tip, displayed asymmetrical Cdc42 activity, a phenotype that is also observed with *gef1Δ* mutants (Das et al., 2015). We find that conserved NDR kinase Orb6 negatively regulates Cdc42 GEF Gef1, an orthologue of mammalian TUBA/DNMBP. A mutated form of Cdc42 GEF Gef1 that cannot be phosphorylated by Orb6 kinase can bypass the microtubule-mediated requirement for NETO. Furthermore, the levels of Orb6-mediated phosphorylation of Gef1 are reduced upon over-expression of Tea4. Thus, the microtubule cytoskeleton opposes Orb6-mediated Gef1 phosphorylation, either by protecting Gef1 from interacting with Orb6, or through the recruitment of a phosphatase needed to dephosphorylate Gef1. These results suggest the presence of a self-organizing microtubule cytoskeleton that regulates Gef1 phosphorylation and Cdc42 activity during morphology control.

Y524 Spindle pole body assembly into the nuclear envelope in budding and fission yeast. *Sue L. Jaspersen*[1,2], Andrew J. Bestul[1], Jingjing Chen[1]. 1) Stowers Inst Med Res, Kansas City, MO; 2) University of Kansas Medical Center.

The double lipid bilayer of the nuclear membrane serves as a physical barrier to restrict access of macromolecules to genetic material inside the nucleoplasm in all eukaryotes. Compartmentalization is at the heart of transcriptional and translational control. However, the nuclear envelope (NE) represents an obstacle to cells during mitosis since the cytoplasmic cytoskeleton must interact with nuclear DNA to ensure accurate chromosome segregation. In most higher eukaryotes, the spindle forms after NE fragmentation in early mitosis, but, in fungi the NE remains intact throughout cell division and yeast microtubule organizing centers, known as spindle pole bodies (SPBs), gain access to chromosomes via insertion into the NE. Membrane insertion of SPBs is thought to be mechanistically similar to that of de novo nuclear pore complex (NPC) assembly, yet how both complexes interact with the NE to promote cell cycle specific changes in its structure is poorly understood. Central to addressing this question is the ability to 1) visualize assembly intermediates and 2) detect proteins that localize permanently or transiently to these structures. Because *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* SPBs are roughly ten times larger than the NPC, they were ideal to investigate how complexes are assembled into the NE. Using biochemical and genetic approaches

combined with super-resolution microscopy, we have identified factors that localize transiently or permanently to SPBs as they are inserted into the NE. These proteins are leading candidates to convert the planar NE into a highly curved pore membrane. Using epistasis and co-localization studies, we have put factors into an assembly pathway and have used mutant alleles to provide evidence that some lead to localized changes in NE structure. Determining how SPBs interact with the NE will provide insight into the ways that their metazoan counterparts (centrosomes) regulate their numbers and control events such as NE breakdown.

Y525 Investigating the Role of Septin Phosphorylation in Controlling of Septin Organization at Cytokinesis. *M. McQuilken*[1], A. Grassetti[1], A. Verma[2], G. Harris[2], S. Gerber[1], S. Abrahamsson[3], R. Oldenbourg[2], A. Gladfelter[1]. 1) Dartmouth College, Hanover, NH; 2) Marine Biological Laboratory, Woods Hole, MA; 3) The Rockefeller University, New York, NY.

Septins are conserved filament-forming proteins that act in cytokinesis, membrane remodeling, cell polarization, and migration. They closely associate with membranes and, in some systems, components of the cytoskeleton. Although septin function is critical for diverse cell events, it is not well understood how they assemble *in vivo* or how they are remodeled throughout the cell cycle. In budding yeast, septins assemble initially as a patch and ring that then flips into a collar at the mother-bud neck as the bud emerges. Late in the cell cycle the collar splits into two rings that generally will be disassembled prior to start of the next cell cycle. The orientation of the dipole moment of GFP has been well established, and thus, constraining GFP to an endogenous septin allows for an assessment of septin organization *in vivo* by polarization microscopy. Polarized fluorescence microscopy analysis has previously shown that septins are arranged in ordered, paired filaments and undergo a coordinated 90° reorientation during cytokinesis *in vivo*. We hypothesized that the reason for this reorganization is the presence of two unequal and differentially arranged septin populations within a septin collar at the mother-bud neck and that over the course of the cytokinesis, the dominant population is no longer enriched in the higher order structure. Data from our lab and others have confirmed a decrease in septin concentration in the collar at mother-bud neck during cytokinesis and we see the kinetics of the decrease depend on known septin regulators including the kinase Gin4. Our goal is to understand the mechanisms regulating changes in septin abundance at septin ring splitting. We have used polarization microscopy to screen mutant yeast strains with abnormally organized septins and have identified septin interactors important for distinct aspects of the assembly, stability, and reorganization of septins. We predicted that these identified regulators control post-translational modifications to trigger reorganization of a subset of septins during cytokinesis. We therefore performed mass spectroscopy on septins synchronized at different stages of the cell cycle and found that there is a difference in the phosphorylation of the N- and C-terminal septin-interacting interfaces depending on whether septins are in collars or split rings. These data suggest that selective phosphorylation of septins can control their polymerization and localization in a cell cycle dependent manner at cytokinesis.

Y526 The respiration/fermentation switch in yeast requires protein aggregation. *Kobi Simpson-Lavy*[1], Mark Johnston[2], Martin Kupiec[1]. 1) Tel Aviv University, Tel Aviv, IL; 2) University of Colorado-Denver, Aurora, CO, USA.

Amyloidogenic proteins (such as Huntingtin) and prions are sequestered under stress conditions or their overexpression into special particles/compartments called IPOD and JUNQ. This process involves the chaperones Hsp104, Btn2 and Cur1. Utilization of non-fermentable carbon sources requires the activity of the Snf1 protein kinase. Snf1 is active in the absence of glucose and regulates the expression and activity of proteins involved in respiration. We have identified two new regulators of Snf1 activity in *S. cerevisiae.* These new regulators (Vhs1 and Sip5) control the aggregation of the Snf1 activator Std1 in response to glucose via the asparagine-rich region of Std1. Interestingly, aggregated Std1 localizes to the IPOD under ambient conditions and this utilizes the same chaperones used by amyloidogenic proteins and prions under pathological or stressful conditions. Thus, amyloidogenic aggregation is a normal, non-pathological physiological state that can be used to regulate central metabolic processes. These results shed light on the evolutionary role of protein aggregation in eukaryotes and have implications for our understanding of cancer and neurodegenerative diseases.

Y527 SNX-BAR proteins contribute to autophagy via trafficking of lipids required for autophagosome-vacuole fusion. *R. J. Chi*[1], M. Ma[2], S. Kumar[2], J. Wang[2], M. Babst[3], C. Burd[2]. 1) University of North Carolina at Charlotte, Charlotte, North Carolina; 2) Yale School of Medicine New Haven, Connecticut; 3) University of Utah, Salt Lake City, Utah.

The yeast *SNX4* family of sorting nexins containing a BAR domain (SNX-BARs), Snx4/Atg24, Snx41, and Snx42/Atg20, are components of endocytic recycling and autophagy trafficking pathways. We show that these proteins possess membrane remodeling activity in vitro and coat endosome-derived transport carriers in vivo. Endosomes decorated by Snx4 proteins are also decorated with retromer SNX-BARs, but each SNX-BAR coats a distinct carrier that buds from the endosome, indicating that, in contrast to current models, the *SNX4* and retromer pathways originate from a common endosome. Cells lacking Snx4 family proteins display a modest deficiency in autophagy that is severely exacerbated when mitochondrial phosphatidylethanolamine synthesis is ablated. Under these conditions, the lipid content of endosome-related organelles is perturbed and autophagosomes accumulate within the cytoplasm as a result of deficient fusion with the lysosome-like vacuole. SNARE-mediated autophagosome-vacuole fusion is restored by increasing phosphatidylethanolamine biosynthesis via alternative pathways, revealing a role for sorting nexins in controlling local pools of glycerophospholipids.

Y528 Rewiring of lipid metabolism in a yeast mutant devoid of the major membrane lipid phosphatidylcholine. *A. de Kroon*[1], X. Bao[1], F. Holstege[2], M. Mari[3], C. Klose[4]. 1) Utrecht University, NL; 2) UMCU, Utrecht, NL; 3) UMCG, Groningen, NL; 4) Lipotype GmbH, Dresden, FRG.

The composition of the membrane lipid matrix determines the physical properties of a biological membrane including membrane surface charge, membrane fluidity and membrane intrinsic curvature. For proper membrane function it is essential that these parameters are maintained in the appropriate range. Yeast is the eukaryote of choice for investigating the regulatory mechanisms governing membrane lipid

composition because the organism is extremely tolerant to manipulation of membrane lipid biosynthesis. Phosphatidylcholine (PC) is a highly abundant membrane lipid in most eukaryotes, and is generally considered essential. With the goal of elucidating the function(s) of PC we have been studying the yeast mutant *cho2opi3* that lacks the methyltransferases for converting phosphatidylethanolamine (PE) into PC and relies on supplementation with choline for PC synthesis by the CDP-choline route. Recently, we isolated *cho2opi3* suppressor (*cho2opi3*S) clones that lost the auxotrophy for choline. These clones exhibit decent growth on fermentable carbon source in the complete absence of choline or choline substitutes. Lipidome analysis demonstrated the absence of PC and revealed that the suppressors exhibit strongly increased synthesis of fatty acids and triglycerides. This is accompanied by a shortening of the average acyl chain length and increased acyl chain desaturation. The changes in fatty acid profile are consistent with the maintenance of membrane physical properties since they reduce the non-bilayer propensity of PE, which has replaced PC as most abundant membrane lipid. At the ultrastructural level, EM revealed massive lipid droplet accumulation and aberrant mitochondrial and vacuolar structure in *cho2opi3*S cells. After return to choline-supplemented medium the suppression of choline auxotrophy is gradually lost, indicating that it is not caused by a mutation. Whole genome sequencing confirmed the lack of common SNPs between suppressors, but instead revealed that the suppressors acquired aneuploidy. Genome-wide transcript profiling is underway to obtain additional clues as to the molecular mechanism of the adaptation that renders PC biosynthesis redundant. Based on the results obtained so far, the hypothesis is put forward that the aneuploidy confers increased synthesis of fatty acids, which is required to shorten the average acyl chain length and thus sustain yeast growth in the absence of PC.

Y529 The lysine acetyltransferase complex NuA4 regulates cellular phosphatidylinositol-4-phospahate and phospholipid metabolism. *L. Dacquay*, M. Kennedy, A. Flint, J. Butcher, A. Stinzi, K. Baetz. Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Canada.

Understanding the regulation of phosphatidylinositol-4-phospahate (PI-4-P) homeostasis is important due to its critical roles in trafficking and proliferation. In Saccharomyces cerevisiae, the conserved lipid-binding proteins Sec14 and Osh4 regulate Golgi-localized PI-4-P homeostasis by either increasing or decreasing its levels respectively. It remains poorly understood how cells compensate in response to changes in the steady-state levels of PI-4-P. Our recent findings suggest that lysine acetyltransferase (KAT) regulation of transcription are contributing to the cellular response to PI-4-P deficiencies. Through a systematic analysis of all KAT deletion mutants, we found that mutants of the NuA4 KAT complex, including *eaf1*, are hypersensitive to *SEC14* inactivation and *OSH4* overexpression, two conditions where Golgi PI-4-P levels are depleted. Through transcriptome profiling, we determined that in PI-4-P deficient conditions, NuA4 regulates the transcription of the essential genes *CDS1*, which catalyzes CDP-DAG biosynthesis, and *BCP1*, which regulates the localization of the PI-4,5-P_2 kinase Mss4. Genetic analysis and microscopy analysis confirms that NuA4-dependent regulation of *CDS1* and *BCP1* is essential to compensate for defects in Sec14 and decreases in PI-4-P. Altogether, we have discovered a new role for the lysine acetyltransferase NuA4 as a regulator of PI-4-P and phospholipid metabolism.

Y530 An unexpected role for casein kinases in glucose sensing and signaling. *C. Snowdon*, M. Johnston. University of Colorado, Aurora, CO.

Saccharomyces cerevisiae prefers glucose as its carbon source, and intercellular glucose acts as an important signaling molecule to regulate many physiological processes, so yeasts have evolved sophisticated signaling pathways to regulate the uptake and metabolism of glucose. The Snf3/Rgt2-Rgt1 (SRR) glucose-sensing pathway enables yeast cells to detect extracellular glucose and transmit an intercellular signal that induces expression of hexose transporter (hxt) genes. The yeast casein kinases (YCKs) are key players in this signaling pathway, and are thought to be responsible for transmission of the glucose signal. The current model of the SRR signaling pathway suggests that binding of glucose to the glucose sensors activates YCKs to phosphorylate the transcriptional co-repressors Mth1 and Std1, targeting them for degradation and thereby relieving repression of *Hxt* genes. However, we have evidence for an upstream role for YCKs in the SRR signaling pathway: (i) overexpression of Rgt2 rescues glucose signaling in a strain lacking YCK; (ii) the C-terminal tail of Rgt2 is phosphorylated by YCK, and (iii) Rgt2 phosphorylation is necessary for signaling; (iv) Rgt2 phosphorylation promotes interaction of the Mth1 and Std1 co-repressors with the Rgt2 glucose sensor. These results lead us to a model in which the YCKs act upstream of the glucose sensors to phosphorylate the C-terminal tail of the glucose sensors to establish a functional interaction site for the co-repressors, bringing them to the glucose sensor to receive and transmit the glucose signal.

12TH INTERNATIONAL CONFERENCE ON ZEBRAFISH DEVELOPMENT AND GENETICS

Plenary and Platform Session Abstracts

12Th International Conference On Zebrafish Development And Genetics

SCHEDULE AT-A-GLANCE

Wednesday, July 13		
2:00pm-9:30pm	Speaker Ready Room Open	Hall of Cities - Anaheim
7:00pm-9:00pm	Scientific Session: Regeneration and Stem Cells	Grand Ballroom 7A
9:00pm-11:00pm	Opening Mixer with Exhibits	Cypress Ballroom
Thursday, July 14		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities -Anaheim
7:45am-10:00am	Genetics and Determinants of Health Joint Plenary Session	Palms Ballroom
8:00am-4:00pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:30am-12:30pm	Scientific Session: Early Development and Morphogenesis Neural Circuits, Neurophysiology and Behavior	Grand Ballroom 7A Grand Ballroom 7B
12:30pm-1:30pm	Mentoring Roundtables #1	North Tower - Harbor Beach
12:30pm-1:30pm	Speaking Up for Genetics and Model Organism Research	Crystal Ballroom H
1:30pm-3:30pm	Poster Presentations 1:30pm-2:30pm: Even-numbered posters 2:30pm-3:30pm: Odd-numbered posters	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center and Job Fair	Cypress Ballroom 1C
4:00pm-6:00pm	Concurrent Scientific Sessions: Cardiac Development Gene Regulation and RNA Biology	 Grand Ballroom 7A Grand Ballroom 7B
4:00pm-6:00pm	Plenary Session and Workshop for Undergraduate Researchers	North Tower - Sawgrass
7:45pm-9:45pm	Scientific Session: Neurobiology	Grand Ballroom 7A
10:00pm-11:30pm	*Science Cafe Event	Palms Ballroom Sabal
Friday, July 15		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-9:30am	Concurrent Scientific Sessions: t Models of Human Disease Evolution	 Grand Ballroom 7A Grand Ballroom 7B
8:00am-4:30pm	Exhibits Open	Cypress Ballroom
9:00am-8:00pm	Demo Room: MOD (FlyBase, MGI, SGD, WormBase, Zfin) Demo Room Open	Palms Ballroom Canary 3-4
10:00am-12:00pm	Concurrent Scientific Session: Emerging Technologies Imaging Signaling Organogenesis (Mesoderm, Endoderm, Ectoderm)	 Grand Ballroom 1-2 Grand Ballroom 7B Grand Ballroom 7A
12:00pm-1:30pm	*Editor's Panel Discussion and Roundtable	North Tower - Harbor Beach

** Ticketed Event*

#TAGC16

Friday, July 15 (continued)		
1:30pm-3:30pm	Poster Presentations 1:30pm-2:10pm: "A" poster authors present 2:10pm-2:50pm: "B" poster authors present 2:50pm-3:30pm: "C" poster authors present	Cypress Ballroom
1:30pm-3:30pm	GeneticsCareers Center	Cypress Ballroom 1C
2:00pm-2:45pm	GeneticsCareers Workshop - Nailing the Job Talk	Cypress Ballroom 1B
4:00pm-6:00pm	Scientific Session: Highlighted Talks, Awards Ceremony and Community Meeting	Grand Ballroom 7A
6:00pm-7:30pm	*Women in Genetics Panel and Networking	North Tower - Harbor Beach
7:30pm-9:30pm	Development and Evolution Joint Plenary Session	Palms Ballroom
Saturday, July 16		
7:00am-5:00pm	Speaker Ready Room Open	Hall of Cities - Anaheim
8:00am-10:00am	Workshops: See topics and descriptions under the Workshop Section	Multiple locations
8:00am-12:00pm	Exhibits Open	Cypress Ballroom
8:00am-9:00am	Trainee Bootcamp Workshops: Session 1	North Tower
9:00am-10:00am	Trainee Bootcamp Workshops: Session 2	North Tower
10:00am-12:00pm	Poster Presentations 10:00am-11:00am Odd-numbered posters 11:00am-12:00pm Even-numbered posters	Cypress Ballroom *(Posters must be removed by 1pm)*
10:00am-12:00pm	GeneticsCareers Center	Cypress Ballroom 1C
10:30am-11:15am	GeneticsCareers Workshop	Cypress Ballroom 1B
12:15pm-1:45pm	*Mentoring Roundtables #2	North Tower - Harbor Beach
1:45pm-3:45pm	Concurrent Scientific Sessions: Neural Development and Regeneration Cancer	 Grand Ballroom 7A Grand Ballroom 7B
4:00pm-6:00pm	Scientific Session: Models of Human Disease	Grand Ballroom 7A
7:30pm-9:30pm	Concurrent Scientific Session: Haematopoiesis and Vascular Biology Cell Biology and Polarity	 Grand Ballroom 7A Grand Ballroom 7B
Sunday, July 17		
8:00am-10:00am	Scientific Sessions: Genome Editing	Grand Ballroom 7A
10:30am-12:30pm	Technology and its Application Joint Plenary Session	Palms Ballroom

** Ticketed Event*

Z531 Modulation of tissue repair by regeneration enhancer elements. *Junsu Kang*[1], Jianxin Hu[2], Ravi Karra[1], Amy L. Dickson[1], Valerie A. Tornini[1], Gregory Nachtrab[1], Matthew Gemberling[1], Joseph A. Goldman[1], Brian L. Black[2], Kenneth D. Poss[1]. 1) Duke University, Durham, NC; 2) UCSF, San Francisco, CA.

How tissue regeneration programs are triggered by injury has received limited research attention. Here we investigate the existence of enhancer regulatory elements that are activated in regenerating tissue. Transcriptomic analyses reveal that leptin b (lepb) is highly induced in regenerating hearts and fins of zebrafish. Epigenetic profiling identified a short DNA sequence element upstream and distal to lepb that acquires open chromatin marks during regeneration and enables injury-dependent expression from minimal promoters. This element could activate expression in injured neonatal mouse tissues and was divisible into tissue-specific modules sufficient for expression in regenerating zebrafish fins or hearts. Simple enhancer-effector transgenes employing lepb-linked sequences upstream of pro- or anti-regenerative factors controlled the efficacy of regeneration in zebrafish. Our findings provide evidence for 'tissue regeneration enhancer elements' (TREEs) that trigger gene expression in injury sites and can be engineered to modulate the regenerative potential of vertebrate organs.

Z532 A screen for epigenetic regulators reveals a requirement for Ing4 in HSC specification and function. *K. L. Kathrein*[1], V. Binder[2], M. Ammerman[1], S. Yang[1], E. M. Durand[1], L. I. Zon[1,3]. 1) Stem Cell Program and Division of Hematology/Oncology, Children's Hospital Boston, Boston, MA; 2) Department of Hematology and Oncology, Dr. von Hauner Children's Hospital, Ludwig-Maximilians University, Munich, Germany; 3) Howard Hughes Medical Institute, Boston Children's Hospital/Harvard Medical School, Boston, MA.

Hematopoietic stem cells (HSCs) are capable of self-renewal and differentiation into all mature hematopoietic lineages. This is regulated by transcription factors and chromatin factors to orchestrate chromatin structure and establish an epigenetic code that facilitates gene expression. To uncover chromatin factors that are necessary for the establishment of HSCs, we conducted a reverse genetic screen in the zebrafish using morpholinos to target 488 chromatin remodeling factors. We identified 29 genes that alter HSC marker expression, *c-myb* and *runx1*, upon knockdown. Several components of chromatin remodeling complexes already known to regulate hematopoiesis were required for HSC development in our screen. We also found components from complexes that have no known role in HSCs, including the iSwi and Hbo1 complexes. Four members of the Hbo1 complex, Ing4, Phf16, Hbo1, and Brd1, show loss of HSC marker expression upon knockdown. This complex regulates gene expression through the binding of H3K4me3 by Ing4, which results in localized histone acetylation by Hbo1. Ing4 has also been shown to negatively regulate the transcription factor NF-kB through sequestration of the RelA component of NF-kB. To test the function of the complex in HSCs, we used low doses of morpholinos to partially inhibit expression of each complex member. With modestly reduced levels of *ing4*, *brd1*, and *jade3* individually, we see no effect on HSC specification. However, when combined we see a loss of marker expression, suggesting they genetically interact to regulate HSC specification. Using ChIP-seq for ING4 in human CD34+ cells, we show that ING4 is bound to many regulators of blood development including *c-myb*, *lmo2*, *runx1* and *ikaros*. Ing4 is also bound to several NF-kB target genes including *il-1b, il-6, il-8, and il-20*, as well as a subset of *ifna* associated genes. Zebrafish lacking *ing4* expression show an increase in NF-kB target gene expression, suggesting that loss of Ing4 results in an overabundance of NF-kB target gene signaling. Mice lacking Ing4 also have altered hematopoiesis. These mice exhibit a differentiation block in short-term HSCs (ST-HSCs) and increased NF-kB target gene expression in HSCs and progenitor cells. Loss of Ing4 has a cell autonomous effect on HSCs where long-term HSCs are unable to repopulate recipient animals in competitive transplantation assays. Surprisingly, Ing4 null multipotent progenitors (MPPs) cells outperform their wildtype counterparts in competitive transplantation, suggesting that MPPs that overcome the block at the ST-HSC stage are superior in repopulation of the niche. Concomitant loss of Ing4 and NF-kB target genes by morpholino can rescue HSC specification in zebrafish. These results suggest a novel mechanism for HSC regulation by Ing4 and the Hbo1 complex through regulation of inflammatory signaling.

Z533 Zebrafish T cells mediate organ-specific regenerative programs . *K. Kikuchi*, S. Hui, D. Sheng, K. Sugimoto, A. Rajal. Victor Chang CRI, Darlinghurst, NSW, AU.

In mammals, T cells play major roles in response to tissue injury and subsequent repair through diverse functions, including the promotion of tissue growth and scarring. Although the adaptive immune system is highly developed in regenerative vertebrates such as salamanders and fish, little is known about the contributions of T cells to scarless regeneration in these animals. Here, we demonstrate that successful tissue regeneration in the zebrafish requires a T-cell subset that has the capacity to produce tissue-specific growth factors. Using a transgenic reporter strain, we found that T cells infiltrated damaged tissues and closely associated with regenerating parenchymal cells (e.g. new neurons, proliferating cardiomyocytes). Inducible genetic ablation of T cells was associated with elevated inflammation and severely impaired spinal cord and heart regeneration as well as significantly reduced neurogenesis and cardiomyocyte proliferation. We found that spinal cord-infiltrating T cells specifically synthesised neurogenic factors, whereas heart-infiltrating T cells synthesised cardiomyocyte mitogens. Furthermore, neurogenic and cardiogenic factor administrations restored the neurogenesis and cardiomyocyte proliferation defects associated with T cell depletion. Thus, zebrafish T cells elaborate an organ-specific secretory phenotype and directly enhance spinal cord and cardiac regeneration by promoting neurogenesis and cardiomyocyte proliferation, respectively. These findings extend our understanding of the immunological regulation of organ regeneration and suggest T cell-mediated regenerative therapies for the treatment of spinal cord and cardiac damage in humans.

Z534 Production of medaka individuals derived from cryopreserved spermatogonia by allogenic transplantation. *S. Seki*[1,2], K. Kusano[1], S. Lee[1], Y. Iwasaki[1], T. Hiratsuka[1], M. Ishida[1], T. Sasado[3], K. Naruse[3], G. Yoshizaki[1]. 1) Tokyo University of Marine Science and Technology, Tokyo, JP; 2) Akita University, Akita, JP; 3) National Institute for Basic Biology, Okazaki, JP.

Although numerous inbred lines and endangered wild populations of medaka exist, the only method currently available for preserving these resources is to rear live individuals, as protocols for cryopreservation of fish eggs or embryos have not yet been successfully established due to their large size. Our group developed a novel method termed surrogate broodstock technology. By transplanting donor germ cells into recipient fish of another species, the donor germ cells mature into sperm or oocytes in recipient gonads. Importantly, the cryopreserved germ cells can be differentiated into functional oocytes. After examining cryobiological properties of medaka spermatogonia, we established a vitrification method for medaka whole testis including spermatogonia, and further established that this method can be applied for cryopreservation of zebrafish whole testis.

When vitrified spermatogonia from the *olvas-GFP* transgenic strain (body color: orange) were transplanted into larvae of a non-transgenic strain (body color: black), female recipients produced oocytes showing green fluorescence. In their gonads, there were recipient-derived oocytes together with donor-derived green fluorescent oocytes. Then, infertile triploids were used as the recipients in the next experiment. When cryopreserved spermatogonia were transplanted into triploid larvae, the mature recipients produced only sperm and eggs showing green fluorescence, which is the donor-derived characteristic. Moreover, the body colors of the offspring obtained by mating the recipient males and females were orange (100%, 1169/1169), suggesting that all of them were derived from cryopreserved spermatogonia. Thus, we succeeded in producing the donor-derived offspring by allogenic transplantation of cryopreserved spermatogonia in medaka. Finally, we confirmed that this system can be applied on vitrified testes for the production of an inbred line, or endangered wild populations (Tokyo-medaka). These techniques will facilitate the establishment of a stable and reliable system for preserving valuable medaka strains semi-permanently.

Z535 Zebrafish heart regeneration requires alleviation of cardiomyocyte genomic stress by BMP signaling. *Gilbert Weidinger*, Mohan Dalvoy, Chi-Chung Wu. Ulm University, Ulm, Germany.

In contrast to adult mammals, zebrafish can regenerate heart injuries via dedifferentiation and proliferation of differentiated cardiomyocytes in the wound border zone. Cell cycle re-entry of non-cycling cells can result in replication stress and thus DNA damage, a phenomenon that has been linked to failed homeostasis and regeneration of organ systems in aged mammals. Surprisingly, we found that a large fraction of cardiomyocytes in the regenerating heart of young zebrafish experience DNA damage, as indicated by accumulation of the phosphorylated histone variant H2a.x (gammaH2a.x). DNA damage is likely the result of replication stress caused by injury-induced cardiomyocyte cell cycle re-entry since no gammaH2a.x is detected in proliferating cardiomyocytes during physiological heart growth but occurs in the same temporal and spatial profile as cardiomyocyte cell cycle re-entry during heart regeneration. Importantly, inhibition of DNA damage response pathways results in blockage of regenerative cardiomyocyte proliferation, indicating that replication stress needs to be resolved for heart regeneration to occur.

We have recently shown that BMP signaling is activated in cardiomyocytes at the wound border and that BMP signaling is required for heart regeneration by promoting cardiomyocyte proliferation (Wu et al, *Dev. Cell* 2016). Since BMP signaling is not required for cardiomyocyte proliferation during physiological heart growth, it might regulate regeneration-specific cellular processes in cardiomyocytes, which are prerequisites for proliferation. Intriguingly, we found that inhibition of BMP signaling increases the number of gammaH2a.x positive cardiomyocytes, while pathway overactivation alleviates DNA damage, suggesting that BMP signaling is required for protection from replication stress or repair of DNA damage.

Our results surprisingly indicate that the elevated heart regenerative capacity of zebrafish might depend on efficient means to deal with replication stress and DNA damage, which are thought to impair organ regeneration in aged mammals. We propose that BMP signaling promotes heart regeneration by protecting cells from genomic stress or by augmenting DNA damage repair, a novel function of the pathway that could be conserved in other systems.

Z536 Autophagy Activation via FGF Signaling Regulates Cytoplasmic Remodeling of Regenerating Adult Zebrafish Myocytes. *Alon Kahana*, Alfonso Saera-Vila, Phillip Kish, Ke'ale Louie, Daniel Klionsky. University of Michigan, Ann Arbor, MI.

Cell identity involves both selective gene activity and specialization of cytoplasmic architecture and protein machinery. Similarly, reprogramming differentiated cells requires both genetic program alterations and remodeling of the cellular architecture. While changes in genetic and epigenetic programs have been well documented in dedifferentiating cells, the pathways responsible for remodeling the cellular architecture and eliminating specialized protein complexes are not as well understood. Here, we utilize a zebrafish model of adult muscle regeneration to study cytoplasmic remodeling during cell dedifferentiation. We describe activation of autophagy early in the regenerative response to muscle injury, while blocking autophagy using chloroquine, atg5 or beclin knockdown reduced the rate of regeneration with accumulation of sarcomeric debris. We further identify Fgf signaling through Erk2 as an important activator of autophagy in dedifferentiating myocytes. We conclude that autophagy plays a critical role in cell reprogramming by regulating cytoplasmic remodeling, facilitating the transition to a less differentiated cell identity.

Z537 Macrophages mediate the repair of brain vascular rupture through direct physical adhesion and mechanical traction. *Lingfei Luo*[1], Chi Liu[1], Chuan Wu[1], Qifen Yang[1], Jing Gao[2], Li Li[1], Deqin Yang[2]. 1) School of Life Sciences, Southwest University, Chongqing, China; 2) The Affiliated Hospital of Stomatology, Chongqing Medical University, Chongqing, China.

Hemorrhagic stroke and brain microbleeds are caused by cerebrovascular ruptures. Fast repair of such ruptures is the most promising therapeutic approach. Due to a lack of high resolution ***in vivo*** real-time studies, the dynamic cellular events involved in cerebrovascular repair remain unknown. Here, we have developed a cerebrovascular rupture system in zebrafish using multi-photon laser, which generates a lesion with two endothelial ends. ***In vivo*** time-lapse imaging showed that a macrophage arrives at the lesion and extended filopodia or lamellipodia to physically adhere to both endothelial ends. This macrophage generated mechanical traction forces to pull the endothelial ends and facilitate their ligation, thus mediating the repair of the rupture. Both depolymerization of microfilaments and inhibition of phosphatidylinositide 3-kinase or Rac1 activity disrupted macrophage-endothelial adhesion and impaired cerebrovascular repair. Our study reveals a hitherto unexpected role for macrophage in mediating repair of cerebrovascular ruptures through direct physical adhesion and mechanical traction.

Z538 Four and a Half LIM Domains 1b (Fhl1b) Is Essential for Regulating the Liver versus Pancreas Fate Decision and for beta-Cell Regeneration. *C. Shin*[1], J. Xu[1], J. Cui[2], A. Del Campo[2]. 1) School of Biology and the Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia, United States of America; 2) Max Planck Institute for Polymer Research, Mainz, Germany.

The liver and pancreas originate from overlapping embryonic regions, and single-cell lineage tracing in zebrafish has shown that Bone morphogenetic protein 2b (Bmp2b) signaling is essential for determining the fate of bipotential hepatopancreatic progenitors towards the liver or pancreas. Despite its pivotal role, the gene regulatory networks functioning downstream of Bmp2b signaling in this process are poorly understood. Through transcriptome profiling of endodermal tissues exposed to increased or decreased Bmp2b signaling, we have discovered the zebrafish gene *four and a half LIM domains 1b* (*fhl1b*) as a novel target of Bmp2b signaling. *fhl1b* is primarily expressed in the prospective liver anlage. Loss- and gain-of-function analyses indicate that Fhl1b suppresses specification of the pancreas and induces the liver. By single-cell lineage tracing, we showed that depletion of *fhl1b* caused a liver-to-pancreas fate switch, while *fhl1b* overexpression redirected pancreatic progenitors to become liver cells. At later stages, Fhl1b regulates regeneration of insulin-secreting beta-cells by directly or indirectly modulating *pdx1* and *neurod* expression in the hepatopancreatic ductal system. Therefore, our work provides a novel paradigm of how Bmp signaling regulates the hepatic versus pancreatic fate decision and beta-cell regeneration through its novel target Fhl1b.

Z539 A Meiotic-Vegetal Center Couples Oocyte Polarization with Meiosis. *Y. M. Elkouby*, A. Jemieson-Lucy, M. C. Mullins. UPenn Perelman School of Medicine, Philadelphia, PA.

Vertebrate oocyte polarity along the animal-vegetal (AV) axis has been observed for two centuries, but how it is generated was unknown. AV oocyte polarity is established by the Balbiani body (Bb), a structure conserved from insects to humans, that contains an aggregate of specific mRNAs, proteins, and organelles. The Bb specifies the oocyte vegetal pole, which is key to forming the embryonic body axes as well as the germ line in most vertebrates. How Bb formation is regulated and how its asymmetric position is established were unknown. Using quantitative image analysis, we traced oocyte symmetry breaking in zebrafish to a nuclear asymmetry at the onset of meiosis called the chromosomal bouquet. The bouquet is a universal feature of meiosis where all telomeres cluster to one pole on the nuclear envelope, facilitating chromosomal pairing and meiotic recombination. We show that Bb precursor components first localize with the centrosome to the cytoplasm adjacent to the telomere cluster of the bouquet. They then aggregate around the centrosome in a specialized nuclear cleft that we identified, assembling the early Bb. We found that the bouquet nuclear events and the cytoplasmic Bb precursor localization are mechanistically coordinated by microtubules. Thus the AV axis of the oocyte is aligned to the nuclear axis of the bouquet. We show that the symmetry breaking events lay upstream to the only known regulator of Bb formation, the Bucky ball protein. Our findings link two universal features of oogenesis, the Bb and the chromosomal bouquet, to oocyte polarization. We propose that a cellular organizer that we term the meiotic–vegetal center, couples meiosis and oocyte patterning. Our findings reveal a novel mode of cellular polarization in meiotic cells whereby cellular and nuclear polarity are aligned. We further revealed that oocytes are organized in cysts where meiotic–vegetal center formation and polarization is synchronized as shown by both live time-lapse and fixed sample data. Moreover, intercellular cytoplasmic bridges remain between oocytes in the cyst and coincide with the location of the centrosome meiotic–vegetal center, suggesting that the last mitotic oogonial division plane positions the centrosome and the meiotic–vegetal center. These results provide the first evidence for a link between polarity and cyst organization. As we show here, the zebrafish ovary provides an excellent genetic model for vertebrate germ cell differentiation, ovarian development and female reproduction.

Z540 Investigating the function of the yolk cell microtubules during zebrafish epiboly. *A. Bruce*, Z. Fei, K. Bae. University of Toronto, Toronto, Ontario, Canada.

Zebrafish epiboly involves the vegetal movement of the blastoderm and the yolk syncytial layer to enclose the yolk cell. An elaborate longitudinal microtubule array extends from the yolk syncytial layer towards the vegetal pole of the yolk cytoplasmic layer and shortening of this array is proposed to provide a vegetally directed pulling force during epiboly. Despite experimental evidence implicating yolk microtubules in normal epiboly movements, their exact function remains unclear. Furthermore, most studies have examined yolk microtubules in fixed specimens, thus little is known about their dynamics. To investigate yolk cell microtubule function, we examined the movements of the microtubule plus end associated protein EB3 fused to GFP (EB3-GFP), which binds to actively polymerizing microtubules. The current model is that the microtubule array is established before epiboly and shortens during epiboly and thus predicts very little polymerization during early epiboly stages. In contrast to this model, EB3-GFP tracking revealed widespread microtubule polymerization in the yolk syncytial layer and extensive vegetally directed microtubule growth in the yolk cytoplasmic layer from high stage to 60% epiboly. Strikingly, after 60% epiboly, EB3-GFP was not detected in the yolk cytoplasmic layer, suggesting that the yolk microtubules might be stabilized at 60% epiboly. To investigate this

further, we performed antibody staining for tyrosinated and de-tyrosinated tubulin, which are markers of dynamic and stabilized microtubules, respectively. The marker for dynamic microtubules was present at early but not late epiboly stages, while the marker for stabilized microtubules was detected at late but not early epiboly stages. We also performed FRAP experiments on embryos with fluorescently labeled microtubules and found that the recovery times were faster at early epiboly stages than at late epiboly stages. Our data are consistent with the hypothesis that yolk cell microtubules are more dynamic at early epiboly stages than at late epiboly stages, suggesting that they may perform distinct functions at these stages. Previous work suggested that the yolk cell microtubules might pull the yolk syncytial layer nuclei towards the vegetal pole starting at 60% epiboly. Thus, the yolk cell microtubules might need to be stabilized before they can function in yolk syncytial layer nuclear migration, a possibility that we are currently investigating. Overall, we found that the yolk microtubules appear to undergo a rapid growth phase during early epiboly followed by a more stabilized phase that correlates with downward movement of the yolk syncytial layer nuclei. Intriguingly, the two phases of microtubule dynamics are consistent with the timing of the initiation and progression phases of epiboly.

Z541 *Myo1D*, an unconventional myosin regulates kupffer's vesicle lumenogenesis in zebrafish. *M. Saydmohammed*, H. Yagi, T. Feinstein, D. Kostka, CW Lo, M. Tsang. University of Pittsburgh, Pittsburgh, PA.

During zebrafish development, a transient ciliated organ known as the kupffer vesicle (KV) forms and contributes to establishing laterality in the embryo. The activity of motile cilia in the KV results in asymmetric flow of extra embryonic fluid and contributes to the left-right arrangement of visceral organs. The molecular mechanisms that contribute to the formation of KV lumen still remain elusive. Recent work in *Drosophila* has identified a novel *situs inversus* gene encoding unconventional type 1D Myosin (*myo1D*). Here, we show that zebrafish *myo1D* is essential for KV lumenogenesis. Targeted mutation in the *myo1D* gene by TALENs affected the formation of KV lumen and randomized heart and gut looping. Confocal imaging of the KV formation revealed failure of the KV lumen to expand. Disrupted KV lumenogenesis in *myo1D* mutants was due to mislocalization of E cadherin and β-catenin that resulted in loss of apical basal polarity in cells that form the KV. *myo1D* is expressed maternally and only the maternal-zygotic (MZ) mutant showed defective KV formation. Defective KV lumenogenesis phenotype was recapitulated by injecting with antisense morpholino (MO) targeting the *myo1D* gene. In summary, *myo1D* is one of the earliest unconventional myosin motors expressed in zebrafish that regulate KV lumenogenesis and critical for proper left right patterning in a vertebrate.

Z542 Combinatorial signaling interactions pattern the dorsal-ventral mesodermal axis by controlling bHLH transcription factor activity. *B. L. Martin*[1], R. Row[1], G. H. Farr III[2], L. Maves[2]. 1) Stony Brook University, Stony Brook, NY; 2) Seattle Children's Research Institute, Seattle, WA.

Newly formed mesoderm is patterned into dorsal-ventral subtypes through combinatorial interactions of paracrine signaling pathways. How these signals are integrated to induce specific dorsal-ventral cell fates is not well understood. The vertebrate tailbud provides a simplified model to understand in vivo mesodermal patterning. Newly formed mesoderm in the zebrafish tailbud undergoes a binary decision to become either dorsal paraxial mesoderm, or ventral endothelial tissue. We show that tailbud mesoderm is patterned by interactions between canonical Wnt, FGF, and BMP signaling, where Wnt and FGF induce paraxial fate, and BMP promotes endothelial fate. The mechanism of signal integration of these three independent pathways is at the level of basic helix loop helix (bHLH) transcription factor activity, which produces a binary cell fate output. High bHLH activity induces paraxial fate, while low bHLH activity results in endothelial induction. Consistent with predictions from this model, we show that endothelium is the default fate of newly generated tailbud mesoderm. We extend our analysis to show that the same mechanism is responsible for the dorsal-ventral patterning of the entire mesodermal germ layer during gastrulation. Unexpectedly, this mechanism only affects dorsal-ventral mesodermal patterning and has no effect on anterior-posterior pattern, providing evidence for the molecular uncoupling of dorsal-ventral and anterior-posterior patterning downstream of Wnt, FGF, and BMP signaling.

Z543 Functional conservation of the zebrafish germ plasm organizer Bucky ball and *Drosophila* Oskar. *P. Krishnakumar*[1], S. Riemer[1], T. Lingner[1], F. Bontems[2], R. Dosch[1]. 1) University Of Goettingen, Goettingen, Niedersachsen, DE; 2) University of Geneva Genève, GE, Switzerland.

The germline is vital for the continuation of a species. Many animals specify their germ cells by deposition of a maternal RNA granule termed germ plasm. The inheritance of germ plasm by an embryonic cell initiates its development into a primordial germ cell. To this end, two proteins are known in animals which program germ cell development *in vivo*: Oskar, which is specific to insects and Bucky ball (Buc), which we discovered in vertebrates. These two proteins are termed germ plasm organizers because their loss of function results in the failure to form germ plasm.

We discovered in the zebrafish embryo that both germ plasm organizers possess the fascinating ability to transform a somatic cell into a primordial germ cell. This result indicates that both proteins act through a conserved biochemical network to specify the germ cell fate. Comparing the amino acid sequence of the two organizers, we found that *Drosophila* Oskar and Zebrafish Bucky Ball showed no conserved domains to explain their similar function. To shed light on the biochemical interaction network of Bucky ball, we carried out co-immunoprecipitation experiments. We found that Buc interacts *in-vivo* with the canonical germ plasm factor Vasa as described for Oskar.

To explain the deviation from the traditional structure-function paradigm by the two proteins, we discovered that both encode intrinsically disordered proteins (IDP). As IDPs quickly change their sequence during evolution, we propose that Buc is an Oskar homolog, which exerts its conserved role through similar biophysical properties. In summary, these data describe the first example of proteins, which were previously classified as "novel" or "species-specific", but which are indeed functional homologs from distant species.

Z544 *Fibroblast growth factor 24* is required for early somatic gonad development in zebrafish. *D. M. Leerberg*[1], K. Sano[2], B. W. Draper[1]. 1) University of California, Davis, Davis, CA; 2) 2Department of Materials and Life Sciences, Sophia University, Tokyo, Japan.

The vertebrate gonad consists of two cell populations: germ cells (GCs), which produce the gametes, and somatic gonad cells (SGCs), which serve two important functions. First, SGCs create an environment that protects GCs and nurtures their development through cell signaling. Second, a subset of SGCs secrete hormones required for secondary sexual development, and therefore defects in SGC development result in disorders of sexual development. Despite the known importance of SGCs for fertility, little is known how the early somatic gonad forms or which genes are required for its formation. We have discovered that a mutation in the zebrafish Fibroblast growth factor (Fgf) ligand *fgf24* results in a reduction of GCs during larval development, at a time that precedes sexual differentiation. The phenotype persists into adulthood, and most mutant adults are sterile. Upon examination of the 10 days post fertilization (dpf) gonad by transmission electron microscopy (TEM), we found that wild-type fish have multilayered gonads, where the SGCs have already begun to separate into two distinct layers and the GCs are arranged in the center, protected by the SGCs. We have determined that *fgf24* is expressed in the outer layer of epithelial-like SGCs. In contrast, a known Fgf-responsive gene, *ets variant 4* (*etv4/pea3*), is expressed in the population of mesenchymal-like SGCs more internally located. Based on the TEM data and Laminin immunostaining, we have found that these layers are separated by a basement membrane. In contrast, the gonads of *fgf24* mutants have only one layer of SGCs enveloping the GCs, lack a basement membrane, and fail to express *etv4*. Furthermore, we asked whether genes known to be important for SGC function were expressed in *fgf24* mutants: *gata4*, a transcription factor required for mouse gonadogenesis; *cyp19a1a*, an aromatase that converts androgens to estrogens; and *amh*, a TGF-β ligand required for male sexual development in many vertebrates. In accordance with our hypothesis, *in situ* hybridization showed that these genes were expressed in the mesenchymal SGC population of wild-type larval fish, but their expression was greatly reduced or completely absent in *fgf24* mutant gonads. These results support our overall hypothesis that Fgf24 functions primarily to promote development of the mesenchymal SGCs and overall morphogenesis of the somatic gonad, and that the loss of GCs in *fgf24* mutants is a secondary consequence of a somatic gonad defect.

Z545 Wnt signaling regulates progenitor cell identity and collective cell migration in the lateral line. *H. F. McGraw*[1], A. Forbes[1], Y. Xie[2], R. I. Dorsky[2], A. V. Nechiporuk[1]. 1) Oregon Health & Science University, Portland, OR; 2) University of Utah, Salt Lake City, UT.

Collective cell migration, which is movement of cells as a cohesive group, is a critical process during embryonic organ formation, wound healing and is inappropriately coopted during the invasion of certain cancers. Although many of the cellular hallmarks of collective cell migration have been defined, the genetic pathways that regulate these processes are not well understood. Development of the zebrafish lateral line has proven to be an elegant model for studying collective cell migration, as it is amenable to live imaging and genetic manipulation. The posterior lateral line (pLL) forms from the posterior lateral line primordium (pLLP), a cohort of ~100 cells which collectively migrate along the trunk of the developing zebrafish embryo. The pLLP is comprised of proliferative progenitor cells and organized epithelial cells that will form the hair cell-containing mechanosensory organs of the pLL. Wnt signaling is active in the leading progenitor zone of the pLLP and regulates cellular proliferation, survival and maintenance. Here we examine the downstream targets of Wnt signaling and their role in mediating pLLP progenitor cell behavior. We used RNA-sequencing to identify genes that are altered in zebrafish embryos carrying mutations in members of the canonical Wnt pathway that are known to regulate pLL formation, i.e. *lef1* and *kremen1*, as compared to wild-type controls. One of the genes we selected for further analysis is the tumor suppressor Fat1b, which is strongly expressed in the wild-type pLLP and is downregulated following loss of Wnt signaling. CRISPR-Cas9-mediated mutation of Fat1b function results in failed pLLP migration in a manner that is similar to previously described canonical Wnt signaling mutants. These data suggest that our approach will allow us to refine our understanding of how canonical Wnt signaling pathway regulates various cellular behaviors.

Z546 Migratory neural crest is required for patterning and morphogenesis of the embryonic optic cup. *C. D. Bryan*, K. M. Kwan. University of Utah, Salt Lake City, UT.

Extrinsic, migratory mesenchymal cells are essential for the development and morphogenesis of many epithelial organs. In mouse, early eye defects have been described in a neural crest mutant, but the cellular and molecular mechanisms underlying aberrant optic cup morphology and patterning are unknown. Using zebrafish molecular genetics and multidimensional timelapse imaging, we sought to determine when and how neural crest migrates around the early eye, as well as exactly when and how eye defects arise in the absence of neural crest.

We find that at the earliest stages of optic vesicle formation, sox10-positive neural crest cells are in direct contact with the dorsal, posterior optic vesicle; these neural crest cells then migrate ventrally and anteriorly to enwrap the developing optic cup. By the end of optic cup morphogenesis, neural crest derived cells encapsulate the optic stalk and all but the distal, lens-facing side of the optic cup. Therefore, neural crest is present at the right time and place to influence patterning and morphogenesis of specific regions of the developing eye.

To determine precisely when and where eye defects arise in the absence of neural crest, we utilize the zebrafish *tfap2a*^{ts213}*;foxd3*^{zdf10} double mutant which exhibits a complete loss of neural crest. In the absence of neural crest, we find dramatic optic cup morphogenesis defects including a misshapen retina and coloboma, suggesting that neural crest is required for optic cup invagination and formation of the optic fissure. Similar results were seen using an independent neural crest mutant (*paf1*^{z24}). In addition to the morphological defects, we began to characterize defects in tissue patterning: as in mouse *Tcfap2a* knockouts, *tfap2a;foxd3* mutant zebrafish display an aberrant expansion of Pax2a expression into ectopic dorsal and posterior domains. This suggests an evolutionarily conserved role for neural crest in optic stalk and optic cup patterning and control of morphogenetic movements critical for optic cup formation.

Using computational cell tracking tools combined with our multidimensional imaging datasets, we are now pinpointing the precise cell

movements disrupted by loss of neural crest as well as examining the specific signaling pathways dysregulated in the absence of neural crest. These results highlight the diverse roles mesenchyme can play in epithelial organ development and morphogenesis.

Z547 Neuronal connectivity analysis of wild-type and mutant zebrafish with transsynaptic virus and 3D brain mapping. *Manxiu Michelle Ma*[1], Owen Randlett[2], Stanislav Kler[1], Kristin Ates[1,3], Avirale Sharma[1], Tong Wang[1], Constance Cepko[4,5], Florian Engert[2], Alexander F. Schier[2], Yuchin Albert Pan[1,6]. 1) Department of Neuroscience and Regenerative Medicine, Medical College of Georgia, Augusta University, Augusta, GA; 2) Department of Molecular and Cellular Biology, Center for Brain Science, Harvard University, Cambridge, MA; 3) MD/PhD Program and Graduate Program in Neuroscience; 4) Howard Hughes Medical Institute; 5) Department of Genetics, Harvard Medical School, Boston, MA; 6) Department of Neurology and James & Jean Culver Vision Discovery Institute, Medical College of Georgia, Augusta University, Augusta, GA.

The unique combination of small size, translucent brain, and powerful genetics makes zebrafish an ideal vertebrate model system to investigate normal and pathological brain functions. However, it remains challenging to map out the myriad of connections between neurons and identify cellular level connectivity changes during development or disease. We recently found that vesicular stomatitis virus (VSV) can effectively infect zebrafish neurons and label connected neurons, paving the way for rapid brain mapping (J Comp Neurol. 2015; 523:1639-63). In this study, we combined multiple types of recombinant transsynaptic VSV, neurotransmitter phenotyping, and semi-automated 3D brain mapping to analyze the connectivity patterns between the retina and the brain in wild-type and mutant zebrafish. In wild-type fish, we observed VSV labeled retinorecipient cells near the visual afferent terminals in the diencephalon and the optic tectum, consistent with previous studies. Next, we analyzed zebrafish mutants of dscaml1, a conserved neuronal transmembrane molecule implicated in human autism spectrum disorder. dscaml1 homozygous mutants show greatly reduced connections, and the decrease is non-uniform among different brain regions. The reduced connectivity to the optic tectum, a critical region for saccade generation, may contribute to the reduced saccadic eye movements observed in mutants. Together, these results suggest novel roles for dscaml1 in establishing neuronal connectivity and visual behaviors. This approach will likely be useful for neuronal connectivity analysis in other zebrafish mutants or disease models.

Z548 Assembling the MET complex in sensory hair cells: Tomt regulates the trafficking of Tmc proteins to the site of mechanotransduction. *Timothy Erickson*[1], Elisabeth Busch-Nentwich[2], Jennifer Olt[3], Katherine Hardy[3], Reo Maeda[1], Rachel Clemens-Grisham[1], Alex Nechiporuk[1], Walter Marcotti[3], Teresa Nicolson[1]. 1) Oregon Health and Science University, Portland, OR; 2) Wellcome Trust Sanger Institute, Cambridge, UK; 3) University of Sheffield, Sheffield, UK.

The mechanoelectrical transduction (MET) complex is a multimeric transmembrane protein assembly that allows sensory hair cells to convert mechanical stimuli into electrical signals. Members of the complex include the tip link proteins PCDH15 and CDH23, as well as MET channel subunits LHFPL5, TMIE and TMC1 / 2. Although the identity of the ion channel that mediates MET is controversial, genetic and molecular evidence shows that Transmembrane channel-like proteins (TMC1 and TMC2) play an essential role in the MET complex, possibly acting as the pore-forming subunits of the channel. How the TMCs and other MET proteins assemble into a functional unit is a major outstanding question in the field of hearing research.

Transmembrane O-methyltransferase (*TOMT* / *LRTOMT*) is a human deafness gene responsible for non-syndromic deafness DFNB63. However, the specific role that TOMT plays in hair cells is not known. We found the zebrafish ortholog of *tomt* (*mercury*) in a mutagenesis screen for deafness and balance mutants in zebrafish. Tomt-deficient hair cells have normal morphology, but do not have functional mechanotransduction. GFP-tagged Tomt is enriched in the Golgi, and excluded from the site of MET in the apical hair bundle of hair cells. Since Tomt is not a part of the MET complex itself, we reasoned that it functions in the Golgi to regulate the trafficking of other MET components to the hair bundle. Tomt is not required for the normal trafficking of Pcdh15a, Lhfpl5a, or Tmie. However, we found that GFP-tagged Tmc1 and Tmc2b proteins are specifically excluded from the hair bundle in Tomt mutants. Transgenic expression of Tomt is sufficient to restore MET to mutant hair cells, and to restore Tmc localization in the hair bundle. Thus, we propose a model of MET complex assembly where Tomt methyltransferase activity is required for the correct trafficking of Tmc proteins in sensory hair cells.

Z549 Asymmetric activation of the dorsal habenulae correlates with larval recovery from electric shock. *E. R. Duboué*[1], E. Hong[2], A. Muto[3,4], K. Kawakami[3,4], M. E. Halpern[1]. 1) Carnegie Institution for Science, Baltimore, MD; 2) Laboratoire Neurosciences Paris Seine, INSERM UMRS 1130, UMR 8246 Université Pierre et Marie Curie 75252 Paris, France; 3) Division of Molecular and Developmental Biology, National Institute of Genetics, Mishima, Shizuoka, Japan; 4) Department of Genetics, The Graduate University for Advanced Studies (SOKENDAI) Mishima, Shizuoka, Japan.

Owing to their differences in size, gene expression and connectivity, the dorsal habenulae (dHb) of larval zebrafish can be used to assess the significance of left-right (L-R) asymmetry of the brain on behavior. In particular, we discovered a differential response between the left and right dHb to a fearful stimulus, mild electric shock. Following shock, wild-type larvae freeze for a short period of time (10-15 sec) before swimming again. Whole body cortisol levels, as measured in groups of larvae, also increase in response to electrical stimulation. Optogenetic activation of the dHb decreases the length of freezing, indicating that this brain region expedites the recovery from an aversive stimulus. Using *in vivo* calcium imaging in head affixed larvae, we identified an asymmetric population of neurons, whose activation is temporally correlated with recovery from shock. Consistent with more neurons in the left dHb modulating the response, unilateral severing of a major axonal pathway from the left but not the right dHb results in increased freezing behavior. Larvae with L-R reversed asymmetry or with right isomerized dHb also show a significant increase in freezing duration, as well as higher cortisol levels compared to controls. Moreover, neural activation in response to shock is not detected in the dHb by calcium imaging of these larvae. By contrast, larvae with left isomerized dHb exhibit a similar

period of freezing as their wild-type siblings but an equivalent number of neurons are activated in both dHb. The data suggest that a neuronal population, which predominates in the left dHb, mediates fear responses in larval zebrafish.

Z550 Linking function to cell type in the optic flow responsive circuit in zebrafish larva. *A. Kramer*, D. Förster, H. Baier, F. Kubo. Max Planck Inst. of Neurobiology, Martinsried, DE.

The accessory optic system (AOS) integrates binocular visual information to drive compensatory eye and body movements in response to optic flow. Recently, we showed, by optical imaging in the AOS homolog of larval zebrafish, the pretectal area, the existence of different types of direction-selective (DS) neurons (Kubo *et al*., 2014). So-called "simple cells" responded to moving gratings presented to one or two eyes, either nasal- or temporalward, or a combination of these stimuli. "Complex cells", on the other hand, encoded either translational or rotational motion. Simple responses can be generated by feedforward monocular DS inputs (e. g. from retinal ganglion cells). Complex response types, however, combine information from both eyes, requiring DS inhibitory connections. From this analysis, Kubo *et al*. (2014) were able to predict a wiring diagram of the optic-flow responsive circuit.

To test the predictions, we established a novel optogenetic method, combining functional characterization with morphological analysis of individual cells. In FuGiMA (Function-guided inducible morphological analysis), a cell of a specific response type is identified by regressor-based correlation of *in vivo* GCaMP6s imaging data with the expected neuronal activity. Next, paGFP is activated in the identified cell, enabling the visualization of its morphology.

We successfully reconstructed neurons with simple and complex response types. Registering the cells to a reference brain, we are now exploring if cells of the same response type have similar projection patterns. In parallel, we are also mapping inhibitory and excitatory cells in the pretectal area.

Z551 A forward genetic screen identifies the G-protein coupled calcium receptor CaSR as a regulator of simple decision-making. *R. A. Jain*[1], M. Wolman[2], K. Marsden[3], J. Nelson[3], H. Shoenhard[3], H. Bell[3], J. Skinner[3], M. Granato[3]. 1) Haverford College, Haverford, PA; 2) U Wisconsin-Madison, Madison, WI; 3) U Pennsylvania, Philadelphia, PA.

Animals appropriately respond to their constantly shifting environment through the critical process of decision-making: the selection of one behavioral response from a set of alternatives, each expected to produce different outcomes. While decision-making can involve complex cognitive processing, even simple responses can be dynamically biased and modulated, representing a more tractable system to study the underlying genetic and cellular decision-making mechanisms.

We have developed a simple decision-making paradigm in larval zebrafish using the evolutionarily conserved acoustic startle response. Larvae respond to acoustic stimuli with one of 2 kinematically, neuronally, and genetically distinct behaviors: a Short-Latency C-bend (SLC) initiated 4-15 ms post-stimulus, or a less vigorous Long-Latency C-bend (LLC) initiated 20-80 ms post-stimulus. Individual larvae can respond to acoustic stimuli with either behavior, yet bias their responses toward SLCs following intense stimuli and toward LLCs following weak stimuli. Importantly, individuals incorporate prior experience in selecting their behavioral output, shifting their response bias from SLCs to LLCs following repeated identical strong stimuli. Thus, the basic dynamic aspects of complex cognitive decision-making are present in this simple SLC/LLC decision-making paradigm.

To identify genes and pathways critical for the development and function of startle decision circuits, we performed a small molecule screen and a forward genetic screen. The small molecule screen results demonstrate that as in more complex cognitive assays, serotonergic modulation through the 5-HT1A receptor is critical for acoustic decision-making. Through our forward genetic screen we identified 10 mutants with specific defects in SLC/LLC bias, the first vertebrate mutants specifically isolated based solely on decision-making deficits. Using whole-genome sequencing we have identified mutations in both the *calcium-sensing receptor* (*CaSR*) gene and a regulator of CaSR endocytosis that both modify decision-making behavior. Furthermore, we have demonstrated an acute role for CaSR during decision-making and have identified the G-protein signaling pathways regulating appropriate bias through specific signaling agonists and antagonists. Finally, we have identified changes in the activity of key circuit components underlying these decision-making alterations through functional neural imaging to elucidate how serotonergic signaling and *CaSR* gene function influence simple decision-making.

Z552 Feeding state modulates behavioral choice and processing of prey stimuli in the zebrafish tectum. *Alessandro Filosa*, Alison Barker, Marco Dal Maschio, Herwig Baier. MPI of Neurobiology, Martinsried, DE.

Animals scan their visual environment to respond to threats and locate food sources. The neural computations underlying the selection of a particular behavior, such as escape or approach, require flexibility to balance potential cost and benefit to the animal's survival. For example, avoidance of a visual object reduces predation risk, but negatively affects foraging success. Zebrafish larvae approach small, moving dots ('prey') and avoid large, looming dots ('predator'). We found that this binary classification of objects by size is strongly influenced by feeding state. Changes in behavior correlate with shifts in neuronal responses to prey cues in the optic tectum. Both behavior and tectal function are modulated by signals from the hypothalamic-pituitary-interrenal axis and the serotonergic system. Our study has revealed a neuroendocrine mechanism by which the motivation to eat influences the perception of food in a vertebrate visual system.

Z553 Impact of circadian protein Period2 on glucocorticoid signaling and depression regulation. *Mingyong Wang*[1,2], Han Wang[1,2]. 1) Center for Circadian Clocks, Soochow University, Suzhou 215123, Jiangsu, China; 2) School of Biology & Basic Medical Sciences, Medical College, Soochow University, Suzhou 215123, Jiangsu, China.

The circadian clock as an endogenous time-keeping mechanism modulates fundamental life processes from molecular, biochemical, cellular, physiological to behaviorial with a period of approximately 24 hours. Previous studies showed that circadian abnormalities often lead to human moody disorders. However, little is known about any role of circadian protein Period2 (Per2) in depression pathogenesis. Here a series of behavioral assays show that TALEN-generated zebrafish *per2* mutant fish display lower locomotor activities, more time staying in the center region of the tank and less time for social interaction, in comparison with wild types, indicating a clear depression phenotype of the *per2* mutant zebrafish. Quantitative RT-PCR showed that glucocorticoid receptor (*gr*) is significantly down-regulated in both the *per2* mutant larvae and adult male fish brain. Luciferase reporter assays showed that Per2 can enhance Rorα-mediated expression of *gr*, and ChIP assays showed that Per2 binds to the three RORE elements in the *gr* promoter, suggesting that Per2 positively regulates *gr* in zebrafish. Further, cortisol, and expression of *pomc*(*proopiomelanocortin*) and *crh* (*corticotropin releasing hormone*) are significantly up-regulated in the *per2* mutant male fish, implicating disruptive activities of the hypothalamic-pituitary-adrenal (HPA) axis of the *per2* mutant fish. Taken together, our findings demonstrate that Per2 acts through glucocorticoid receptor signaling to impact activities of the hypothalamic-pituitary-adrenal (HPA) axis and to contribute to depression pathogenesis, shed light on a novel circadian role in moody disorders.

Z554 Visualizing Inhibitory Structural Synaptic Plasticity During Day and Night. *I. Elbaz*[1], D. Zada[1], A. Tovin[1], T. Tsur[1], T. Lerer-Goldshtein[1], G. Wang[2], P. Mourrain[2,3], L. Appelbaum[1]. 1) Bar-Ilan University, Ramat-Gan, IL; 2) Stanford University, Palo Alto, CA; 3) Ecole Normale Supérieure Paris, France.

Sleep is tightly regulated by the circadian clock and homeostatic mechanisms. The function of sleep is debated although it is established that the sleep/wake cycle is associated with structural and physiological synaptic changes that benefit the brain. Continuous imaging of single neuronal circuits in live animal is vital to understand the role of sleep in regulating synaptic dynamics. The hypothalamic hypocretin/orexin (Hcrt) neurons regulate various functions including feeding, reward, sleep and wake. In the zebrafish model, few Hcrt neurons regulate sleep and wake, and its transparency enables time-lapse imaging of single synapses during both day and night. Here, we established the Gephyrin (Gphn) protein, a central inhibitory synapse organizer, as a fluorescent post-synaptic marker of inhibitory synapses. A transgenic zebrafish that expresses the fusion protein Gphn-EGFP in specific neurons of interest was generated. Double labeling showed that Gphn-tagRFP and Collybistin-EGFP clusters co-localized in dendritic inhibitory synapses. In the dendrites of Hcrt neurons, inhibitory synapse number is increased during development and is stabilized at 6 days post fertilization (dpf). To determine the effect of sleep on inhibitory synapse number, we performed two-photon live imaging of Gphn-EGFP clusters in Hcrt neurons during day and night, under light/dark and constant light and dark conditions, and following sleep deprivation. We found that synapse number is increased during the night and decreased during the day. These changes in synaptic number are eliminated under constant conditions and in sleep-deprived larvae. These results suggest that rhythmic structural plasticity of inhibitory synapses in Hcrt dendrites is independent of the circadian clock and is regulated by sleep pressure.

Z555 Identifying novel regulators of early cardiac development in zebrafish using single-cell mRNA-seq and ATAC-seq. *X. Yuan*[1,2], M. Song[3], A. Aleksandrova[1], W. Devine[4], B. Bruneau[4,5], M. Wilson*[1,2], I. Scott*[1,2], *co-corresponding authors. 1) The Hospital for Sick Children, Toronto, ON, Canada; 2) University of Toronto, Toronto, ON,Canada; 3) Peking University, Beijing, China; 4) Gladstone Institute, San Francisco, CA, USA; 5) UCSF, San Francisco, CA, USA.

Heart disease remains a leading cause of death worldwide. A comprehensive understanding of the mechanisms underlying heart formation is crucial for uncovering causes of congenital heart disease and developing regenerative therapies. However there is currently a lack of markers that will enhance our understanding of the very earliest steps of vertebrate heart development, prior to the onset of *nkx2.5* expression in the cardiac crescent. Recently a mouse enhancer (*Smarcd3*-F6) was shown to label cardiac progenitor cells (CPCs) *in vivo*. Here we asked whether this mouse *Smarcd3*-F6 enhancer could serve as an early CPC marker in zebrafish. We created a stable zebrafish *Smarcd3-F6:EGFP* transgenic line and characterized the labeled population using immunostaining, RNA-seq and ATAC-seq (genome-wide profiling of open chromatins). The *Smarcd3*-F6 enhancer was active at early gastrula stage and enriched for CPCs. Despite sharing no sequence conservation with zebrafish, *Smarcd3-F6:EGFP* activity required the cardiac master regulator Gata5. Both RNA-seq and ATAC-seq results showed cardiac development-related pathways were enriched in *Smarcd3-F6:EGFP* labeled cells. Several ATAC-seq peaks near known cardiac genes drove early cardiac expression when tested in embryos, suggesting they could be novel markers facilitating early cardiac development studies. In order to further dissect the *Smarcd3*-F6+ cell population, we conducted single-cell mRNA-seq and identified a cluster of cells co-expressing known cardiac markers at the end of gastrulation, which represented the potential CPC lineage. Using *in situ* hybridization we have tested the expression of over 20 novel genes identified from the single-cell cardiac cluster and confirmed the vast majority of them showed early expression in the cardiac domain of zebrafish embryos. Currently we are performing single-cell mRNA-seq at multiple time points during gastrulation to assess the dynamics of CPC development. On-going experiments will also characterize the roles the novel genes play in cardiac development via CRISPR/Cas9 mutagenesis. In addition to improving our understanding of early cardiac gene regulation in zebrafish, our work underscores the conserved regulatory logic of vertebrate heart development.

Z556 PDGF signaling directs cardiomyocyte movement toward the midline during heart tube assembly. *D. Yelon*, J. Bloomekatz, A. C. Dunn, M. Vaughan. University of California, San Diego, La Jolla, CA.

Communication between neighboring tissues plays a central role in directing cell movement during organ morphogenesis. Heart tube assembly, for example, begins with the movement of bilateral populations of cardiomyocytes toward the midline, where they merge together in a process called cardiac fusion. Prior studies have established that cardiac fusion is regulated by interactions between the lateral plate mesoderm and the adjacent endoderm, but the molecular underpinnings of the relationship between these tissues remain unclear. Here, we reveal a new role for platelet-derived growth factor (PDGF) signaling in mediating tissue communication during heart tube assembly. We find that mutation of the zebrafish *pdgfra* gene, encoding PDGF receptor alpha, causes cardia bifida, a failure of cardiac fusion. Cell tracking experiments demonstrate that this defect is the consequence of misdirected cell movements, indicating a specific role of PDGF signaling in guiding cardiomyocytes toward the midline. Intriguingly, the PDGF ligand gene *pdgfaa* is expressed within a portion of the endoderm that is positioned just medial to the *pdgfra*-expressing lateral plate mesoderm. Overexpression of *pdgfaa* throughout the embryo interferes with cardiac fusion, revealing an instructive role for PDGF signaling during this process. Together, our data suggest a novel mechanism through which endodermal-myocardial communication directs the stereotyped patterns of cell movement that initiate cardiac morphogenesis.

Z557 Convergence of FGF and Nodal signals on the actin cytoskeleton controls cardiac cell migration in zebrafish. *M. Grant*, J. Rowland-Williams, D. Grimes, R. D. Burdine. Princeton University, Princeton, NJ.

Nearly 40,000 infants are born each year with Congenital Heart Defects, structural abnormalities arising from aberrant asymmetric heart development. As formation of the zebrafish heart also relies upon proper asymmetric morphogenesis, we use these organisms as a model to understand the necessary signals and cellular events that underlie this process. In zebrafish, the first asymmetric event in the heart, cardiac jogging, results in the repositioning of atrial precursors to the left and anterior of ventricular precursors. The process of jogging occurs simultaneously with the conversion of a structure called the cardiac cone into a linear heart tube. We previously discovered that the Nodal signaling pathway contributes to the asymmetry of the zebrafish heart tube by increasing the velocities of cells on the left of the cardiac cone.

Microarray analysis on isolated cardiac cells reveals upregulation of regulators of the actin cytoskeleton, FGF receptors, and FGF transcriptional targets in the heart in response to Nodal during jogging. In our preliminary data, we find evidence of the convergence of the Nodal and Fibroblast Growth Factor (FGF) pathways on the actin cytoskeleton. We also find that reduction of actin polymerization or FGF signaling mimic loss of nodal: under these conditions, tube formation is disrupted and tube extension is perturbed. Time-lapse confocal microscopy reveals a significant decrease in cell migration rates in embryos in which FGFR signaling or actin polymerization is reduced. Finally, epistasis analysis suggests that FGFR signaling functions parallel to Nodal to affect cell migration. Thus, we propose that FGFR signaling acts as a general pro-migratory signal for cardiac cells and cooperates with Nodal, which exerts an asymmetric motogenic effect on cardiac precursors by acting on the cytoskeleton.

Z558 Cardiomyocyte fusion in zabrafish. *S. Sawamiphak*, Z. Kontarakis, S. Reischauer, D. Stainier. MPI Heart and Lung Research, Bad Nauheim, DE.

Cells can sacrifice their individuality and fuse to acquire specialized functions. The generation of multinucleated syncytia in the developing skeletal myocytes by cell fusion is thought to be essential for effective contractility. However, the prevalence and significance of cell fusion in different tissues under developmental or physiological conditions is not well described. Here, we introduce a transgenic zebrafish reporter line that allows fluorescent labeling of polyploid cells. Surprisingly, besides skeletal muscles, some cardiomyocytes turn on the fluorescent reporter. This cell population largely contributes to the proliferating cardiomyocytes at this stage. Therefore, we hypothesized that, rather than cytokinesis failure, membrane fusion is the mechanism that activates the polyploidy reporter in these cells. Correspondingly, we observed a reduction of polyploidy reporter-activated cardiomyocytes in the cell fusion-compromised *jam3b* mutants, accompanied by a decrease in cardiomyocyte proliferation. Regenerative response of injured adult heart, conversely, increases polyploidy reporter-activated cells. Furthermore, blastula transplantations show a significant number of cardiomyocytes that harbor both donor- and host-derived transgenes, further suggesting the occurrence of cardiomyocyte fusion in the larval heart. Together, our findings not only uncover a novel cellular process occurring in the developing heart, but also highlight its possible role in cardiomyocyte proliferation in the vertebrate heart.

Z559 HDAC1 repression of retinoic acid-responsive genes promotes second heart field development. *Y. Song*[1,2], A. Rydeen[1,2], T. Dohn[1,2], J. Waxman[2]. 1) University of Cincinnati, Cincinnati, OH; 2) Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Outflow tract (OFT) defects make up a significant portion of congenital heart defects (CHDs), the most common birth malformations. The vertebrate OFT is derived from later differentiating second heart field (SHF) progenitors, necessitating a greater understanding of the mechanisms directing proper SHF development. In a screen for effectors of heart development, we identified the *cardiac really gone* (*crg)* mutant, which displays a smaller OFT and reduced ventricular cardiomyocytes. Furthermore, we observe a loss of SHF marker gene expression after cardiac differentiation occurs from the earlier differentiating first heart field, suggesting that SHF development is specifically affected in *crg* mutants. Positional cloning of *crg* revealed that it is a novel *histone deacetylase 1* (*hdac1)* mutant allele, with a mutation in a nucleotide of the splice donor site for the 7th exon. Consistent with HDAC1 being required to promote SHF addition, embryos treated with the HDAC inhibitor trichostatin A (TSA) or depleted for HDAC1 with morpholinos also display a similar specific loss of ventricular cardiomyocytes and SHF progenitor markers. Interestingly, the specific effects on SHF development are similar to what we have recently found in Cyp26 deficient embryos, which have an increase in retinoic acid (RA) levels. Although HDACs are known to interact with RA receptors (RARs), there are few

examples of RARs being required to mediate transcriptional repression during development. Hence, we performed RNA-seq on *crg* and Cyp26 deficient embryos and identified that the expression of *ripply3*, a transcriptional co-repressor of TBX transcription factors, is similarly increased in the hearts of both conditions, suggesting that HDAC1-mediated repression of ripply3 may be necessary for SHF development. Consistent with this hypothesis, we find that overexpression of Ripply3 can restrict cardiac development. Altogether, our study reveals a novel mechanism whereby the epigenetic regulator HDAC1 promotes SHF development through repressing the expression of the RA-responsive gene *ripply3*.

Z560 Epigenetic control of zebrafish cardiogenesis by TET2/3. *Y. Lan*[1], C. Li[1,2], M. Goll[2], T. Evans[1]. 1) Weill Cornell Medical College, New York, NY; 2) Memorial Sloan Kettering Cancer Center, New York, NY.

The targeted addition and removal of DNA methylation marks are major epigenetic regulatory mechanisms essential for normal development. Ten-eleven translocation (TET) enzymes (TET1/2/3) mediate methylcytosine (5mC) hydroxylation, which can facilitate DNA demethylation and altered gene expression. Functions for TET enzymes during development of complex organs including the heart have not been explored. Using zebrafish strains with targeted mutations in TET genes, we identified Tet2 and Tet3 as the major 5mC dioxygenases during zebrafish embryogenesis and observed specific defects in cardiogenesis in tet2/3$^{-/-}$ double mutant larvae. Morphological, molecular, and reporter strain analyses indicate defects in both epicardium migration and atrioventricular canal (AVC) development in tet2/3$^{-/-}$ double mutants. Co-culture experiments suggest that the epicardial defect may be indirectly caused by an altered myocardial program. To investigate the molecular mechanism, we compared transcript profiles of embryonic hearts isolated from wild type or double mutant embryos, and identified several candidate pathways that could impact epicardial and AVC development. Genome-wide MeDIP-seq and hMeDIP-seq experiments are ongoing to correlate DNA methylation and gene expression changes between mutant and wild types. This analysis should elucidate for the first time essential DNA epigenetic modifications that govern gene expression changes during cardiac development.

Z561 Multicolor mapping of the cardiomyocyte proliferation dynamics that construct the atrium. *M. J. Foglia*, J. Cao, V. A. Tornini, K. D. Poss. Duke University Medical Center, Durham, NC.

The orchestrated division of cardiomyocytes assembles heart chambers of distinct morphology. To understand the structural divergence of the cardiac chambers, we determined the contributions of individual embryonic cardiomyocytes to the atrium in zebrafish by multicolor fate-mapping, and we compare our analysis to the established proliferation dynamics of ventricular cardiomyocytes. We find that most atrial cardiomyocytes become rod-shaped in the second week of life, generating a single-muscle-cell-thick myocardial wall with a striking webbed morphology. Inner pectinate myofibers form mainly by direct branching, unlike delamination events that create ventricular trabeculae. Thus muscle clones assembling the atrial chamber can extend from wall to lumen. As zebrafish mature, atrial wall cardiomyocytes proliferate laterally to generate cohesive patches of diverse shapes and sizes, frequently with dominant clones that comprise 20-30% of the wall area. A subpopulation of cardiomyocytes that transiently express amhc (myh6) contributes substantially to specific regions of the ventricle, suggesting an unappreciated level of plasticity during chamber formation. Our findings reveal proliferation dynamics and fate decisions of cardiomyocytes that produce the distinct architecture of the atrium.

Z562 Spatiotemporal regulation of cell size and nuclear content during regeneration of the epicardium. *Jingli Cao*, Jinhu Wang, Kenneth Poss. Duke University, Durham, NC.

Adult zebrafish possess a remarkable capacity for cardiac regeneration without significant scarring. The epicardium, a mesothelial cell layer enveloping the heart, is activated by cardiac injury and enables new muscle regeneration through paracrine effects and as a multipotent cell source. Recent work revealed that the epicardium is highly regenerative, and that its presence is required for muscle regeneration. Yet, the cellular and molecular mechanisms by which epicardial cells respond to cardiac injury require elucidation. In this study, we created a panel of transgenic lines employing *tcf21* regulatory sequences to visualize subcellular components (LifeAct-EGFP, Histone H2A-EGFP) and cell cycle phases (FUCCI) within epicardial cells. Combining these reagents with an *ex vivo* system for live imaging of epicardial regeneration, we found that endoreplication and hypertrophy are prominent in cells at the leading edge of the regenerating epicardial sheet. By contrast, epicardial cells in the lagging regions complete cytokinesis and are responsible for increased cell density. Endoreplication (including both endocycling and endomitosis), but not cell fusion, leads to hypertrophic epicardial cells. These cells are further eliminated through apoptosis after full recovery of the ventricular epicardium. Through chemical screening we found that inhibition of Tgf-beta signaling blocked epicardial cell proliferation, led to smaller numbers of cells and higher incidence of hypertrophy, suggesting a key role in balancing sheet dynamics. Our findings indicate an unexpected role for endoreplication and hypertrophy in regeneration of the cardiac mesothelium.

Z563 The zebrafish embryo mRNA interactome reveals distinct roles for hnRNP A1 during the maternal to zygotic transition. *K. Neugebauer*, V. Despic. Yale, New Haven, CT.

In all metazoans, early embryogenesis is driven by maternal RNAs until zygotic transcription begins several hours later. During this maternal-to-zygotic transition (MZT), mRNA translation and stability regulation is essential. Thus, early embryogenesis is governed entirely by RNA biology; yet the RNA-binding proteins (RBPs) that interact with RNA are largely unknown. Here we identify 223 mRNA-bound proteins (mRBPs) in zebrafish embryos before and during zygotic transcriptional onset, including a subset of stage- and species-specific mRBPs. We show that hnRNP A1, an abundant component of the interactome, exhibits unusual 3'UTR binding. This is surprising, because hnRNPs bind mostly to introns in somatic cells. Furthermore, hnRNP A1 associated with zygotic m6A modified pri-miR430 transcripts, implicating hnRNP A1 in the

biogenesis of miR430, which is essential for maternal mRNA degradation during MZT. Our study uncovers core regulators of embryonic RNA and suggests that hnRNP proteins adopt unconventional RNA processing roles to promote vertebrate development.

Z564 Clearance of maternal mRNAs via 3'-end uridylation in vertebrate embryos. *Hyeshik Chang*[1,2], Jinah Yeo[1,2], Jeong-gyun Kim[2], Hyunjoon Kim[1,2], Jaechul Lim[1,2], Hee-Yeon Jeon[2], Hyun Ho Kim[2], Jiyeon Ok[3], Hosung Jung[3], Hyunsook Lee[2], Kyuwon Kim[2], V. Narry Kim[1,2]. 1) Institute for Basic Science, Seoul, KR; 2) Seoul National Univ., Seoul, KR; 3) Yonsei Univ., Seoul, KR.

During the maternal-to-zygotic transition (MZT), maternal transcriptome should be degraded, and replaced by zygotic transcripts in a highly coordinated manner. As transcription is silenced in early stages, transcriptome is regulated by cytoplasmic polyadenylation and RNA decay. We recently developed a new high-throughput sequencing technique, coined TAIL-seq, that profiles the poly(A) length and 3'-end modifications. TAIL-seq enables us to monitor the dynamics of adenylation, deadenylation and nucleotide tagging to the 3'-end of maternal RNAs with single-nucleotide resolution at genomic scale.

In this study, we apply TAIL-seq to early stage embryos of zebrafish to reveal how the RNA regulatory mechanisms influence the transcriptome. Our data confirm that poly(A) tails of most mRNAs elongate shortly after fertilization until the middle of the MZT, while a smaller group of transcripts escapes from this regulation. Surprisingly, most maternal RNAs acquire U tails in addition to poly(A) tails during the MZT. This phenomenon is observed in mouse and *Xenopus laevis* embryos, as well.

We identify Zcchc6 (also known as TUT7) and Zcchc11 (also known as TUT4) as the enzymes responsible for uridylation with further TAIL-seq experiments using morpholino-mediated knockdown. The maternal RNA clearance in zebrafish is significantly delayed during the MZT when Zcchc6 and Zcchc11 are knocked down, indicating that uridylation facilitates mRNA decay. Furthermore, the depletion of those enzymes delays the progression to gastrula in both zebrafish and *Xenopus laevis*. Thus, uridylation is indispensable for early embryogenesis.

We previously showed that human orthologs of Zcchc6 and Zcchc11 are pivotal to uridylation of messenger RNAs for their decay, and prefer short poly(A) tails. Our current data suggest that Zcchc6 and Zcchc11 contribute to selective clearance of molecules with short poly(A) tails. Indeed, maternal RNAs with shorter poly(A) tails elude the degradation in the MZT when the expression of Zcchc6 and Zcchc11 proteins are repressed.

In conclusion, Zcchc6 and Zcchc11 add uridine tails to short poly(A) tails in the vertebrate MZT, which is essential for the precise regulations of maternal factor expression in the early embryogenesis.

Z565 Testing the *in vivo* consequences of splicing and transcriptional crosstalk. *A. De La Garza*, T. Bowman. Albert Einstein College of Medicine, Bronx, NY.

Departments of Developmental and Molecular Biology and Medicine (Oncology), Gottesman Institute of Stem Cell Biology and Regenerative Medicine

Splicing and transcription are tightly intertwined processes that occur simultaneously. Disruption of the spliceosome or inhibition of splicing in cultured cells and yeast leads to transcriptional stalling, suggesting the existence of a transcriptional checkpoint that is linked to splicing. It is unclear if this occurs *in vivo* and if there are specific phenotypic consequences associated with activating a splicing checkpoint. To investigate if disruption of the spliceosome affects transcriptional elongation *in vivo*, we utilized a zebrafish loss-of-function mutant for the core spliceosomal component *sf3b1 (splicing factor, 3b subunit 1)*. In order to detect transcriptional stalling, we conducted Pol II ChIP-seq of wild type and mutant embryos, and are analyzing the results to determine if the position of RNA PolII across gene bodies is altered. To corroborate the Pol II ChIP-seq results, we will also analyze nascent RNA lengths in genes that are misexpressed in *sf3b1* mutants. Additionally, *sf3b1* mutants have defects in hematopoietic stem and progenitor cell (HSPC) formation from hemogenic endothelial cells. HSPCs require high transcriptional levels to arise from endothelium.To determine if there is transcriptional impairment in the hemogenic endothelium in the mutant, we employed flow cytometry to measure the amount of 5-ethynyluridine (EU) incorporation into RNA in *flk1:gfp* positive endothelial cells in wild-type and *sf3b1* siblings. The mutant endothelial cells had two-fold less EU incorporation than their wild-type siblings indicating that loss of *sf3b1* leads to a diminished transcriptional output. These studies will help determine if spliceosomal disruption causes transcriptional stalling *in vivo*, and whether the HSPC formation defects observed in *sf3b1* mutants are caused by transcriptional deficiencies.

Z566 Longterm Regulation of Zebrafish Behavior by Maternal Rest/NRSF is Mediated by *snap25a/b*. *Howard I. Sirotkin*[1], Cara E. Moravec[1], John Samuel[2], Wei Weng[3], Ian C. Wood[4]. 1) Stony Brook University, Stony Brook, NY; 2) Seneca College, Toronto, Canada; 3) Ingenious Targeting Labs, Ronkonkoma, NY; 4) University of Leeds, Leeds, UK.

During embryonic development, regulation of gene expression is key to creating the many subtypes of cells that an organism needs thoughout out its lifetime. Recent work has shown that maternal genetics and environmental factors have lifelong consequences on diverse processes ranging from immune function to stress responses. The RE1-silencing transcription factor (Rest) is a transcriptional repressor that interacts with chromatin-modifying complexes to repress transcription of neural specific genes during early development. In zebrafish, *rest* is maternally expressed and the chromatin modifications mediated by Rest have may exert long-term effects. We found that maternally supplied *rest* regulates expression of target genes during larval development and has lifelong impacts on behavior. Larvae deprived of maternal *rest* are hyperactive and show atypical spatial preferences. As adults, male but not female fish deprived of maternal *rest* present with atypical spatial preferences in a novel environment assay. To identify key targets of maternally supplied *rest,* we utilized transcriptome sequencing and found 158 genes that are repressed by maternal *rest* in blastula stages embryos. Expression analysis revealed that maternal *rest* is required to repress expression of several of these targets for extended periods (up to 6 dpf). Disruption of the RE1 sites in either of two of these genes, *snap25a*

and *snap25b* resulted in behaviors that recapitulate the hyperactivity phenotype caused by absence of maternal *rest*. Both the maternal *rest* mutants and *snap25a* RE1 site mutants have changes to the primary motor neuron architecture that is characteristic of overexpression of *snap25*. These results demonstrate that maternal *rest* represses *snap25a/b* to modulate larval behavior and that early Rest activity has lifelong behavioral impacts.

Z567 TFAP2A drives melanocyte gene expression in parallel with MITF. *H. E. Seberg*[1], E. Van Otterloo[1], S. K. Loftus[2], G. Bonde[1], R. Sompallae[1], J. F. Santana[1], J. R. Manak[1], W. J. Pavan[2], R. A. Cornell[1]. 1) University of Iowa, Iowa City, IA; 2) National Human Genome Research Institute, Bethesda, MD.

Disruption of the transcription factor network governing melanocyte differentiation contributes to the pathogenesis of pigmentation disorders and melanoma. While this network is well-studied, many details are unresolved. Transcription factor activator protein 2 alpha (TFAP2A) is expressed in melanoblasts and mutations in this gene cause pigmentation phenotypes in humans, mice, and zebrafish. However, the the transcriptional targets of TFAP2A, and the extent to which they are shared with MITF, a master regulator of melanocyte biology and melanoma progression, have been unclear. To determine the position of TFAP2A in the melanocyte gene regulatory network, we used microarray analysis in zebrafish and mouse immortalized melanocytes (melan-a cells) to profile genes that are downregulated in the absence of TFAP2A. We then conducted anti-TFAP2A ChIP-seq to create profiles of TFAP2A-bound loci in melan-a cells and human primary melanocytes. Genes at the intersection of the microarray and ChIP-seq profiles are likely direct targets of TFAP2A. These include *mc1r* and several melanin synthesis or melanosome structural genes, most of which are also thought to be direct targets of MITF. Comparison of our TFAP2A ChIP-seq profile with a published MITF ChIP-seq profile showed that TFAP2A peaks overlap MITF peaks at a large fraction of promoters and enhancers active in these cells. In reporter assays, deletion of TFAP2A binding sites decreased activity of a minimal TRPM1 promoter, similar to published results for deletion of MITF binding sites from this element. Furthermore, counts of embryonic melanocytes indicate a genetic interaction between *tfap2a* and *mitfa* in zebrafish. The significance of these findings is, first, they show that TFAP2A and MITF work in parallel to promote gene expression in melanocytes. Second, they show that a widely-expressed transcription factor, TFAP2A, cooperates with a more tissue-restricted transcription factor, MITF, to directly regulate expression of lineage-specific melanocyte genes. Third, they indicate that the reduction of TFAP2A expression levels observed in advanced melanoma is a cause and not an effect of melanoma progression, as such a reduction would be expected to decrease the pro-differentiation effect mediated by TFAP2A.

Z568 Tet-mediated DNA hydroxymethylation is required for retinal neurogenesis. *Pawat Seritrakul*[1,2], Jeffrey Gross[1]. 1) University of Pittsburgh, PA; 2) University of Texas at Austin, TX.

Tet dioxygenases convert 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC), epigenetic marks that are thought to regulate gene expression. How 5mC and 5hmC facilitate gene expression that underlies cellular differentiation during vertebrate eye development has not been resolved. The zebrafish retina is composed of neuronal and glial cells derived from a common pool of seemingly indistinguishable retinal progenitor cells (RPCs), serving as an ideal structure in which to study the epigenetic regulation of cellular differentiation. Gene expression analyses reveal that *tet2* and *tet3* are expressed in the developing retina. To functionally test the roles of these enzymes, we generated loss-of-function mutations in these genes. Homozygous mutant embryos deficient in either *tet2* or *tet3* showed no visible phenotype. However, double mutant (*tet2*$^{-/-}$*; tet3*$^{-/-}$) embryos developed ocular defects; they are microphthalmic, lack an optic nerve, and possess reduced numbers of differentiated neurons. BrdU incorporation assays showed elevated numbers of proliferative RPCs, and in situ hybridization revealed expanded RPC marker gene expression. Immunohistological and gene expression analyses revealed that the neurogenesis phenotype is likely due to failure of retinal neurons to terminally differentiate. RNA-seq analyses of gene expression in dissected eye tissues revealed reduced expression of terminal differentiation markers and upregulation of genes not normally expressed in the eye, suggesting that RPCs may be mis-specified when tet protein function is disrupted. To independently test this model, we generated inducible transgenic tet overexpression lines to artificially drive 5mC to 5hmC conversion. Embryos expressing the transgenes show increased global levels of 5hmC and defects in RPC differentiation. At the genome-wide level, virtually nothing is known about how the 5mC/5hmC landscape changes during the RPC to neuron transition. To profile these marks at single nucleotide resolution, we performed bisulfite sequencing, hydroxymethylation profiling, and RNA-seq using pure RPCs isolated from *vsx2*:GFP embryos, which express GFP in RPCs prior to differentiation. Initial analyses revealed reduced 5mC levels and enrichment of 5hmC at regulatory regions of known RPC genes, while genes expressed in other cell types maintain high 5mC levels. This correlates with RNAseq data, where RPC-specific genes are highly expressed. Ongoing work is aimed at profiling both 5hmC and 5mC at a genome-wide level during neurogenesis, utilizing a similar strategy to isolate pure populations of differentiated neuron types. Together, our work is the first evidence that tet proteins and 5hmC are required for normal retinal development.

Z569 Genomic dissection of conserved transcriptional regulation in intestinal epithelial cells. *Colin Lickwar*[1], J. Gray Camp[2], Matthew Weiser[3], David Kingsley[2], Shehad Sheikh[3], John Rawls[1]. 1) Duke University, Durham, NC; 2) Stanford University, Stanford, CA; 3) University of North Carolina at Chapel Hill, Chapel Hill, NC.

The intestinal epithelium serves as a major site of dietary nutrient absorption and microbial interactions. These important roles for intestinal epithelial cells (IECs) are shared among all vertebrates, but underlying transcriptional regulatory mechanisms are unresolved. Here we use a comparative functional genomics approach to test the hypothesis that conserved IEC functions are achieved using conserved transcriptional regulatory mechanisms across vertebrate species. We generated genome-wide adult IEC RNA-seq and open chromatin data (FAIRE-seq or DNase-seq) from four vertebrate species: Zebrafish, Stickleback, Mouse, and Human. Despite the evolutionary distance between these species,

we observe moderate correlations between relative IEC mRNA levels of 1to1to1to1 orthologs and identify a core subset of orthologous genes comprising a vertebrate IEC signature. We also compare existing expression data of mouse and zebrafish and find additional evidence for transcriptional and functional conservation regionally along the length of the intestine. Despite conservation of expression patterns, we find that only a few open chromatin regions overlapping conserved noncoding elements (CNEs) are specifically accessible in IECs. Using transgenic reporter assays to test the regulatory potential of these regions, we identified uncharacterized CNEs neighboring orthologous *hes1/her6* loci that are sufficient to drive IEC expression in zebrafish. However, these highly conserved IEC-specific open chromatin regions are very rare, and do not explain the tissue-specific expression of most IEC genes. In contrast, we find that many non-conserved regions neighboring orthologous genes in different genomes can still drive highly similar IEC expression in zebrafish. This indicates that traditional sequence conservation metrics do not sufficiently capture cis-regulatory information that is similar between species. In accord, we identify a set of transcription factor binding motifs that are similarly enriched in open regulatory regions near IEC signature genes despite a lack of traditional sequence conservation, suggesting a conserved transcriptional regulatory network. This work establishes a genomic resource for comparing and dissecting these transcriptional regulation networks that underlie shared intestinal structure and function in vertebrate species.

Z570 Constructing gene regulatory networks underlying fate specification of multipotent progenitors in the zebrafish neural crest. *K. Petratou*[1], T. Subkhankulova[1], G. Aquino[2], H. Schwetlick[1], A. Rocco[2], R. N. Kelsh[1]. 1) University of Bath, Bath, GB; 2) University of Surrey, Guildford, GB.

The gene regulatory networks (GRNs) underlying specification of multipotent progenitors to different lineages remain poorly characterised. Using the zebrafish neural crest (NC) and its derivative pigment cell lineages as a model and employing a previously established systems biology approach, we set out to construct GRNs governing fate segregation of multipotent NC progenitors. Here, we present data used to construct the core specification GRN for the iridophore, one of the zebrafish chromatophore lineages. Mutant phenotypes identified the transcription factors Sox10, Tfec and Mitfa as well as the receptor tyrosine kinase Ltk as key players. Regulatory interactions were derived using chromogenic *in situ* hybridisation on wild-type and mutant embryos, but also by overexpressing each gene and measuring resulting changes in gene expression by qRT-PCR. Our data supported the crucial role of a *sox10*-dependent positive feedback loop between *tfec* and *ltk*, driving iridophore specification. Establishment of the core network topology was followed by mathematical modelling using a system of differential equations solved numerically in MATLAB. The biological relevance of the predicted gene expression dynamics was experimentally assessed and testable hypotheses were derived to improve the model's accuracy. Using cycles of theoretical testing and experimentation, we improved and expanded the GRN by identifying previously overlooked regulatory mechanisms and additional factors. For example, we propose that a threshold level of Ltk signalling is required to enable *tfec* upregulation. Moreover, we discovered that *sox10* expression remains upregulated throughout iridophore development, leading us to investigate candidate repressors of its melanocyte-specific downstream target, *mitfa*. Although Foxd3 is reportedly suppressing *mitfa*, we present *in situ* hybridisation, as well as *ltk*/*foxd3* co-expression data obtained by the novel and highly sensitive fluorescent labelling RNAscope technique, that surprisingly challenge the proposed ongoing role of Foxd3 in the lineage. We conclude that Foxd3 is unlikely to be the repressor of *mitfa* in this context, while the lack of iridophores observed in *foxd3* mutant embryos likely results from a role in earlier NC progenitors. Overall, our studies shed light on NC development and demonstrate the value of integrating experimental and mathematical approaches when investigating complex networks.

Z571 Emergence of Patterned Activity in the Developing Zebrafish Spinal Cord. *Y. Wan*, Z. Wei, S. Druckmann, P. Keller. HHMI Janelia Research Campus, Ashburn, VA.

Spontaneous, patterned neuronal activity has been closely linked to developmental mechanisms shaping the early nervous system and is suggested to play a key role in the fine-tuning of developing circuits. However, little is known about how patterned activity emerges *de novo* and what factors control this maturation process. Here, we use Simultaneous Multi-view (SiMView) Light-sheet Microscopy to image the emergence of patterned activity in the developing spinal cord of embryonic zebrafish. We developed imaging assays and computational tools to record embryogenesis at the cellular level and systematically track cellular dynamics and lineage relationships in the developing spinal cord. By seamlessly transitioning from developmental imaging to high-speed volumetric functional imaging, we furthermore mapped calcium activity in all post-mitotic neurons for a large fraction of the developing spinal cord at a temporal resolution of 4 Hz. These data show that spinal cord neurons undergo a rapid transition from sporadic single-neuron activity to ipsi-laterally correlated and contra-laterally anti-correlated activity between 18 and 22 hours post fertilization. We developed a computational model to reconstruct the maturation process of this spinal cord circuit from our image data at the single-neuron level and characterize dynamic changes in functional connectivity as a function of time. We found that early functional communities are first established by spatially neighboring neurons, and neighboring communities subsequently merge by synchronization to form a patterned network. The time of recruitment of neurons to the spinal cord circuit follows, on average, a gradient from the anterior to the posterior spinal cord. Finally, we identified the cell types of active neurons with genetic markers and found that different types of neurons play different roles in circuit maturation. Ventral interneurons and motor neurons appear to serve as pioneers in the emergence of local functional communities, whereas dorsal commissural neurons may play a key role in establishing and maintaining the phase-locked state between left and right hemi-segments of the spinal cord.

Z572 Automated 3D cellular-resolution phenotyping of whole zebrafish with *in situ* RNA probe libraries. *Y. Wu*[1], A. Allalou[1], M. Rezaie[1,2], P. Eimon[1], M. Yanik[1,2]. 1) Massachusetts Institute of Technology, Cambridge, MA; 2) ETH, Zurich, Switzerland.

To enable quantitative high-content phenotyping in zebrafish using large *in situ* RNA probe libraries, we have developed an optical projection

tomography platform capable of imaging complex anatomical structures and gene expression patterns rapidly and affordably in three dimensions and at high resolution. In contrast to standard 3D imaging modalities--which require expensive hardware, are often not amenable to high-throughput sample handling, and are restricted to fluorescent readouts--our system can be assembled using commercially available components and enables 3D imaging of gene expression patterns at cellular resolution in non-embedded zebrafish larvae using standard chromogenic *in situ* protocols. Image acquisition time is less than 7 seconds per embryo and the system can be paired with automated fluidics such as the Vertebrate Automated Screening Technology (VAST) system we previously developed for higher-throughput larvae handling. We have additionally developed algorithms to automate all aspects of 3D reconstruction and registration to stage-specific anatomical atlases, allowing for rapid visualization of morphology and gene expression patterns throughout early development. By applying correlation analysis to a diverse library of brain-specific probes, we are able to quantify subtle changes in gene expression patterns between experimental groups throughout the entire brains of intact animals. Applying this approach to the well-characterized *tof/fezf2* mutant, we are not only able to automatically extract virtually all previously described early developmental defects, but also to detect novel aspects of the phenotype. Quantitative phenotypic data sets such as these allow labs to more easily compare phenome-level observations and will be an invaluable tool for analyzing the numerous mutant lines being generated through the rapid adoption of genome editing tools.

Z573 A conserved role for Lef1-mediated Wnt signaling in hypothalamic neurogenesis and anxiety. *Y. Xie*[1], S. Panahi[1], H. Xue[2], C. Fung[1], E. Levine[1], H. Coon[1], R. Dorsky[1]. 1) University of Utah, Salt Lake City, UT; 2) University of Iowa, Iowa City, IA.

We have previously shown that Wnt signaling through the transcriptional effector Lef1 is required for hypothalamic neurogenesis in zebrafish. However, the specific cellular and molecular functions of Lef1 remain unknown. We find that in zebrafish, Lef1 cell autonomously promotes the differentiation of multiple periventricular neuronal subtypes from Wnt-responsive progenitors. RNAseq analysis of zebrafish *lef1* mutants supports these results, showing decreased expression of known Wnt target genes and markers for defined neuronal subtypes that regulate anxiety. Indeed, we find that zebrafish *lef1* mutants display enhanced anxiety in a novel tank diving test, and gain weight more slowly than control siblings, which is consistent with enhanced anxiety. Analysis of a conditional hypothalamic *Lef1* knockout mouse shows similar neurogenesis defects, as well as decreased expression of orthologous genes that are co-expressed with *Lef1* in the mouse hypothalamic posterior periventricular and premammillary nuclei. Conditional *Lef1* knockout mice also gain weight more slowly than controls, and we are currently testing their behavior. Finally, we have found a human *LEF1* variant that is significantly enriched in Utah suicide cases, compared to equivalent control populations. Together, these data suggest a conserved role for Lef1 in regulating the development of hypothalamic circuits that mediate anxiety. We believe that our genetic models may prove useful in clinical medicine for the diagnosis and treatment of anxiety-related mental disorders.

Z574 Injury-induced *ctgfa* directs glial bridging and spinal cord regeneration in zebrafish. *Maryssa H. Mokalled*[1], Chinmoy Patra[2], Amy L. Dickson[1], Toyokazu Endo[1], Didier Y. Stainier[2], Kenneth D. Poss[1]. 1) Duke University Medical Center, Durham, NC; 2) Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany.

Unlike mammals, adult zebrafish efficiently regenerate functional nervous system tissue after major spinal cord injury. Whereas glial scarring is a major roadblock for mammalian spinal cord repair, glial cells in zebrafish form a bridge across severed spinal cord tissue and facilitate regeneration, a process about which little is known. Here, we performed a genome-wide profiling screen for secreted factors that are induced during zebrafish spinal cord regeneration. We find that *connective tissue growth factor a* (*ctgfa*) is induced in and around the glial cells that participate in initial bridging events. Mutations in *ctgfa* permit development to adulthood but disrupt spinal cord repair, whereas transgenic *ctgfa* overexpression and local recombinant protein delivery accelerate bridging and functional regeneration. Our study reveals Ctgf as a pro-regenerative factor that is necessary and sufficient to stimulate glial bridging and natural spinal cord regeneration.

Z575 Light-dependent regulation of sleep/wake states by prokineticin 2 in zebrafish. *D. Prober*[1], S. Chen[1], S. Reichert[2], J. Rihel[2]. 1) California Institute of Technology, Pasadena, CA; 2) University College London, London, UK.

Light affects sleep/wake behaviors indirectly by providing a cue that entrains circadian rhythms but also via direct regulation of behavior through a phenomenon known as masking. While circadian entrainment by light is well characterized at the molecular level, genes that underlie masking are largely unknown. Using zebrafish, a diurnal vertebrate, we found that both overexpression and mutation of the neuropeptide prokineticin 2 (Prok2) affect sleep/wake behaviors in a light-dependent but circadian-independent manner. We also found that the Prok2 overexpression phenotype requires *prokineticin receptor 2* and that Prok2 overexpression induces expression of *galanin*, a known sleep-inducing peptide, in the hypothalamus in a light-specific manner. These results suggest a novel genetic and neuronal circuit that links light via Prok2 to a known sleep-promoting center.

Z576 A forward genetic screen identifies Huntingtin-interacting protein 14 as an *in vivo* regulator of zebrafish habituation learning. *J. C. Nelson*[1], R. Jain[2], M. Granato[1]. 1) University of Pennsylvania, Philadelphia, PA; 2) Haverford College, Haverford, PA.

Learning reflects the ability of animals to modify behavioral responses using new information obtained from the environment. Habituation is learning in its simplest form: animals cease to respond to repeated but non-threatening visual, tactile or acoustic stimuli. In humans, habituation and other sensory thresholding processes are disrupted in multiple psychiatric and neurodegenerative disorders. Despite its importance, the genetic programs and molecular mechanisms that govern the assembly and function of the neuronal circuits critical for habituation learning are not well understood. Zebrafish exhibit numerous complex behaviors relevant to the study of neuropsychiatric

disorders, including acoustic startle habituation, with kinematic and pharmacodynamic parameters identical to those observed in adult zebrafish and mammals (Wolman et al, PNAS 2011).

To identify the genetic programs that govern vertebrate habituation learning, we have conducted the first forward genetic screen for genes critical for zebrafish startle habituation. This screen has yielded fifteen mutants with deficits in habituation learning, and through whole genome sequencing (WGS) we identified the PAPPAA metalloprotease as a novel regulator of IGF-dependent habituation learning (Wolman et al, Neuron 2015). Using WGS, we have since mapped 3 additional habituation mutants, identifying presumptive null mutations in *ap2s1*, *cacna2d3* and *zdhhc17/hip14*, implicating diverse cellular processes from Clathrin-mediated endocytosis to protein palmitoylation in modulating habituation learning. HIP14, or Huntingtin-Interacting Protein 14, is a palmitoyl acyl transferase that physically interacts with the Huntington's disease-associated Huntingtin protein. We will present behavioral analyses of all mutants, as well as a characterization of neuronal and synaptic morphology in known circuit components in *hip14* mutants. Together, these genes represent an unique opportunity to probe the cell and molecular mechanisms critical for the formation and function of the circuits underlying vertebrate learning behaviors.

Z577 Larval zebrafish show individual left/right bias in movement direction during local light-search behavior. *Eric Horstick*, Yared Bayleyen, Harold Burgess. NIH, Bethesda, MD.

Motivated states allow plasticity of an animal's behavior, facilitating adaptive responses to fluctuating homeostatic states and external challenges. A nearly universal motivated drive exists for finding resources. These goals are achieved by active modulation of sensory responsiveness and locomotor patterns. Despite the importance of these goal-directed behaviors for survival, the underlying neural mechanisms are still poorly understood. Here we report the identification of a novel light-search state in zebrafish that surprisingly includes an individual preference for left or right-ward movement. Using spatial analysis we found that after loss of illumination, larvae first show movement patterns consistent with a local light-search behavior that transitions to an outward exploratory phase. Each phase of the search exhibits distinct patterns of sensory responsiveness and movement trajectories that allow efficient navigation to local or remote sources of illumination respectively. Zebrafish *otp* mutants, null for the transcription factor Orthopedia, and previously demonstrated to have a reduced visual motor response, failed to transition out of local-search. Orthopedia is required for mid-brain dopaminergic (DA) neurons, neurosecretory neurons, and opn4a expressing deep-brain photoreceptors. Using mutants we found that the *otp* phenotype was not due to DA neuron loss, and was at least partially due to opn4a deep-brain photoreceptors. Next we sought to resolve the circuitry underlying the lateralized turning behavior. During local search, individuals show a robust preference for turning either left-ward or right-ward. Repeatedly testing individuals showed that an individual's left/right turn bias persisted for at least 2 days. We have excluded genetic inheritance, sensory input, and retinal circuitry as the source of the bias. To locate circuitry establishing the bias we genetically ablated 40 different Gal4 enhancer trap labeled neuronal populations and tested for turn preference. Ablation of the xa170 enhancer trap abolished lateralized turning behavior. The xa170 expression pattern labels several populations of neurons including pineal projection neurons and hypothalamic neurons. Functional whole brain activity mapping confirmed increased pineal activity during lateralized turning implicating pineal output in the maintenance of lateralized behavior. Thus, loss of illumination initiates a behavioral state consistent with conserved search strategies observed in numerous species including *C. elegans*, *Drosphila* and mammals. This state is temporally regulated by Orthopedia specified neurons. In addition, the initial phase of the behavior exhibits a persistent left/right preference. Using this novel model we aim to dissect the circuitry of goal-mediated responses and motor asymmetry and how these impact behavioral choice.

Z578 Neuro-taxonomy: Towards a complete parts list of the zebrafish central nervous system. *H. Baier*, M. Kunst, E. Laurell. Max Planck Institute of Neurobiology, Martinsried, DE.

One major obstacle to progress in neuroscience is the lack of knowledge about the neuron classes that make up the CNS. How many discrete cell types exist, and what is the "morphospace" of each of these types? In order to tackle this problem, we propose a combined genetic and anatomical approach to neuron classification at a large scale using larval zebrafish (*Danio rerio*). Zebrafish have ideal properties for this approach since they are translucent and easily amenable to genetic modification. Their brains are relatively small (ca. 90,000 neurons at 6 days post-fertilization) but display the basic architecture that is conserved across vertebrates. The small size permits this approach to reach full brain coverage in the near future. We are using a genetic tool that stochastically labels individual neurons with membrane-targeted fluorophores. Optical access and visualization are further facilitated by prior tissue clearing with a streamlined Clarity protocol. Labeled neurons are imaged by confocal microscopy at high resolution and reconstructed for quantitative morphological analysis. Because the method is based on the Gal4/UAS system, it will be possible, in the future, to target defined neuronal populations in order to combine genetic identity with morphological analysis. A variation of this tool tags pre-synaptic sites with a second, genetically encoded fluorophore, which gives information about the direction of information flow between neighboring cells and between interconnected brain regions. Currently, we have reconstructed >1,000 neurons from a pan-neuronal expression pattern and recorded their morphologies. Future efforts in our laboratory will be directed at automating image acquisition and establishing an image-processing pipeline, which will greatly accelerate our throughput. In order to compare neurons between individual fish, we are aligning them to a high-resolution, age-matched standard brain. This reference brain will consist of several different markers giving other researchers the flexibility of using a reference stain that best suits their experimental paradigm. Furthermore, combining all these markers into one standard brain will give us further insight into the functional modules that make up the larval zebrafish brain. This new database of zebrafish neurons will initially be populated by their morphological properties, anatomical location and transmitter type, but can be expanded to include functional, developmental and gene expression data. With support from a local supercomputing center, it is planned to make the database a web-accessible resource for the community and link it to ZFIN. This will give

zebrafish neuroscientists the ability to browse the stored information, upload data from their own experiments and carry out similarity searches, analogous to BLAST or PDB.

Z579 Identifying roles for Pbx factors in heart development and congenital heart defects using zebrafish genome engineering. *L. Maves*[1,2], G. Farr[1], D. Pouv[1,2], R. Kao[1], K. Magham[1,2], M. Majesky[1,2]. 1) Seattle Children's Research Institute, Seattle, WA, USA; 2) University of Washington, Seattle, WA, USA.

Work from our group and others has implicated Pbx homeodomain transcription factors in myocardial differentiation and outflow tract development. However, the mechanisms underlying Pbx function in heart development are not understood, and it is not known whether Pbx factors causally contribute to human congenital heart defects. We have used the CRISPR-Cas9 system to engineer zebrafish strains for a variety of pbx gene alleles to determine the roles of Pbx factors in heart development. First, we have used null alleles of the pbx2 and pbx4 genes to show that zebrafish embryos lacking both pbx2 and pbx4 show severe defects in early myocardial differentiation, myocardial morphogenesis, and outflow tract formation. Second, we have engineered in-frame deletions within the pbx4 gene to demonstrate critical functions in heart development for a domain conserved in Pbx proteins. Third, we have engineered a zebrafish strain carrying a Pbx gene variant that has been associated with congenital heart defects in human patients. We are using this strain to identify causative effects of this variant on heart and outflow tract development. Our zebrafish pbx gene alleles now allow us to test whether Pbx proteins function through transcriptional activation or repression in myocardial differentiation. Our studies provide novel examples of using genome engineering for structure-function analyses of critical heart transcription factors as well as for testing human variant functions in heart development.

Z580 An essential splice site mutation in flt1 protects against early-stage atherosclerosis in zebrafish larvae. *Marcel den Hoed*[1], Manoj bandaru[1], Lisa Conrad[1], Anastasia Emmanouilidou[1], Petter Ranefall[2], Carolina Wählby[2], Anders Larsson[3], Erik Ingelsson[1]. 1) Department of Medical Sciences, Molecular Epidemiology and SciLifeLab, Uppsala University, Sweden; 2) Department of Information Technology, Division of Visual Information and Interaction and SciLifeLab, Uppsala University, Sweden; 3) Department of Medical Sciences, Biochemical structure and function, Uppsala University, Sweden.

Objective: Genome-wide association studies (GWAS) identified 56 loci that are robustly associated with the risk of coronary artery disease (CAD). Only a handful of genes in these loci are currently known to influence CAD. We previously showed that: 1) CAD-associated loci exert their effect at least in part by influencing atherosclerotic plaque formation; 2) zebrafish larvae represent a robust model system for high-throughput, image-based screens of early-stage atherosclerosis. We now aim to identify causal genes in GWAS-identified loci for CAD using zebrafish model systems.

Methods: Heterozygous carriers of nonsense (sa1516 in cxcl12b; fh336 in col4a1) or essential splice site (sa1504 in flt1) mutations were in-crossed, and offspring were fed on a normal or high amount of a normal or high cholesterol diet from 5 to 10 days post-fertilisation. Vascular lipid deposition (monodansylpentane cadaverase) and co-localisation of lipids with macrophages (*Tg:mpeg1-mCherry*) and/or neutrophils (*Tg:mpo-EGFP*) were subsequently visualised using an automated positioning system, fluorescence microscope and CCD camera, and objectively quantified using a custom-written image analysis pipeline in ilastik, CellProfiler and ImageJ. After imaging, whole-body total cholesterol and triglyceride levels were assessed using enzymatic assays, and larvae were genotyped using KASP technology. A multilevel (hierarchical) mixed model analysis was performed to examine the effect of the mutations (additive model) on atherogenic traits, while adjusting for diet and time-of-imaging, as well as for batch (random factor).

Results: In data from up to 253 larvae, sa1504 in flt1 was associated with less vascular lipid deposition (P=2E-5), less co-localisation of lipids and macrophages (P=9E-3), and lower whole body total cholesterol levels (P=2E-2). No significant effects were identified for the sa1516 and fh336 mutations (N up to 223 and 344, respectively).

Discussion: Our results represent the first mutant model demonstrating that flt1 plays a role in early-stage atherosclerosis, implying that *FLT1* is the culprit for CAD in the 13q12 locus. Based on the results of *a priori* performed bioinformatics analyses, we believe that *CXCL12* and *COL4A1* remain the most promising candidates in their respective loci. The lack of effect for these genes in our screen may reflect genetic redundancy, adaptation and/or a late onset effect.

Z581 Mutations in COL22A1 cause a loss of vascular integrity that result in intracranial aneurysms. *Quynh V. Ton*[1], Daniel Leino[1], Joseph Broderick[2], T. Foroud[2], Allison Lubert[1], J. Farlow[2], D. Woo[2], Saulius Sumanas[1]. 1) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) University of Cincinnati, Cincinnati, OH.

Intracranial saccular aneurysms (IAs) are balloon-like or small berry defects in the wall of the major intracranial artery. Both environmental and genetic factors have been linked to the formation of IAs, but genetic risk factors of IAs are largely unknown. To identify additional genes responsible for IA development, whole exome sequencing of the patients from seven selected families was performed. One of the identified variants found in all five affected members of one family was a missense mutation in the highly conserved triple-helix region of the collagen *COL22A1* isoform, the function of which has not been previously known. This mutant variant was examined in zebrafish embryonic and adult models. We demonstrated that *COL22A1* is expressed in adjacent to the eyes, in the cranial tissues, in the myotendinous junctions, and in perivascular cells in the head region of the embryos. Early global overexpression of the human mutant but not wild-type *COL22A1* in the zebrafish embryos interfered with epiboly movements during gastrulation due to the retention of the mutant protein in the cytoplasmic domain that led to an induction of Endoplasmic reticulum stress response. Inducible overexpression of the human mutant protein at a later stage resulted in the increased percentage of embryos with hemorrhages compared with the wild type protein. These data further suggest that

the mutation has a deleterious dominant effect. We then used TALEN-engineered nucleases to generate a loss-of-function mutant in zebrafish *col22a1*. The homozygous null mutants were viable as adults but exhibited increased abnormal blood accumulations in the eyes and cranial regions, suggestive of hemorrhages. In addition, hemorrhages were present in the trunks of these adults caused by rupture of blood vessels. Furthermore, adult mutants are more susceptible to excessive forced overload cardiac stress than wild type siblings and have increased number of hemorrhages. We also demonstrated that the homozygous embryos show increased sensitivity to cardiovascular stress and exhibit greater frequency of intracranial hemorrhages, which can be partially rescued by the transient expression of wild type human COL22A1. The mutant embryos also exhibited increased vascular permeability as demonstrated by 10 kDa dextran injection into the circulation of 3 and 4-day post fertilization embryos. Our results argue that the function of COL22A1 is important in maintaining vascular integrity and that mutations in COL22A1 are one of genetic causes for intracranial aneurysms in humans.

Z582 Cure modeling human genetic skeletal muscle disorders. *N. Umemoto*, M. Urban, R. Harm, M. McNulty, K. Predmore, M. Serres, C. Daby, H. Sizek, C. Bullard, D. Hunter, X. Xu, K. Clark, S. Ekker. Mayo Clinic, Rochester, MN.

Background: Approved treatments are available for only ~5 percent of the thousands of known rare diseases. In particular, almost all neuromuscular disorders are considered orphan diseases caused by single gene defect. However, there are few approved drugs for the disorders. To overcome the bottleneck in drug discovery for these rare diseases, we hypothesize that the use of a new concept we call "cure modeling" can be used to augment traditional disease modeling such as gene knock-out animals.

Methods: To explore and identify the molecular mechanisms and signatures of health from these genetic skeletal muscle diseases, we have deployed our genome engineering technology to construct reversible mutant alleles, Protein Trap Gene-break Transposon (GBT) system in zebrafish. The main features of the GBT system are; i) GBT constructs are integrated in zebrafish genome using transposons and mapped using next gen sequencing; ii) >95% of the time, GBT alleles show 99% knockdown of the tagged endogenous transcripts; iii) mRFP reporter expression shows specific localization of the gene product; iv) Cre recombinase can be used to revert the mutant locus to wild-type function.

Results and Discussion: We have created 5 GBT cure models that have the mutant locus in orthologous zebrafish genes (*ano5a*, *ryr1b*, *dnajb6b*, *casq1a* and *gyg1a*) associated with human genetic disorders in skeletal muscle. To assess functional phenotypes of skeletal muscle in the GBT fish lines compared with or without exercise, we are screening the alteration of tcap expression, a molecular marker rapidly induced by skeletal muscle activity. Here, we report details about our findings from this unique functional phenotypic assay. These initial revertible mutants exhibit how cure modeling can represent an idealized therapy to treat a tested genetic disease at the level of transcripts, proteins, metabolites and other molecular components.

Z583 Fmrp interacts with Adar and regulates RNA editing, synaptic density and locomotor activity in zebrafish. *A. Shamay-Ramot*[1,2], K. Khermesh[1], H. Porath[1], M. Barak[1], Y. Pinto[1], C. Wachtel[1], A. Zilberberg[1], T. Lerer-Goldshtein[1,2], S. Efroni[1], E. Levanon[1], L. Appelbaum[1,2]. 1) The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, IL; 2) The Leslie and Susan Gonda Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan, IL.

Fragile X syndrome (FXS) is the most frequent inherited form of mental retardation. The cause for this X-linked disorder is the silencing of the fragile X mental retardation 1 (*fmr1*) gene and the absence of the fragile X mental retardation protein (Fmrp). The RNA-binding protein Fmrp represses protein translation, particularly in synapses. In *Drosophila*, Fmrp interacts with the adenosine deaminase acting on RNA (Adar) enzymes. Adar enzymes convert adenosine to inosine (A-to-I) and modify the sequence of RNA transcripts. Utilizing the *fmr1* zebrafish mutant (*fmr1*-/-), we studied Fmrp-dependent neuronal circuit formation, behavior, and Adar-mediated RNA editing. By combining behavior analyses and live imaging of single axons and synapses, we showed hyperlocomotor activity, as well as increased axonal branching and synaptic density, in *fmr1*-/- larvae. We identified thousands of clustered RNA editing sites in the zebrafish transcriptome and showed that Fmrp biochemically interacts with the Adar2a protein. The expression levels of the *adar* genes and Adar2 protein increased in *fmr1*-/- zebrafish. Microfluidic-based multiplex PCR coupled with deep sequencing showed a mild increase in A-to-I RNA editing levels in evolutionarily conserved neuronal and synaptic Adar-targets in *fmr1*-/- larvae. These findings suggest that loss of Fmrp results in increased Adar-mediated RNA editing activity on target-specific RNAs, which, in turn, might alter neuronal circuit formation and behavior in FXS.

Z584 Pyruvate carboxylase functions in astrocytes to regulate habituation learning. *M. Wolman*, C. Jennings, L. Johnson. University of Wisconsin, Madison, WI.

Animals constantly update their behavior by evaluating the significance of current sensory input and then integrating this information with knowledge gained from prior experiences. To filter irrelevant input and focus attention towards high priority stimuli, all animals exploit a fundamental form of learning, called habituation. Habituation is observed by a progressive response decline to repeatedly experienced, yet insignificant stimuli and provides a behavioral measure of a neural circuit's ability to balance synaptic excitation, inhibition, and plasticity. Habituation deficits are observed in schizophrenia, autism, and addiction, and contribute strongly to the patients' overall dysfunction. Despite its conservation and clinical relevance, the genetic and cellular mechanisms that mediate habituation remain poorly understood.

To identify genes critical for habituation, we recently performed an unbiased, genome-wide screen for zebrafish mutants with reduced habituation of the acoustic startle response. This screen identified a functional gene set for habituation, including a gene previously unknown for a role in habituation: *pyruvate carboxylase a*. Pyruvate carboxylase (PC) is a mitochondrial enzyme that converts pyruvate to oxaloacetate to stimulate the TCA cycle, and hence, the synthesis of metabolic products with diverse biological functions. In the brain, PC is not thought to be active in neurons, but instead, functions in oligodendrocytes to promote myelination and in astrocytes to replenish neurons with

neurotransmitters. Although both glial cell types are known to support synaptic plasticity, it remains unclear how signaling pathways in either cell type directly regulate habituation.

Through molecular-genetic, pharmacological, and behavioral analyses, we will provide data showing that astrocytic PC activity supports glutamatergic neurotransmission to promote habituation. This work defines a novel molecular mechanism underlying habituation and reveals a novel cellular locus of plasticity for habituation: astrocytes!

Z585 Introgression in Zebrafish and related species was mediated by genome structure. *Braedan M. McCluskey*, Peter Batzel, Jason Sydes, John H. Postlethwait. University of Oregon, Eugene, OR.

Introgression is increasingly appreciated as a major contributor to species diversification. Using exome sequencing for zebrafish and ten related species, we show that introgression played a major role in the formation of several *Danio* species, including the zebrafish, *Danio rerio*. We find introgressed loci primarily in genomic regions with high recombination rate, which is strongly impacted by chromosome structure in zebrafish. Due to varying levels of introgression across the genome and a likely major chromosomal rearrangement, the closest historical relative of zebrafish varies for different genomic regions. These varied relationships across the genome give general insight into the effects of genome structure on introgression and the importance of incorporating structural genomic information into phylogenomic studies involving taxa with high levels of introgression. These results also provide specific insight into the complex speciation process in the recent history of a major model organism. Future studies investigating the phenotypic differences between zebrafish and other danios will need to account for this complex speciation process.

Z586 Regulation of actinodin1 in embryonic fins via tissue-specific cis-acting regulatory elements: a potential mechanism for the loss of these genes during the fin-to-limb transition. *R. Lalonde*, D. Moses, J. Zhang, N. Cornell, M.-A. Akimenko. University of Ottawa, Ottawa, CA.

The fin-to-limb transition is an important evolutionary step in the colonization of land and diversification of terrestrial vertebrates. Our lab has identified a gene family in zebrafish, termed *actinodin* (*and*), which codes for structural proteins in the teleost fin. These proteins are crucial for the formation of rigid fibrils known as actinotrichia. Actinotrichia support the fin fold, a structure formed from the extension of the apical ectodermal ridge (AER) during pectoral fin development. During limb development, the AER is maintained relatively longer prior to eventual regression. Interestingly, this gene family is absent from all tetrapod genomes examined to date, suggesting that it was lost during limb evolution. We propose that the disappearance of *and* genes resulted from modification in their regulation and we therefore characterized the regulatory elements of *actinodin1 (and1)*, one of two longer *and* paralogues (*actinodin1/2)* in zebrafish. Within a 2kb fragment immediately upstream, we have identified tissue-specific *cis*-acting regulatory elements responsible for *and1* expression in the developing zebrafish median and pectoral fin folds. Using transgenic reporter lines, we have identified and isolated the regulatory elements responsible for *and1* activation in the ectodermal and mesenchymal cell populations, respectively. To test their functionality in tetrapods, a transgenic LacZ reporter mouse line was made. In contrast to the zebrafish transgenic line, reporter expression is restricted to the ectoderm of the fore- and hindlimb buds, localizing within the autopodal domain. One possibility suggests that while the regulatory network for *and1* ectodermal activation remains intact in mice, the mechanisms for mesenchymal activation have been lost or altered during tetrapod evolution. Putative transcription factor binding sites were identified via TRANSFAC and tested for enhancer activity using site-directed mutagenesis. Removal of tcf and 5' hoxA/D binding sites lead to the loss of ectodermal and mesenchymal enhancer activation, respectively. Dissecting the regulatory mechanisms of this gene family is crucial for uncovering the mechanisms of *and* loss during tetrapod evolution. Finally, *and1 cis*-acting regulatory elements enable us to perform tissue-specific lineage tracing and ablation of *and1*-expressing cells. We are currently performing lineage tracing experiments on both ectodermal and mesenchymal cell populations using the Cre-LoxP system, in addition to observing the effects of fin fold mesenchyme ablation on fin development using the Nitroreductase/Metronidazole system. This work is supported by a NSERC grant to M.-A.A.

Z587 The MITF family member *tfec* functions in zebrafish neural crest pigment cell fate diversification. *J. Lister*, S. Spencer. Virginia Commonwealth University, Richmond, VA.

In organisms such as fish and reptiles where the neural crest produces more than one type of pigment cell, the existence of a lineage-restricted precursor of all pigment cell types has been speculated upon but not conclusively demonstrated. In zebrafish, null mutations in the *microphthalmia-associated transcription factor* (*MITF*) ortholog *mitfa* lack all neural crest-derived melanocytes but retain the other two neural crest pigment cell types, xanthophores and iridophores. In fact, *mitfa* mutants display an increased number of iridophores compared to wild-type, suggestive of a possible cell fate switch, and cell lineage experiments indicate the existence of a bipotent iridophore/melanocyte precursor. We have begun to examine another member of the zebrafish MITF family, *tfec*, which is expressed in premigratory and migrating neural crest cells and later in differentiating iridophores. Knockdown of *tfec* with antisense morpholino oligonucleotides, or by a CRISPR/Cas9 approach indicates that *tfec* is necessary for the development of iridophores. Intriguingly, knockdown of *tfec* in *mitfa* mutant embryos additionally eliminates the third pigment cell type, xanthophores. These data indicate that all three pigment cell lineages in zebrafish are dependent upon one or a combination of MITF family proteins, and suggest that perhaps the choice between these cell fates may depend in part on their relative levels. Moreover, they provide genetic evidence for a distinct pan-pigment precursor in the hierarchy of zebrafish neural crest cell fate diversification.

Z588 Evolutionary Conservation of Tcf12 and Twist1 Function in Coronal Suture Development. *C. Teng*, G. Crump, R. Maxson. University of Southern California, Los Angeles, CA.

In a normal developing child, skull bones are connected by fibrous sutures that allow pressure to be released as the brain grows and expands. Craniosynostosis is the premature fusion of these sutures in newborns and infants. In Saethre-Chotzen syndrome, a disorder characterized by synostosis of the coronal suture and other facial irregularities, heterozygous mutations in the basic helix-loop-helix (bHLH) transcription factors *TWIST1* or *TCF12* account for the majority of diagnoses. We hypothesize that Tcf12 functions as a suture-specific heterodimerization partner of Twist1 that promotes its ability to maintain osteoblast progenitors in an undifferentiated state. In support of such an interaction, loss of one allele of *Tcf12* enhances coronal synostosis in *Twist1+/-* mice. In order to understand how early changes in osteoblast differentiation may account for suture defects in individual animals, we have developed a zebrafish model of Saethre-Chotzen syndrome by generating null alleles for *tcf12* and the two zebrafish Twist1 homologs (*twist1a* and *twist1b*). Remarkably, one particular combination of alleles, *tcf12-/-*; *twist1b-/-*, survives to adulthood and displays a near complete and specific loss of the coronal suture. Whereas *twist1a*; *twist1b* double mutants have much earlier defects in specification of the neural crest-derived ectomesenchyme that generates the head skeleton, further loss of *tcf12* partially restores early craniofacial development and allows survival to adulthood. These findings suggest that Twist1 may have different binding partners during ectomesenchyme and suture development, with Tcf12 acting as a Twist1 binding partner during later suture development. While mouse coronal sutures lie at a boundary between neural crest-derived frontal bones and mesoderm-derived parietal bones, zebrafish coronal sutures lie between the mesoderm-derived portions of the frontal and parietal bones. Our data thus provide strong evidence for deep evolutionary homology of the coronal suture between fish and mammals, as the role of Tcf12/Twist1 interactions is preserved regardless of tissue origin. We are presently conducting live imaging of osteoblast differentiation over time in our zebrafish model to better understand how early defects might result in later craniosynostosis.

Z589 Fish synovial joints as new models for joint development and disease. *Joanna Smeeton*[1], Amjad Askary[1], Sandeep Paul[1], Simone Schindler[1], Ingo Braasch[2,3], Nicholas A. Ellis[4], John Postlethwait[2], Craig T. Miller[4], Gage Crump[1]. 1) University of Southern California, Los Angeles, CA; 2) University of Oregon, Eugene, OR; 3) Michigan State University, East Lansing, MI; 4) University of California, Berkeley, CA.

Synovial joints are the most common and flexible type of joint in mammals. These freely moveable joints are characterized by the presence of a synovial cavity, articular cartilage and joint capsule. A widely held view is that synovial joints first evolved in tetrapods, with fish lacking this type of joints. Surprisingly however, we found that joints in the jaw and pectoral fin of zebrafish, stickleback and spotted gar share the characteristics of mammalian synovial joints. Histological analysis and live imaging of fluorescent transgenic zebrafish from 3 days post fertilization to 12 months of age demonstrated that the zebrafish jaw joint has a synovial cavity and is wrapped in a thin joint capsule. Cartilage cells that have flattened morphology just like mammalian articular chondrocytes cover the surface of juxtaposed skeletal elements at the zebrafish jaw and fin joints. In situ hybridization analysis of genes expressed in mammalian synovial joints revealed that, similar to mammals, the zebrafish synovial joint articular cells do not express collagen type II or X, aggrecan or matrilin1, and instead express a joint lubricating proteoglycan, lubricin, encoded by the gene *prg4b*. Lubricin is secreted from the articular cells of the joint to lubricate the joint surface and its expression is one of the hallmarks of a synovial joint. To determine whether the jaw joint *prg4b* expression pattern is a conserved feature throughout ray-finned fish species, we investigated *prg4* expression in a distantly related teleost fish, the threespine stickleback (*Gasterosteus aculeatus*), and a basal ray-finned fish, the spotted gar (*Lepisosteus oculatus*). Similar to zebrafish, *prg4* homolog expression is restricted to the jaw joint articular surface in juvenile stickleback and spotted gar. Finally, genetic deletion of zebrafish *prg4b* demonstrated a conserved requirement for *prg4b* gene function in the age-related maintenance of joint cartilage. Our data represent the first molecular and functional evidence that ray-finned fish have lubricated synovial joints, supporting a model that lubricated synovial joints evolved at least in the last common ancestor of all bony vertebrates. Moreover, our results establish the zebrafish as a novel model organism to investigate synovial joint development and repair, and opens up the powerful genetic tools of the zebrafish to the field of arthritis research.

Z590 Cavefish evolution as a natural model for metabolic diseases. *N. Rohner*[1], A. C. Aspiras[2], C. J. Tabin[2]. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Harvard Medical School, Boston, MA.

Understanding the genetic basis of adaptation has broad implications not only for a basic understanding of evolution, but also for human pathologies given that many human diseases are a consequence of mis-adaptation to modern societies. The emerging cavefish model system *Astyanax mexicanus* has become an important fish species to address adaptation to extreme environments due to its unique ecology and the availability of genetic tools (e.g. QTL mapping) and genomic resources. Despite 180 million years divergence to zebrafish, cavefish is currently the closest related sequenced fish species on Ensembl.

Cave environments are typically dark and as a consequence nutrient deprived. We have previously shown that cavefish acquired impressive adaptations such as hyperphagia (increased appetite), starvation resistance and altered feeding behaviors to cope with these harsh conditions. Here, we have focused on the fatty livers and symptoms reminiscent of diabetes these fish develop. Interestingly, we detected only very low insulin levels in cavefish (compared to surface or zebrafish) partially due to lower numbers of beta-insulin producing cells in the pancreas. In addition, cavefish display strong insulin resistance when administered with ectopic insulin. Despite the consequential elevated and highly fluctuating blood glucose levels, cavefish live long and healthy lives, probing the question whether they have acquired mechanisms allowing them to cope with extreme nutritional levels.

Taking advantage of the newly available genome of *Astyanax mexicanus* and tissue specific RNA-Seq data, we identified mutations in the insulin receptor of cavefish most likely responsible for the observed insulin resistant phenotype. Importantly, the same mutations were found

in cases of Type-II diabetic patients in human populations. Our findings in independently derived cavefish populations suggest that cavefish are inherently insulin resistant, potentially as an additional strategy to acquire better starvation resistance. We are currently using genome editing to functionally test these and other candidate mutations in zebrafish and cavefish itself to study in detail the molecular mechanisms underlying the adaptation of cavefish to the extreme and nutrient poor environments, thereby providing potential new insights into human health.

Z591 *in toto* imaging of osteoblast cell cycle dynamics in regenerating zebrafish scales. *B. Cox*[1], V. Tornini[1], A. Puliafito[2], S. Di Talia[1], K. Poss[1]. 1) Duke University, Durham, NC; 2) Candiolo Cancer Institute - FPO, IRCCS, Candiolo, Torino, Italy.

The adult zebrafish can regenerate many tissues, but not all are amenable to live imaging due to their size, location, or time required to regenerate. Zebrafish scales, like fins, contain osteoblasts and epidermis, but they regenerate much more quickly after being plucked or lost during the natural course of the fish's life, recapitulating their gross structure within three to four days of loss of the original tissue. Thus, they would make ideal models for live imaging of regeneration. We have developed a method for long-term (up to twenty-four hours) live imaging of adult fish using low concentrations of anesthetic, giving us previously unprecedented access to spatiotemporal information about individual and collective cell behavior during regeneration. Through live imaging of transgenic fish expressing the FUCCI cell cycle reporter in osteoblasts (*osx*:Venus-hGeminin/*osx*:mCherry-zCdt1), we have identified a transition during regeneration from early uniform proliferation to later growth, which is spatially restricted to the borders of regenerating scales. We have developed computational methods to segment and track hundreds to thousands of osteoblasts, analyze their cell cycle and division patterns over long periods, and infer how individual cell cycle changes underlie tissue-wide growth patterns.

Z592 Plasticity & Robustness in Gastrulation: Siamese Zebrafish. *A. Ortiz*[1], S. de Man[1,2], N. Peyrières[1]. 1) BioEmergences (USR3695), CNRS, 91190 Gif-sur-Yvette, France; 2) University of Amsterdam, 1012 WX Amsterdam, Netherlands.

Embryogenesis is remarkably dynamic & precisely regulated, and the underlying morphogenetic processes are still largely unknown. We aim at understanding the plasticity & robustness of morphogenetic processes in deuterostome early embryogenesis using as a model Siamese zebrafish. Specifically, we expect insights from the quantitative analysis of the: 1) respective contributions of ingression & involution to the formation of the endo-mesodermal layer in endogenous & induced axes; 2) convergence-extension movements in Siamese compared to normal embryos; 3) dynamics of the underlying gene regulatory network. The injection of mRNA encoding a constitutive form of a Nodal pathway receptor (acvr1ba*) into a single blastomere at the 16-cell stage was used to induce a secondary axis. The co-injection of mRNA encoding H2B-mCherry was used to trace the induced axis. Also, we utilized the gsc:egfp transgenic fish line with staining under the control of the goosecoid transcription factor regulatory sequences to reveal the embryonic shields & their derivatives: notochord, prechordal plate & pharyngeal endoderm. Embryos were sorted at shield stage according to the position of the induced shield relative to the endogenous one. According to previous studies, we expected random positioning of the induced shield and an even distribution of embryos across 7 categories, position 0 being at the level of the endogenous shield and 6 being opposite to it. With current categorization of up to 600 embryos, we confirmed this hypothesis. Embryos in opposite positions, class 6, were rare suggesting the requirement for the precise balance of opposing forces, and displayed the most complete duplication of embryonic structures compared to other configurations leading to more or less extensive regulation. Answering the questions above and assessing the balance of biomechanical forces was achieved using cell tracking obtained by the automated analysis of in toto 3D+time imaging. Our best time-lapse data was obtained by 2-Photon microscopy imaging with a horizontal microscope (LaVisionBiotech). We present our most recent results in terms of cell behaviors for class 6 specimens. Our strategy toward the integration of cellular & genetic dynamics relied on the expression of transgenic reporters. Expression of the gsc transgenic reporter in the embryonic shield derivatives revealed unexpected variability with mosaic staining of notochord cells not observed in the endogenous axis. Systematically exploring transgenic reporter expression along the cell lineage is expected to reveal molecular & cellular integrated regulation mechanisms at stake in the robustness of gastrulation morphogenetic processes.

Z593 Spatiotemporal regulation of metalloprotease activity in DV patterning. *Francesca Tuazon*, Mary Mullins. University of Pennsylvania, Philadelphia, PA.

Bone Morphogenetic Proteins (BMPs) act as a morphogen to pattern dorsoventral (DV) axial tissues in invertebrates and vertebrates. The shape of the BMP gradient spatially across the DV axis is critical: precise amounts of BMP signaling at discreet DV positions specify distinct cell fates. DV patterning occurs from late blastula through gastrula stages, so the BMP gradient is also maintained temporally during gastrulation. Gastrulation and dorsal convergence generate the embryonic germ layers and body axis by rapidly reorganizing a large number of cells, challenging the mechanisms that shape the BMP gradient in space and time. To determine the shape of the BMP gradient, we developed a quantitative immunofluorescence assay of nuclear phosphorylated Smad1/5, the direct intracellular readout of BMP signaling. Using this assay, we discovered that the shape of the BMP gradient significantly changes during gastrulation: the BMP gradient steepens both dramatically and rapidly between mid- and late gastrulation stages.

Extracellular modulators of BMP ligand availability shape the BMP gradient. An essential extracellular modulator is Chordin, a BMP antagonist that binds BMP to inhibit signaling. Chordin is central to generating the initial BMP signaling gradient and continually regulates the BMP gradient during gastrulation. Chordin itself is regulated by two key classes of proteins: (i) the highly homologous metalloproteases Tolloid and Bmp1a, which cleave and inactivate Chordin, and (ii) the metalloprotease inhibitor Sizzled.

We discovered that *tolloid (tld)* and *sizzled (szl)* mutants display distinct alterations in the shape of the BMP gradient at the end of gastrulation, suggesting region-specific roles for Tld and Szl. Our data indicate that Tld maintains the steepness of the late BMP signaling

gradient, while Szl restricts BMP signaling in lateral regions. These results support a novel requirement for metalloprotease regulation during late gastrulation to steepen the BMP gradient and correctly pattern posterior tissues of the zebrafish embryo. Unlike *tld, bmp1a* is maternally deposited. We found that while MZ-*bmp1a* mutants have no DV patterning defects, MZ-*bmp1a* embryos depleted of Tld are severely dorsalized. The severity of dorsalization indicates that Tld and Bmp1a are redundant and essential to establish the BMP gradient at late blastula stages. These data support fundamental, stage-specific roles for Bmp1a/Tld to differentially shape the BMP gradient, putting forth spatiotemporal metalloprotease regulation as a central mechanism for the spatiotemporal regulation of BMP signaling.

Z594 Automated approaches to sample handling and high-throughput behavioral screening of zebrafish. *Ravindra Peravali*[1], Anna Popova[1], Daniel Marcato[1], Eduard Gursky[1], Johannes Stegmaier[2], Ruediger Alshut[4], Helmut Breitwieser[2], Robert Geisler[1], Christian Pylatiuk[2], Jos van Wezel[3], Pavel Levkin[1], Ralf Mikut[2], Uwe Straehle[1]. 1) Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany; 2) Institute for Applied Computer Science, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany; 3) Steinbuch Centre for Computing, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany; 4) MCC - Measure , Check & Control GmbH & Co. KG, Karlsruhe, Germany.

The zebrafish (*Danio rerio*) has emerged as a robust model organism owing to its transparency, fecundity and genetic capabilities including transgenesis and gene knock-out. In recent years, zebrafish behavioral phenotyping has been increasingly used for studying toxicological and teratogenic effects, neurological disorders and certain diseases. However, to fully exploit the advantages of the zebrafish model novel sample handling, imaging and data analysis and storage platforms are required. The Screening Facility linked to the European Zebrafish Resource Center (EZRC) has developed high-throughput behavioral screening platforms to systematically characterize zebrafish embryonic behavior. The "zebrafish sorting robot" automatically pipettes single or multiple zebrafish eggs or embryos into standard microtiter plates. This robot can operate in complete darkness and thereby facilitates experiments that demand the absence of ambient light. The "Droplet-Microarray Sandwiching Chip" is an array of superhydrophilic spots separated by superhydrophobic borders. By rolling a suspension of zebrafish embryos on the surface of the chip using a pipette we are able to spread zebrafish embryos in one-embryo-per-droplet manner without need for pipetting. This device has been successfully used to screen compound libraries and performs comparably to standard microtiter plates. Moroever, we developed a high-throughput Photomotor Response (PMR) imaging system that measures the response of zebrafish embryos at 30 – 40 hours post fertilization to flashes of bright light. This PMR platform can be completely remotely controlled thereby ensuring no human interference and extraneous sound and vibrations. Another behavioral platform quantifies the startle response using an automated acoustic vibration system combined with a very high frame rate high-resolution camera. Finally, a robotic imaging platform was developed that can assess long term locomotion and feeding behavior in an unsupervised way. In addition to the development of the imaging systems, specialized real-time and quantitative image analysis and processing pipelines have been established. These high-performance computing pipelines extract locomotor kinematics and behavioral dynamics. Furthermore, since behavioral experiments are data intensive (often leading to 7 – 10 tera bytes of data per experiment per day), a dedicated large scale data facility that has more than several peta bytes of storage and archival capacity has been established.

References: Marcato, D., et al. An Automated and High-throughput Photomotor Response Platform for Chemical Screens Proc., 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBS), 2015.

Z595 Quantitative *in vivo* optical tomography of cancer progression and vasculature development in adult zebrafish. *N. Lockwood*[1], S. Kumar[2], M. C. Ramel[1,2], T. Correia[1], M. Ellis[3], Y. Alexandrov[2], N. Andrews[2], R. Patel[1], L. Bugeon[2], M. J. Dallman[2], S. Brandner[3], S. Arridge[1], M. Katan[1], J. McGinty[2], P. M. W. French[2], P. Frankel[1]. 1) University College London, London, GB; 2) Imperial College London, London, GB; 3) UCL Institute of Neurology, London, GB.

We describe a novel approach to study tumour progression and vasculature development *in vivo* via global 3-D fluorescence imaging of live adult zebrafish utilising angularly multiplexed optical projection tomography with compressive sensing (CS-OPT). This method bridges a "mesocopic" imaging gap between established high resolution 3-D fluorescence microscopy techniques with whole body planar imaging and diffuse tomography.

Our new approach has been developed for imaging at multiple excitation wavelengths of non-pigmented adult zebrafish fish up to 3 cm in length. Using unfocussed illumination with two multiplexed imaging channels and iterative reconstruction of sparsely sampled OPT data, our approach requires only ~100 seconds per spectral channel for image acquisition. Using CS-OPT we imaged an inducible genetic model of liver cancer in adult transparent and fluorescent vasculature expressing zebrafish (TraNac *Tg (KDR:mCherry:Fabp10-rtTA:TRE-eGFPKRAS^{V12})*). In this disease model, addition of a chemical inducer (doxycycline) drives expression of eGFP tagged oncogenic KRAS^{V12} in the liver of immune competent animals. We show that our novel OPT methodology enables non-invasive quantitative imaging of the development of tumour and vasculature throughout the progression of the disease and validate our results against established methods of pathology including immunohistochemistry. We have validated this new imaging approach against established methods of pathology including immunohistochemistry and further demonstrated the potential of CS-OPT through a longitudinal study of vascular development in the same zebrafish from early embryo to adulthood.

To increase the metastatic potential of our cancer model we are employing novel approaches using gene editing techniques including TALENs and CRISPRs in an attempt to produce inducible knockouts in adult zebrafish, which are currently unavailable.

We believe this imaging modality with its associated analysis and data management tools constitute a new platform for *in vivo* cancer studies and drug discovery in zebrafish disease models.

Z596 Pancellular, whole-organism tissue microCT as a basis for organismal phenomics. *K. C. Cheng*[1], Y. Ding[1], A. Y. Lin[1], X. Xin[1], S. Y. Wang[2], P. La Riviere[3]. 1) Penn State College of Medicine, Hershey, PA; 2) Motorola Corp, Chicago, IL; 3) University of Chicago, Chicago, IL.

For multicellular model systems, comprehensive morphological phenotyping ideally includes assessments of every cell type in every organ system. We report the ability to use synchrotron-microCT to create 3D images of whole, fixed and metal-stained zebrafish at cellular resolutions such that virtually every cell type can be visualized and phenotyped. Sample diameters may be as large as about 1.5 mm for 0.743 micron voxel resolution and about 2.9 mm for 1.43 micron resolution. Full volumes of 3 or 4 day old zebrafish larvae at the higher resolution or 1 cm long juvenile fish at the lower resolution yield files of about 100 GB. Computer-based visualizations can be optimized for visualization of different cellular structures such as nerve tracts, blood vessels, bone, and melanocytes. This pancellular form of microCT allows volumetric measurements, the creation of slab thicknesses of any multiple of the voxel resolution, and any angle of cut. Analysis of a pola2 mutant in zebrafish shows that we are able to detect abnormalities at the cellular level including atypical nuclei, nuclear fragments, and abnormal mitoses. Since large scale projects will require far greater throughput than is currently possible, we are pursuing optimizations and standardization for sample generation and handling, instrumentation, optics, reconstruction, and visualization, under the auspices of a Synchrotron MicroCT Imaging Resource for Biology. We are recruiting partners as we plan for broad usage of this tool for samples of mm length scale, including whole worms, and flies, and parts of larger models including Arabidopsis and mammals. Automated image processing tools for phenotypic analysis are being developed in parallel with rapid imaging capabilities. Through the creation of these imaging capabilities for the research community, we hope to facilitate a far more rapid and comprehensive understanding of organismal diversity, gene function, organismal toxicity, and disease than is currently possible.

Z597 Automated Segmentation and Morphological Characterization of Neuronal Cell Nuclei in Synchrotron MicroCT Images of Whole Zebrafish. *Y. Ding*, A. Lin, K. Cheng. Penn State College of Medicine, Hershey, PA.

Our group is developing the use of synchrotron micro-computed tomography (microCT) to examine whole zebrafish in three-dimensions. The image volumes acquired from synchrotron microCT have sub-micron isotropic resolution, enabling visualization of individual cells in the context of the whole organism. Our lab is currently engaged in the design and construction of higher-throughput instrumentation for microCT imaging, allowing many zebrafish to be imaged and characterized for genetic or chemical screens. In preparation for the analysis of the resultant data, we are engaged in the parallel pursuit of analytical tools for phenotyping imaged zebrafish at the tissue and cellular levels. Due to intrinsically digital nature of the images, automated image analysis tools can be applied to enable quantitative phenotyping of the whole zebrafish. Here, we show an automated segmentation method to detect and segment neuronal cell nuclei in both wild-type and mutant zebrafish. The mutant zebrafish, *huli hutu (hht)*, causes cytological and nuclear atypia in multiple organs. First, wild-type and mutant 3-, 4-, and 5-day post-fertilization zebrafish were fixed, stained, and embedded in resin before being imaged at Beamline 2-BM at Advanced Photon Source in Argonne National Laboratory (Lemont, IL). From the synchrotron microCT images, 100 neuronal cell nuclei were manually segmented and averaged to create a template for typical neuronal cell nuclei. Next, areas in the zebrafish volumes that are most similar to the average neuronal cell nuclei are extracted for further analysis. Multiple templates can be used to detect neuronal cell nuclei of different sizes. Once the cell nuclei are extracted, morphological characteristics (i.e size, shape) of the cell nuclei are compared between wild-type and *hht* zebrafish. The zebrafish volumes can also be registered to existing digital zebrafish brain atlases, enabling the segmentation of the zebrafish brain into different regions. The morphological features of the cell nuclei are then compared among the different brain regions, in both wild-type and *hht* zebrafish. Overall, the results show that there are fewer neuronal cell nuclei in the mutant zebrafish. The size distribution of the cell nuclei is also different between *hht* and wild-type zebrafish, with *hht* having an increased fraction of smaller objects of nuclear density, consistent with nuclear fragmentation/apoptosis. The work presented here shows that automated tools can be used for quantitative phenotyping based on synchrotron microCT images of whole zebrafish. Ongoing research is focusing on extending this method to segment other cell types, organ systems, and tissue phenotypes.

Z598 Diverse structures and functions of pharyngeal teeth in teleost (zebrafish, medaka, carp, snowflake moray) live-imaged by synchrotron X-ray cinematography. *K. Hatta*[1], S. Shiomoto[1], S. Nomura[1], T. Inoue[1], K. Kuwabara[1], K. Uesugi[2], A. Suzu[3], K. Fujita[1], T. Harada[1], T. Ikenaga[1,3]. 1) University of Hyogo, Akou-gun, Hyogo, JP; 2) JASRI, Sayou-gun, Hyogo, JP; 3) Kagoshima University, Kagoshima, JP.

Many teleost have teeth not only on the jaws but also in the pharynx. The latter is called pharyngeal teeth, and their size, shape, and distribution are highly diversified. For example, zebrafish has no oral teeth, but has 10-20 'cone-shaped' pharyngeal teeth only on the 5th pharyngeal bones. Medaka, on the other hand, have oral teeth and 'more than 1000 small, cone-shaped' pharyngeal teeth distributed on the 2nd / 4th dorsal and the 5th ventral pharyngeal bones. Although the molecular, cellular and genetic mechanisms of their development have been studied, how the pharyngeal teeth are used for feeding in these species had been unknown since they are hidden deeply inside. Previously, by X-ray micro-computed tomography and cinematography at SPring-8 (BL20B2), we have succeeded in visualizing not only their 3D structures but also the movement during feeding, for the first time (2007B1795, 2008A1754, 2009B1911). Here we obtained additional movies at higher resolution and analyzed their motions during feeding (2012A1113, 2013A1161, 2014B1377). We also succeeded in live-imaging the movement of pharyngeal teeth in several other teleost species including carp, which is a close relative of zebrafish but has ‘molar-shaped’ teeth, eating snails, and snowflake moray, which has a distinct ‘tongs-like second jaws’, eating a slice of squid or a living zebrafish (2014A1475, 2015B1531). We are hoping that these data provide basic information to elucidate how such diverse 3D morphologies and functions of pharyngeal teeth arised during teleost evolution.

Z599 Calcium signalling mediated by *tmem33* is essential for endothelial tip cell function during angiogenesis in zebrafish. *A. M. Savage*[1,2], H. R. Kim[2], E. Markham[2], E. Honore[3], F. J. M. van Eeden[2], T. J. A. Chico[1,2], R. N. Wilkinson[1,2]. 1) Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Western bank, Sheffield, UK; 2) Bateson Centre, Firth Court, University of Sheffield, Western bank, Sheffield, UK; 3) Institut de Pharmacologie Moleculaire et Cellulaire, Nice, France.

During angiogenesis, endothelial cells (ECs) sprout from pre-existing vessels driven by the response of endothelial tip cells to a gradient of VEGF. Stalk cells, which are determined through lateral inhibition via Notch signalling, are unable to respond to VEGF signals and migrate under the guidance of the tip cell. Calcium signalling is activated in ECs following stimulation by VEGF, which is dependent upon function of the calcium release-activated calcium (CRAC) channel. We have generated a transgenic zebrafish reporter of calcium signalling in ECs (*fli1-gff; uas-gcamp7a)*. Using light sheet microscopy, we are able to visualise real-time EC calcium signalling *in vivo*. Inhibition of TRPV4 reduces calcium oscillations and delays segmental artery (SeA) migration, indicating TRPV4 may play a role in SeA development.

TMEM33 is a 3-transmembrane domain protein and putative component of the calcium signalling pathway which interacts with known calcium channels, PKD1 and PKD2 in mice. Zebrafish *tmem33* morphants exhibit angiogenic branching defects. Furthermore, *tmem33* knockdown attenuates EC calcium signalling, decreases EC filopodial dynamics and delays segmental artery migration, suggesting morphant ECs display a reduced response to VEGF signalling. Overexpression of VEGF mRNA does not rescue the *tmem33* morphant phenotype. Inhibition of VEGF in zebrafish ECs also attenuates calcium signalling. Collectively, these data indicate *tmem33* lies downstream of VEGF. Conversely, inhibition of Notch signalling in zebrafish embryos increases the frequency of calcium oscillations in ECs during angiogenesis. Zebrafish *mindbomb* mutants, which are Notch signalling deficient, exhibit increased *tmem33* expression, while *tmem33* morphants display attenuated Notch signalling, indicating Notch may be involved in negative feedback regulation of *tmem33*.

Interestingly, *tmem33* mutants display nonsense-mediated decay of *tmem33* transcripts, yet exhibit normal angiogenesis. *tmem33* mutants also exhibit resistance to *tmem33* morpholinos, suggesting genetic compensation exists in the *tmem33* mutant.

Z600 Lgr6 Is A Wnt Target That Promotes Support Cell Proliferation In The Regenerating Lateral Line Neuromast. *Jonathan Kniss*, Sofia Robb, Tatjana Piotrowski. Stowers Institute for Medical Research, Kansas City, MO.

Mechanosensory hair cells in the zebrafish, and other nonmammalian vertebrates, are capable of regeneration after damage, whereas mammals are largely unable to regenerate hair cells. However, across vertebrates many of the same pathways are required for hair cell formation: Wnt/β-catenin signaling promotes proliferation of sensory progenitor cells and Fgf and Notch signaling activate and restrict expression of the prosensory transcription factor atoh1. atoh1 is also required for differentiation and hair cell maturation. The zebrafish lateral line is composed of mechanosensory organs (neuromasts) that are functionally analogous to the mechanoreceptors of the mammalian inner ear. Mature hairs cells within each neuromast are constantly replenished during homeostasis, and hair cell regeneration occurs rapidly after damage. We are using the zebrafish lateral line to dissect the roles of Wnt, Notch and Fgf signaling in support cell proliferation and hair cell differentiation during homeostasis and regeneration. Our previous work showed that in mature neuromasts, Notch and Wnt signaling balance support cell proliferation and hair cell differentiation. However, the genes regulated by these pathways during regeneration are still unknown. In mammalian tissues, the leucine-rich repeat-containing G-protein-coupled receptor (LGR) family members Lgr4/5/6 are stem cell markers in the intestinal epithelium, the hair follicle niche and the cochlea. In zebrafish, we have been unable to identify *lgr5* by sequence similarity or phylogenetic analysis, but *lgr4* and *lgr6* are expressed during lateral line development. However, only *lgr6* is expressed in support cells that surround lateral line hair cells in mature neuromasts. Our functional analyses using heatshock inducible and *lgr6* mutant lines demonstrate that *lgr6* is a Wnt target in mature neuromasts. Wnt/β-catenin promotes support cell proliferation after hair cell damage and our results suggest that Wnt/β-catenin regulates proliferation via Lgr6 during hair cell regeneration. Lgr6 is also regulated by Fgf and Notch signaling and our studies reveal a regulatory network between the Wnt, Fgf, Notch and Lgr6 pathways that balance proliferation and differentiation during homeostasis and regeneration. These findings are important for designing therapies to induce hair cell regeneration in mammals.

Z601 *In vivo* ranking of RASopathy MEK1 variants using functional assays in zebrafish and *Drosophila*. *Granton A. Jindal*[1,2,3], Yogesh Goyal[1,2,3], Kei Yamaya[3,4], Alan S. Futran[2,3], Iason Kountouridis[3], Courtney A. Balgobin[4], Trudi Schüpbach[4], Rebecca D. Burdine[4], Stanislav Y. Shvartsman[2,3,4]. 1) These authors contributed equally to this work; 2) Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ; 3) The Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 4) Department of Molecular Biology, Princeton University, Princeton, NJ.

Germline mutations in components of the Ras/MAPK signaling pathway result in developmental disorders called RASopathies, affecting ~1/1000 human births. Advances in genome sequencing are making it possible to identify disease-related mutations, but currently there is no framework for patient-specific predictions of disease progression based on the individual mutations. Here, we focus on all 14 MEK1 mutations found in RASopathies as well as 2 MEK1 mutations found only in cancer. We find that these can be robustly ranked according to their effect on oval shape of the zebrafish embryo at 11 hours post fertilization (hpf) and lethality fraction at 48 hpf using a quantitative, but also quick and inexpensive approach of MEK1 variant overexpression by microinjection of mRNA at the 1-cell stage. We found that mutations identified in cancer were stronger than those identified in both RASopathies and cancer, which, in turn, were generally stronger than those identified only in RASopathies. We also find that this rank extends to heart size at 20 hpf in zebrafish, another Ras/ERK-dependent developmental process. Moreover, this rank is conserved in other fly-specific assays including ectopic wing vein formation and lethality fraction. Finally, we show that this rank is predictive of MEK inhibitor dosages needed to reverse the oval shape defect, although there is a balance between correcting a

particular defect fully and altering other Ras/ERK-dependent developmental processes. These assays can be used to test the strength of newly found MEK1 mutations as well as for RASopathy and cancer mutations in other pathway components.

Z602 Hedgehog Signaling in Choroid Fissure Formation and Coloboma. *H. B. Gordon*, E. Wirik, B. Gaynes, K. M. Kwan. University of Utah, Salt Lake City, UT.

Optic cup morphogenesis is the process by which the nascent optic vesicle undergoes complex cell and tissue movements to transform into the optic cup—proper structure of which is critical for visual function. Coloboma is a structural defect of the eye arising from improper development of the choroid fissure—a transient yet critical optic cup structure through which retinal axons exit the eye and vasculature enters the eye. The Hedgehog (Hh) signaling pathway is a major effector of choroid fissure development: loss of function mutations in the Hh receptor *patched*, which lead to overactive Hh signaling, result in coloboma in zebrafish and humans. Despite the importance of the choroid fissure, we have a poor understanding of the cellular mechanisms underlying its morphogenesis.

Here we combine multidimensional timelapse imaging with custom cell tracking software to establish the cell movements underlying choroid fissure formation in zebrafish. Specifically, we mapped the origin and trajectories of cells of the nasal and temporal choroid fissure margins under wild type conditions, and we determined the precise movements that are disrupted in *patched2*^{tc294z} homozygous mutants. Our results indicate that cells that should be destined for the nasal margin of the choroid fissure stall in their migration, rendering them stuck in the optic stalk. To examine the migration defect at the cellular level, we marked cells using the photoactivatable fluorophore Kaede: in wild type embryos, cells exhibit a bipolar morphology while moving to form the choroid fissure. In *patched2* mutant embryos, these cells fail to maintain their initial bipolar morphology: they take on an aberrant multipolar morphology and cease movement within the optic stalk region. We conclude that overactive Hh signaling causes coloboma by disrupting cell movements underlying formation of the choroid fissure.

Having defined the cellular events underlying choroid fissure formation in wild type and *patched2* mutant embryos, we set out to determine the molecular mechanisms by which loss of *patched2* alters cell behavior. First, we asked in which cells overactive Hh signaling acts to disrupt choroid fissure cell movements. Surprisingly, using blastula cell transplantations, we find evidence for a non-cell autonomous role for overactive Hh signaling in controlling cell migration and morphology. This suggests interaction between migrating cells and the *patched2* mutant environment. We are now determining whether overactive Hh signaling due to loss of *patched2* is acting via canonical or non-canonical downstream signaling pathways to disrupt cell morphology and migration.

Z603 The role of the Nkd EF-hand in modulating Wnt signaling outputs. *A. N. Marsden*[1,2], S. W. Derry[1], T. A. Westfall[1], D. C. Slusarski[1,2]. 1) Department of Biology, University of Iowa, Iowa City, IA; 2) Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA.

The Wnt signaling network plays critical roles in development and is implicated in human disease. Wnts comprise a complex signaling network that, upon ligand binding, activates the phosphoprotein Dishevelled (Dvl), leading to distinct outputs including polarized cell movement (known as planar cell polarity, Wnt/PCP) and stabilization of the transcription factor β-catenin (Wnt/β-catenin). The mechanisms that determine a specific output are not completely understood, especially because they share receptors and cellular effectors, such as Naked-cuticle (Nkd), a Dvl-interacting protein. The Nkd protein contains a myristoylation domain and an EF-hand, a putative calcium binding domain. Genetic evidence in *Drosophila* demonstrates that Nkd acts as a Wnt/β-catenin antagonist, while in contrast, Nkd modulates both branches of Wnt signaling in vertebrates. We hypothesize that the specialized role of Nkd in *Drosophila* is due to a disrupted EF-hand that cannot not bind calcium. Indeed, this change is unique to *Drosophila* and is not present in closely related insects. To test the role of the Nkd EF-hand in Wnt signal integration, we created Nkd with a neutralized EF-hand, as well as a *Drosophila*-like EF-hand, and manipulate Nkd activity in the zebrafish. Using a combination of biochemical and functional assays, we identified a requirement for the Nkd EF-hand in Wnt/PCP but not in Wnt/β-catenin transcriptional outputs. We demonstrate that the *Drosophila*-like Nkd antagonizes Wnt/β-catenin more robustly than zebrafish Nkd. The EF-hand of Nkd is similar to the EF-hand of a known calcium binding protein, Recoverin, a myristoyl-swtich protein that shuttles between the membrane and the cytoplasm depending on its calcium bound state. Consistently, we observe that Nkd^{WT} shows localization changes in the calcium fluxing DFCs versus calcium quiescent cells but not the mutant forms. The Nkd EF-hand may serve to interpret the physiology of a cell receiving multiple cues and provides mechanistic insight into Wnt signal integration in vivo.

Z604 Glycolysis meets Fgf signaling: The glycolytic enzyme PGK1 is required non-autonomously for Fgf-dependent specification of otic neurons in zebrafish. *B. B. Riley*, H. Kantarci, K. Hofstetter. Texas A&M University, College Station, TX.

We conducted a pilot screen in zebrafish to identify ENU-point mutations that alter production of neurons of the statoacoustic ganglion (SAG), which innervates the inner ear. Two SAG-deficient mutations were recovered, termed *sagd1* and *sagd2*. Both are recessive lethal mutations that show no overt morphological defects but both strongly reduce subsets of SAG neurons. *sagd1* mutants, for example, show a specific deficiency in vestibular neurons required for balance, whereas auditory neurons develop normally. Whole-genome sequencing revealed that *sagd1* affects the glycolytic enzyme Pgk1 (Phosphoglycerate kinase-1). Targeted disruption of *pgk1* using CRISPRs yields an identical phenotype. Surprisingly, it appears that Pgk1 acts non-autonomously to promote efficient Fgf signaling. Fgf is normally required for specification of SAG neuroblasts, with vestibular neuroblasts forming first, followed by auditory neuroblasts. Although expression of known Fgf ligands appears normal in *sagd1* mutants, analysis of Fgf-reporters shows that the early phase of Fgf signaling required for vestibular neuroblasts is impaired. Fgf signaling later recovers to support normal development of auditory neuroblasts. Analysis of genetic mosaics shows the requirement for Pgk1 is non-cell autonomous, suggesting that Pgk1 acts outside the cell to facilitate Fgf's role in SAG specification. Other recent studies have shown that Pgk1 is often secreted to perform various "moonlighting" functions required for normal

development, as well as for efficient metastasis of certain aggressive forms of cancer. Because misexpression of Fgf8 from an inducible transgene does not rescue the *sagd1* phenotype, we speculate that secreted Pgk1 directly or indirectly modifies the extracellular matrix to promote efficient Fgf signaling. Experiments are ongoing to determine how Pgk1 influences the Fgf pathway.

Z605 Shh promotes direct interactions between epidermal cells and osteoblast progenitors to shape regenerated zebrafish bone. *K. Stankunas*, B. Armstrong, A. Henner, S. Stewart. University of Oregon, Eugene, OR.

A major challenge of regenerative medicine is to coax therapeutic stem cells to restore both form and function to injured tissues and organs. Zebrafish provide a vertebrate model that impeccably regenerates lost or damaged tissue by innate mechanisms that precisely control the proliferation, differentiation, and position of injury-induced progenitor cells. For example, Hedgehog/Smoothened (Hh/Smo) signaling in progenitor osteoblasts (pObs) is implicated in the re-establishment of stereotypically branched ossified rays during fin regeneration. Using a photoconvertible patched2 reporter line, we resolve active Hh/Smo output to a narrow distal fin regenerate zone comprising pObs and neighboring migratory basal epidermal cells. Hh/Smo activity is driven by epidermal Sonic hedgehog a (Shha) rather than pOb-derived Indian hedgehog a (Ihha), which instead uses non-canonical signaling to support bone maturation. Using high-resolution imaging and BMS-833923, a uniquely effective Smo inhibitor, we show that Shha/Smo promotes fin ray branching by escorting pObs into split groups that mirror transiently divided clusters of Shha-expressing epidermis. Epidermal cellular protrusions directly contact pObs only where an otherwise occluding Laminin B1A-containing basement membrane remains incompletely assembled. These intimate interactions coupled with epidermal movements progressively generate physically separated pOb pools that then continue regenerating independently to collectively form a now branched bony ray.

Z606 A genetic mechanism to sense and respond to enhanced cellular sphingosine levels during development. *T. Evans*, K. Mendelson, T. Hla. Weill Cornell Medical College, New York, NY.

The lipid mediator sphingosine-1-phosphate (S1P) plays multiple critical roles during embryogenesis. The earliest known function is for heart tube formation, revealed by the *mil* mutant, which is caused by a defective S1P receptor gene (*s1pr2*). S1P can only be generated by phosphorylation of a sphingosine precursor, and only one sphingosine kinase gene (*sphk2*) is expressed during zebrafish embryogenesis. Therefore, we generated *sphk2* mutants to determine if there are earlier or receptor-independent functions for S1P. A maternal zygotic mutant for *sphk2* (*sphk2*^{MZ}) phenocopies *mil*, suggesting that *sphk2* transmits essential migrational cues to the precardiac mesoderm during formation of the heart tube. However, we also noticed a slight developmental delay, and that *sphk2*^{MZ} embryos have elevated sphingosine levels. Compared to wildtype embryos, mutant embryos are sensitive to exogenous treatment with sphingosine. This results in a gastrulation defect and early embryonic lethality, which is phenocopied by morpholino knockdown of *sphk2*. We considered that the phenotype could be caused by excessive sphingosine, rather than a lack of S1P, and that *sphk2*^{MZ} embryos might be depleting sphingosine by another mechanism. Indeed, we observed a striking increase in transcript levels of the ceramide synthase 2b gene (*cers2b*) in *sphk2*^{MZ} embryos, but not in morphants. The transcriptional response to increased sphingosine levels is recapitulated on a reporter regulated by the *cers2b* promoter, and we mapped the minimal sequences essential for the sphingosine response. This is the first report of a transcriptional sphingosine response element (SphRE). These results indicate that *sphk2*^{MZ} embryos up-regulate a salvage pathway for sphingosine turnover to protect embryos from enhanced sphingosine levels in the absence of Sphk activity.

Z607 Developmental origin of muscle-associated fibroblasts. *P. Huang*, R. Ma, K. Kocha. University of Calgary, Calgary, Alberta, CA.

Skeletal muscles control many of the essential functions that our bodies constantly perform such as walking, eating and breathing. Defects in muscle function, for instance muscular dystrophy, have profound consequences. Despite extensive studies on muscles, relatively little is known about how muscle-associated cells modulate muscle function. Recently, several types of non-muscle cells have been discovered to interact closely with muscle fibers and play important roles in muscle regeneration and degeneration. Using a zebrafish model, we aim to determine the embryonic origin and functions of muscle-associated fibroblasts. We hypothesize that a subset of muscle-associated fibroblasts originates from the sclerotome, a sub-compartment of the somite. Using lineage tracing, we showed that sclerotome-derived cells undergo stereotypic migration out of each somite to surround the notochord during early development. Hedgehog (Hh) signaling is required in multiple steps during sclerotome formation. Although the initial expression of sclerotome markers can be induced in the absence of Hh signaling, active Hh signaling is essential to maintain their expression in sclerotome-derived cells. Furthermore, cell tracing experiments demonstrated that Hh signaling is required for the migration of sclerotome-derived cells. Finally, using time-lapse imaging, we showed that sclerotome-derived cells contribute to muscle-associated fibroblasts including tenocytes. We are currently investigating the function of muscle-associated cells in muscle regeneration and degeneration.

Z608 Hepatic nuclear receptor 4 alpha mediates microbial control of host gene expression in the zebrafish digestive tract. *James M. Davison*[1,2], Ghislain Breton[3], John F. Rawls[2]. 1) Dept. of Molecular Genetics and Microbiology, Duke University, Durham, NC; 2) Dept. of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC; 3) Dept. of Integrative Biology and Pharmacology, University of Texas Health Sciences Center, Houston, TX.

Intestinal microbiota influence diverse aspects of host nutrient metabolism and immunity in part by controlling tissue-specific transcription of key host genes. However, the host transcriptional regulatory mechanisms mediating microbial control of host gene expression are poorly understood. Microbiota colonization in zebrafish and mice leads to significant reductions in intestinal epithelial expression of Angiopotein-like

protein 4 (*angptl4/fiaf*), a circulating inhibitor of lipoprotein lipase. We previously showed that a discrete DNA cis-regulatory module (CRM) at the zebrafish *angptl4* locus named in3.4 mediates microbial control of transcription in the intestinal epithelium. To identify transcription factors that might regulate in3.4 activity, we used an unbiased yeast 1-hybrid approach and discovered that members of the Hepatic nuclear factor 4 (Hnf4) family of nuclear receptor transcription factors specifically bind to in3.4. Site-directed mutagenesis of in3.4 in yeast 1-hybrid and zebrafish transgenesis assays confirmed the existence of two Hnf4 binding sites in in3.4. Using the CRISPR/Cas9 system, we made targeted mutations in the zebrafish *hnf4a* gene and found that in3.4 enhancer activity was significantly attenuated in the intestinal epithelium of *hnf4a* homozygous mutants. To test the requirement for *hnf4a* on host transcriptional responses to microbiota, we used RNA-seq to compare transcript levels in the digestive tracts of *hnf4a* mutant and wild-type zebrafish raised germ-free or colonized with a conventional microbiota. Zebrafish *hnf4a* mutants displayed reduced expression of genes involved in lipid biosynthesis and metabolism, and increased expression of genes involved in inflammation and response to bacteria. Strikingly, loss of *hnf4a* significantly altered the expression of over half of the 822 genes that displayed differential expression in response to microbiota colonization. Hnf4a is an ancient member of the nuclear receptor family with important known roles in intestinal and hepatic physiology. Our results establish a novel role for Hnf4a in mediating host transcriptional responses to intestinal microbiota, and provide new mechanistic insight into how animal hosts perceive and respond to microbial cues.

Z609 Deciphering the role of Isl1 in enteroendocrine cell differentiation. *M. Voz*, J. Pirson, D. Stern, V. Von Berg, L. Flasse, I. Manfroid, B. Peers. GIGA, Liege, BE.

The gastrointestinal tract contains the largest population of hormone-producing cells in the body. These enteroendocrine cells, scattered throughout the digestive epithelium, secrete over fifteen different hormones, regulating important physiological functions such as glycaemia, pancreatic secretion and food intake. Understanding enteroendocrine cell differentiation is essential for identifying future targets for common disease such as diabetes and obesity.

To get a comprehensive view of the formation of these cells, we determined the transcriptomic landscape of enteroendocrine cells through RNAseq analyses of 4 dpf FACS sorted *pax6b:GFP* enteroendocrine cells. We found that the vast majority of the hormones described to be expressed in the gastrointestinal tract of the mouse are also expressed in the zebrafish gut. This transcriptomic analysis also highlighted the expression in the gut of four hormones not yet reported to be expressed in enteroendocrine cells of any species. Furthermore, we noticed a high similarity between enteroendocrine and pancreatic endocrine cells as many transcription factors expressed in the pancreas are also expressed in the gut. Among them, the LIM transcription factor Isl1 labels only a subset of enteroendocrine cells. The null *isl1*^{sa0029} mutant displays a drastic and specific reduction of the expression of the hormones synthetized by the corresponding enteroendocrine cell types. In contrast, the expression of transcription factors, thought to be important for the formation of the enteroendocrine progenitor cells, like *ascl1a, sox4b and neurod1*, is not perturbed in the *isl1*^{sa0029} mutant. The transcriptomic comparison by RNAseq of *wt* and mutant *isl1*^{sa0029} embryos identified 530 differentially expressed genes and among them, only a very limited number of transcription factors. Altogether, this suggests that Isl1 controls the last steps of the differentiation process of specific enteroendocrine cells.

Z610 Liver-enriched gene 1, a glycosylated secretory protein, binds to FGFR and mediates an anti-stress pathway to protect liver development in zebrafish. *J. R. Peng*[1], M. J. Hu[1], Y. Bai[1], C. X. Zhang[2], F. Liu[2], Z. B. Cui[3], J. Chen[1]. 1) Zhejiang University, Hangzhou, Zhejiang, CN; 2) Institute of Zoology, CAS, Beijing, China; 3) Institute of Hydrobiology, CAS, Wuhan, China.

Unlike mammals and birds, teleost fish undergo external embryogenesis, and therefore their embryos are constantly challenged by stresses from their living environment. These stresses, when becoming too harsh, will cause arrest of cell proliferation, abnormal cell death or senescence. Such organisms have to evolve a sophisticated anti-stress mechanism to protect the process of embryogenesis/organogenesis. However, very few signaling molecule(s) mediating such activity have been identified. *liver-enriched gene 1* (*leg1*) is an uncharacterized gene that encodes a novel secretory protein containing a single domain DUF781 (domain of unknown function 781) that is well conserved in vertebrates. In the zebrafish genome, there are two copies of *leg1*, namely *leg1a* and *leg1b*. *leg1a* and *leg1b* are closely linked on chromosome 20 and share high homology but is differentially expressed. In this report, we generated two *leg1a* mutant alleles using the TALEN technique, then characterized liver development in the mutants. We show that *leg1a* mutant exhibits a stress-dependent small liver phenotype that can be prevented by chemicals blocking the production of reactive oxygen species. Further studies reveal that Leg1a binds to FGFR3 and mediates a novel anti-stress pathway to protect liver development through enhancing Erk activity. More importantly, we show that the binding of Leg1a to FGFR relies on the glycosylation at the 70th asparagine (Asn^{70} or N^{70}) and mutating the Asn^{70} to Ala^{70} compromised Leg1's function in liver development. Therefore, Leg1 plays a unique role in protecting liver development under different stress conditions by serving as a secreted signaling molecule/modulator.

Z611 Program number not assigned.

Z612 Wnt signaling is required for adult zebrafish kidney regeneration. *C. N. Kamei*[1], Y. Liu[1], N. A. Hukriede[2], I. A. Drummond[1,3]. 1) Massachusetts General Hospital, Charlestown, MA; 2) University of Pittsburgh, Pittsburgh, PA; 3) Harvard Medical School, Boston, MA.

Kidney regeneration in zebrafish occurs by repair of existing nephrons as well as by *de novo* generation of new nephrons from adult organ progenitor cells by neonephrogenesis. We report here that Wnt signaling plays an essential role in adult zebrafish kidney regeneration. Newly forming nephron condensates in the developing mesonephros as well as those formed in the adult in response to gentamicin injury express the Wnt receptor *frizzled 9b* (*fzd9b*) and the canonical Wnt target gene *lef1*. In the *Tg(lhx1a:GFP)* nephron progenitor reporter line, both individual

lhx1a-positive adult kidney progenitor cells and nephron condensates are specifically marked by expression of *fzd9b*. Canonical Wnt signaling reporter line *Tg(TCF/Lef-miniP:dGFP)* combined with EdU labeling shows distinct zones of hi vs low Wnt activity and proliferation in new nephron formation. Pharmacological blockade of Wnt signaling using IWR1 and IWP2 led to a decrease in *lhx1a*+ nephron condensates after injury. EdU labeling in *Tg(lhx1a:GFP)* fish after gentamicin injury reveals that newly forming condensates are actively proliferating and this proliferation is blocked by Wnt inhibition. *wnt9b* is strongly induced in collecting duct epithelia after injury, making it a strong candidate for a mediator of the regeneration response. Our results demonstrate an essential role for Wnt signaling in adult zebrafish kidney regeneration. Identification of *fzd9b* as a new marker of adult kidney progenitor cells opens new avenues to investigate their developmental origins and regenerative potential.

Z613 Gain-of-function mutations of *mau*/DrAqp3a influence zebrafish pigment pattern formation through the tissue environment. *Anastasia Eskova*[1], Rosa Garcia-Junco[2], Francois Chauvigné[3], Hans-Martin Maischein[4], Moritz Ammelburg[5], Joan Cerdà[3], Lars Kaderali[6], Christiane Nüsslein-Volhard[1], Uwe Irion[1]. 1) MPI for Developmental Biology, Tübingen, Germany; 2) Grupo de Investigación en Bioloxía Evolutiva (GIBE), Facultade de Ciencias e CICA, Universidade da Coruña, A Coruña, Spain; 3) IRTA-Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas (CSIC), 08003 Barcelona, Spain; 4) Current address: Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany; 5) Current address: Fish & Richardson's, Munich, Germany; 6) Universitätsmedizin Greifswald, Institut für Bioinformatik, Greifswald, Germany.

The characteristic striped pigment pattern in zebrafish is formed by three types of pigment cells: black melanophores, yellow xanthophores, and iridescent iridophores. The patterning process depends on self-organizing properties of pigment cells, as well as extrinsic cues. By now most of the known pigment pattern formation mutations in zebrafish act cell autonomously in pigment cells, and our knowledge of the patterning regulation by surrounding tissues is scarce.

Here, we describe four dominant missense mutations in *mau*/Aquaporin 3a (Aqp3a), which is one of the main aquaporins in zebrafish skin and epithelia, known to transport water, glycerol and some other solutes. All four mutations lead to a similar phenotype: although all three types of pigment cells are present in the skin, the mutants display irregularities and broken stripes. In addition, they have short fins with fewer, but normally sized, fin ray segments. Blastula transplantations indicate that all the pigment cells types of *mau* mutants are capable of forming a wild-type-like pattern, but the mutant tissue environment prevents them from doing so. CRISPR-Cas9 knock-out of *mau*, which leads to no phenotypic consequences, and experiments in *Xenopus* oocytes as well as expression of mutant variants of Aqp3a in mammalian cells and zebrafish embryos indicated that *mau* mutations are neomorphic and their effect is dose-dependent. Based on transcriptome analysis and visualization of calcium distribution in the tissues of *mau* and wild-type fish, we suggest calcium signaling as a factor influencing behavior of pigment cells and their communication with surrounding tissues.

Z614 Evidence for ECM-Sema3d interactions controlling skeletal regeneration in the fin. *M.Kathryn Iovine*, Jayalakshmi Govindan. Lehigh University, Bethleham, PA.

The extracellular matrix (ECM) is much more than an inert material surrounding cells. Rather, evidence suggests that the ECM provides instructional cues regarding the external environment. One way that the ECM may act is as a reservoir for growth factors. However, specific growth factor-ECM interactions are undescribed in many instances. Recent research in the Iovine lab has identified a novel growth factor-ECM interaction that contributes to skeletal morphogenesis during fin regeneration. The fin skeleton is comprised of multiple bony fin rays, and each fin ray is comprised of bony segments separated by joints. Our research on the *short fin* (*sof*) mutant revealed that the gap junction protein Connexin43 (Cx43) coordinates skeletal growth (cell proliferation) and patterning (specification of joints). To provide insight into how Cx43 influences these cell behaviors, we identified downstream mediators of Cx43 function. Included among the Cx43-dependent genes are the secreted signaling molecule Semaphorin3d (Sema3d) and the link protein Hyaluronan and Proteoglycan Link Protein 1a (Hapln1a). Sema3d serves as a secreted growth factor that stimulates signal transduction pathways that promote cell proliferation in blastemal cells and suppress joint specification in skeletal precursor cells. Hapln1a/link protein is a component of the extracellular matrix (ECM) that is known to stabilize interactions between hyaluronan (HA) and proteoglycans (such as Aggrecan and Versican). Prior studies demonstrated that both Sema3d and Hapln1a are molecularly and functionally downstream of Cx43, as knockdown of either recapitulates the *sof* phenotypes (i.e. reduced cell proliferation and short segments/premature specification of joints). Current findings demonstrate that *hapln1a* and *sema3d* interact genetically, suggesting that the Hapln1a and Sema3d gene products function in a common pathway to coordinate skeletal growth and patterning. Moreover, morpholino-mediated knockdown of Hapln1a leads to reduced HA, Aggrecan, and Sema3d protein in regenerating fins. These findings suggest that Hapln1a is needed to stabilize aggregates of HA and Aggrecan, which in turn are required to stabilize Sema3d protein. Therefore, Sema3d protein levels and Sema3d-based signal transduction depend on the presence of HA-Hapln1a-Aggrecan aggregates. Overexpression of Sema3d rescues Hapln1a-knockdown phenotypes, providing further evidence that Hapln1a and Sema3d functionally interact during fin regeneration. These results are the first to demonstrate that Sema3d function requires an intact Hapln1a-ECM. Moreover, these findings reveal tangible connections between the Hapln1a-ECM, Sema3d-dependent signal transduction, and skeletal morphogenesis.

Z615 Positional cloning of *cloche*, a gene that drives endothelial and hematopoietic lineage specification. *Didier Y. R. Stainier*[1,2], Sven Reischauer[1,2], Oliver Stone[1,2], Alethia Villasenor[1,2], Suk-Won Jin[1], Neil Chi[1], Marcel Martin[3], Miler T. Lee[4], Michele Marass[2], Ian Fiddes[1], Taiyi Kuo[1], Won-Suk Chung[1], Sherveen Salek[1], Robert Lerrigo[1], Jessica Alsiö[1], Shujun Luo[6], Björn Nystedt[7], Antonio J. Giraldez[4], Gary P. Schroth[5], Olov Andersson[5]. 1) Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, California, USA; 2) Max Planck

Institute for Heart and Lung Research, Bad Nauheim, Germany; 3) Science for Life Laboratory, Dept of Biochemistry and Biophysics, Stockholm University, Solna, Sweden; 4) Department of Genetics, Yale University School of Medicine, New Haven, Connecticut, USA; 5) Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden; 6) Illumina, San Diego, CA, USA; 7) Science for Life Laboratory, Dept of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden.

Vascular and hematopoietic cells organize into specialized tissues during early embryogenesis to supply essential nutrients to all organs and thus play critical roles in development and disease. At the top of the hemato-vascular specification cascade lies *cloche*, a gene that when mutated in zebrafish leads to the striking phenotype of loss of most endothelial and hematopoietic cells with a substantial increase in overall cardiomyocyte numbers. While this mutant has been analyzed extensively to investigate mesoderm diversification and differentiation and continues to be broadly utilized as a unique avascular model, the isolation of the *cloche* gene has been challenging due to its telomeric location. Using a deletion allele of *cloche*, we identified several new *cloche* candidate genes within this genomic region, and systematically genome-edited each candidate. Through this exhaustive interrogation, we have finally identified the *cloche* gene and will report on its identity and further functional analysis.

Z616 Molecular asymmetry at electrical synapses – at the gap and beyond. *Adam Miller*[1], Alex Whitebirch[2], Arish Shah[2], Cecilia Moens[2]. 1) University of Oregon, Eugene, OR; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

Camillo Golgi and Santiago Ramón y Cajal famously argued over whether the nervous system was one big syncytium or instead a network of independent units. While it is clear that neurons are separate units as Cajal suggested, communication between neurons can occur directly, without chemical intermediary, at electrical synapses, akin to what Golgi envisioned. Electrical synapses are composed of gap junction (GJ) channels between neurons allowing for direct ionic and metabolic communication. Neuronal GJs are often thought to be simple, symmetric structures, with the same protein constituents on either side of the synapse. During GJ formation, both the pre- and postsynaptic neurons contribute hemichannels composed of hexamers of Connexin (Cx) protein to form functional channels between neurons. Despite this apparent simplicity, work from cell culture has found that GJs can contain multiple different Cx proteins and this can affect the functional properties of communication. In a forward genetic screen in zebrafish, which looked for mutations affecting electrical synapse formation in the Mauthner neural circuit, we identified the *Dis3* mutation that disrupts a homologue of mammalian *connexin36* (*cx36*). *cx36* is thought of as the main neuronal GJ gene due to its broad neuronal expression and extensive contributions to electrical synapses throughout the brain,. Through genome gazing we found that zebrafish have four orthologous cx36-like genes, *cx34a* (*Dis3*), *cx34b*, *cx35a*, and *cx35b*. To identify which Cxs were required we developed a CRISPR-based *in vivo* screen and found that only *cx34a* and *cx35a* were necessary for M electrical synapse formation. Using cell transplantations we found that *cx34a* is required exclusively postsynaptically, while *cx35a* is required exclusively presynaptically, for synaptogenesis. Additionally, the CRISPR screen identified a scaffolding molecule, *tjp1b*, that is necessary for electrical synaptogenesis and preliminary evidence suggests *tjp1b* is required exclusively postsynaptically. We conclude that vertebrate electrical synapses can be molecularly asymmetric at the level of the GJ proteins themselves. In addition, our preliminary evidence suggests that such asymmetries can extend beyond the channel to the larger proteome of the electrical synapse. Such asymmetries at the GJ and beyond suggest a molecular mechanism for generating functional asymmetry across the electrical synapse. Moreover, this work suggests that we are just starting to scratch the surface of electrical synapse complexity and future work will continue to reveal the richness of these structures at the molecular and functional level. Also, it hopefully allows Golgi to rest a bit easier.

Z617 Cell proliferation and differentiation are controlled by different Fgf downstream targets during sensory hair cell regeneration. *Mark E. Lush*, Scott Freeburg, Tatjana Piotrowski. Stowers Institute for Medical Research, Kansas City, MO.

Organ regeneration is a complex process involving integration of multiple cell signaling pathways. The zebrafish lateral line contains mechanosensory hair cells that respond to vibrations in the surrounding water and serve as a great model to study the development and regeneration of the auditory system. Lateral line hair cells are surrounded by at least two groups of support cells, which together form a structure called the neuromast. Unlike mammals, the sensory hair cells of the lateral line regenerate after damage. We have previously shown that hair cell regeneration requires Notch down regulation and Wnt-dependent proliferation of a resident support cell population. Other signaling pathways, such as Fgf are also expressed in mature neuromasts. Fgf signaling is required for lateral line development, as it is for the ear of mouse, chicken and zebrafish, but its role in regeneration has not been studied. RNASeq and in situ hybridization analyses show that *fgf3* and *fgf10a*, and the receptors *fgfr1a* and *fgfr2* are expressed in naïve neuromasts and are immediately down regulated after hair cell death. This finding suggests that down regulation of Fgf signaling maybe required to initiate hair cell regeneration. Indeed, in *fgf3* mutants, hair cell regeneration is enhanced due to increased support cell proliferation. This finding is significant, as it suggests that Fgf3 manipulations might be important for inducing regeneration in the mammalian ear. Interestingly, *fgf10a* mutants do not show differences in regeneration potential, suggesting that different *fgf* ligands may activate different receptors or downstream signaling pathways in the lateral line. Increased hair cell regeneration and proliferation is mimicked by low dose treatment with pharmacological inhibitors of Fgf receptors. Surprisingly, high doses of Fgf inhibitors block regeneration, even though proliferation is still increased. These findings suggest that proliferation and hair cell differentiation are regulated via different receptors or downstream targets. Inhibition of Notch signaling during regeneration also leads to increased cell proliferation suggesting that Notch and Fgf might interact. However, Notch inhibition and the *fgf3* mutation affect different downstream genes. Also, in *fgf3* mutants or after inhibition of Fgf signaling, proliferation is even increased in undamaged neuromasts, which is not the case after Notch inhibition. These results show that Notch and *fgf3* initiate divergent downstream signaling events to inhibit

proliferation during homeostasis and regeneration. Therefore, the combined downregulation of these pathways should be explored in mammals to trigger proliferation and regeneration.

Z618 Cilia genes play differing roles in hair cells. *T. Stawicki*[1], L. Hernandez[1], R. Esterberg[1,2], T. Linbo[1], K. Owens[1,2], A. Shah[3], C. Moens[3], E. Rubel[1,2], D. Raible[1,2]. 1) University of Washington, Seattle, WA; 2) Virginia Merrill Bloedel Hearing Research Center, Seattle, WA; 3) Fred Hutchinson Cancer Research Center, Seattle, WA.

Hair cells contain a single primary cilium early in development, known as the kinocilium. This cilium is usually lost in auditory hair cells but maintained in vestibular hair cells. Hair cells also possess rows of actin-based stereocilia that are responsible for responses to mechanical stimuli. The kinocilium is located adjacent to the tallest stereocilia row. In hair cells, the primary function of cilia genes is believed to be in the development of stereocilia polarity indirectly through regulation of kinocilia. Through a screen for mutations that confer resistance to aminoglycoside-induced hair cell death we have identified a number of mutations in cilia genes, showing a novel role of cilia genes in hair cells. We found that mutations in the intraflagellar transport (IFT) genes *ift88, traf3ip, dync2h1* and *wdr35* all lead to strong resistance to neomycin-induced hair cell death. Mutations in the transition zone genes *cc2d2a, mks1,* and *cep290* lead to more moderate protection. These two classes of genes appear to play different roles in aminoglycoside-induced hair cell death. Mutations in IFT, but not transition zone, show a reduction of neomycin uptake into hair cells, loss of kinocilia and a reduction in control hair cell numbers. The individual IFT genes appear to differently affect hair cells as well, as mutations in *ift88, traf3ip* and *dync2h1,* but not *wdr35* show a reduction in hair cell FM1-43 uptake, a process dependent upon mechanotransduction activity. This does not appear to be due to defects in stereocilia morphology. Future work will focus on delineating the mechanisms behind the diverse roles cilia genes play in hair cells.

Z619 The Agrin receptor Lrp4 promotes peripheral nerve regeneration through a novel, MuSK-independent pathway. *K. D. Gribble*[1], J. Bremer[1], J. Y. Kuwada[2], M. Granato[1]. 1) University of Pennsylvania, Philadelphia, PA; 2) University of Michigan, Ann Arbor, MI.

Following injury, peripheral nerves reestablish neuromuscular connections with their developmental targets. While the molecular pathways that govern peripheral nerve development and neuromuscular synapse formation are well understood, it is unclear whether the same pathways are reemployed to promote regeneration. For example, neuromuscular synapses require the evolutionarily conserved Agrin-Lrp4-MuSK signaling pathway to cluster acetylcholine receptors (AChRs) beneath motor axon terminals, yet their *in vivo* role in peripheral nerve regeneration has not been examined. To determine whether this pathway is re-utilized during regeneration, we used a previously established assay that enables us to transect spinal motor nerves and continuously monitor regeneration in live, intact zebrafish (Rosenberg et al., 2012).

We find that animals harboring a TALEN-induced *lrp4* null allele display defects in neuromuscular synapse formation, while motor axon growth and guidance is unaffected. In contrast, after nerve transection, these axons largely fail to regrow to their muscle targets. Using the same assay, we find that the obligate Lrp4 co-receptor MuSK, which is essential for neuromuscular synapse development, is dispensable for motor axon regeneration, demonstrating that Lrp4 promotes axon regeneration through a MuSK-independent pathway. Moreover, using a second *lrp4* mutant allele we find that the Lrp4 transmembrane domain, while critical for neuromuscular synapse development, is dispensable for axon regeneration, consistent with the idea that Lrp4 promotes regeneration through a novel, tether-independent pathway. Restoring *lrp4* expression only to muscle cells in animals otherwise lacking *lrp4* rescues neuromuscular synapse development but does not rescue motor axon regeneration, suggesting that Lrp4 acts in another cell type to promote axon regeneration. We will present ongoing efforts to further characterize this novel MuSK-independent pathway through which Lrp4 promotes peripheral nerve regeneration.

Z620 *pregnancy-associated plasma protein-aa* (*pappaa)* mediates the development and function of distinct retinal circuits. *Andrew Miller*[1], Holly Howe[2], Scott Friedle[2], Marc Wolman[2]. 1) Neuroscience Training Program, University of Wisconsin-Madison; 2) Department of Zoology, University of Wisconsin-Madison.

Neural circuits must rapidly detect and interpret sensory stimuli to accurately guide behavior. In the visual system, retinal circuits begin to distinguish light and dark stimuli at synapses between photoreceptors and ON- and OFF-bipolar cells, respectively. Bipolar cells develop without a need for other retinal cell types or activity, and thus, are thought to rely on autonomous gene expression. Through a genetic screen, we identified an essential role for *pregnancy-associated plasma protein-aa* (*pappaa)* in visually guided behaviors mediated by OFF-bipolar cells. By characterizing the identified *pappaa* mutant, our work describes the first role for *pappaa* in the development of neural circuits and identifies a critical factor in the formation and function of the retinal circuits that specifically guide behavioral responses to dark stimuli.

Despite a grossly normal retina and retinotectal projection, larvae harboring nonsense mutations in *pappaa* show an almost complete failure to initiate an O-bend response to sudden darkness or to turn away from a gradient of darkness. In contrast, *pappaa* mutant larvae facing a light target accurately swim towards the light. Together, the mutants' behavior suggest that the ON-bipolar retinal pathway is intact, but the OFF-bipolar pathway mediating dark induced behavior is disrupted. Indeed, selective pharmacological stimulation of the OFF-bipolar pathway in *pappaa* mutants restores their dark induced behavioral deficiencies. *pappaa* acts as a metalloprotease to stimulate local IGF1 signaling. To gain insight into when *pappaa-IGF1* signaling is required for dark induced visually guided behavior, we used genetic and pharmacological approaches to manipulate IGF1 signaling in *pappaa* wild type and mutant larvae. Induction of a transgenic, dominant negative IGF1R during temporally restricted periods indicated that IGF1 signaling is critical during the period of retinal synaptogenesis for larvae to respond to dark stimuli. Moreover, stimulation of IGF1 signaling in *pappaa* mutants during this stage improved dark induced behavior in the mutants. Throughout this period of retinal synaptogenesis, *pappaa* is exclusively expressed by bipolar cells in the retina and not in the tectum, consistent with a developmental role for *pappaa* in OFF-bipolar cell synaptic connections. Currently, we are performing ERG recordings and high-

resolution anatomical analyses of OFF-bipolar cells to identify the specific requirement of PAPPAA-IGF1 signaling for OFF-bipolar cell synaptic function.

Z621 CachD1 is a novel type I transmembrane protein that regulates the development of habenular asymmetry in zebrafish. *A. F. Faro*[1], H. Stickney[1,3], G. Powell[1,2], G. Gestri[1], I. Leek[1], P. Henriques[1], R. Young[1], T. Hawkins[1], F. Cavodeassi[1], Q. Schwartz[1], G. Wright[2], D. Raible[3], S. Wilson[1]. 1) University College London, London, GB; 2) Wellcome Trust Sanger Institute, Cambridge , GB; 3) University of Washington, Seattle, US.

Structural and functional nervous system asymmetries are conserved throughout the animal kingdom and have been shown to impact cognition and behavior. Despite their prevalence, our understanding of the genetic bases for the development of brain asymmetries is still sparse.

One of the most amenable structures in which to study developmental mechanisms underlying central nervous system asymmetries is the epithalamus. In zebrafish, this structure is composed of the medially positioned pineal complex and the bilateral asymmetric habenular nuclei.

We isolated the *rorschach* (*rch)* mutant through a mutagenesis screen to identify mutations disrupting habenular asymmetry. Mutant embryos display left-isomerized symmetric habenula yet viscera asymmetries are unaffected. Positional cloning has shown that *rch* mutants have a V1122D substitution in the *cache domain containing 1* gene which encodes a novel type I transmembrane protein. The lesion occurs in the gene's single transmembrane domain and disrupts protein localization.

We have performed a screen to identify binding partners for Cachd1 and found that it physically interacts with Frizzled receptors – key components of the Wnt signaling pathway. This is consistent with previous studies showing Wnt signaling has a pivotal role in the elaboration of asymmetries within the habenular nuclei. Temporal and spatial activation of a reporter for β-catenin dependent transcription and expression of *axin2*, a bona-fide Wnt target gene, are affected in the developing diencephalon of *rch* mutants, while epistasis studies show that Cachd1 acts upstream of Axin1.

Taken together these findings suggest that Cachd1 modulates the activation status of Wnt signaling in habenula progenitors to regulate allocation of left-right character to habenular neurons.

Z622 New pathways required for zebrafish brain Left-Right asymmetry and bilateral symmetry. *Michael Rebagliati*[1,2], Merlin Lange[3], Anastasiia Aleksandrova[4], Laure Bally-Cuif[3], Olivier Pourquié[1,2,4,5,6]. 1) Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), CNRS (UMR 7104), Inserm U964, Université de Strasbourg, Illkirch, France; 2) Stowers Institute for Medical Research, Kansas City, Missouri, USA; 3) Paris-Saclay Institute for Neuroscience, CNRS, Univ Paris Sud, Université Paris-Saclay, Gif-sur-Yvette, France; 4) Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, Kansas, USA; 5) Howard Hughes Medical Institute, Kansas City, Missouri, USA; 6) Department of Genetics, Harvard Medical School and Department of Pathology, Brigham and Woman's Hospital, Boston, Massachusetts, USA.

Nodal, a member of the TGF-B superfamily of growth factors, controls visceral organ Left-Right (LR) asymmetry in vertebrates as well as some epithalamic brain LR asymmetries that exist in zebrafish but are not apparent in mammals. But the pathways that operate broadly in all vertebrates to establish brain Left-Right asymmetry and lateralized behaviors are unknown. At least some of these unknown pathways are thought to be Nodal-independent, since lateralized behaviors like handedness and right ear hearing advantage are normal in human patients with Left-Right reversed Nodal signaling. We present evidence that the Rere protein is part of a Nodal-independent pathway needed for zebrafish brain laterality. Rere is a nuclear receptor co-regulator required for optimal Retinoic Acid signaling as well as for modulating other pathways. In humans, *RERE* is a candidate risk gene for schizophrenia and autism spectrum disorders. We find that heterozygosity for the *rerea* mutation, *bab*^{tb210}, disrupts normal population-level laterality of adult zebrafish females without disrupting other behavioral parameters and without perturbing *nodal* (*southpaw*) expression and function. We compare these results to the results for population-level laterality of adult zebrafish males heterozygous for the *bab*^{tb210} allele. We also examine the role of *rerea* and other factors in establishing and/or maintaining bilateral symmetry in the zebrafish embryo. Finally, we discuss the relevance of our results for human-specific traits like handedness and language, as well as the implications for human congenital diseases and neurodevelopmental disorders like schizophrenia and autism, which often have disruptions of bilateral symmetry and/or of LR asymmetry.

Z623 Roundabout2 and exotosin-like 3 promote target specific peripheral nerve regeneration *in vivo*. *P. L. Murphy*, J. Isaacman-Beck, C. Syrett, M. Granato. University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania.

Following injury, axons of the peripheral nervous system regenerate, but only a small fraction reinnervate their original targets. Instead, regenerating axons grow too slowly to reach their original targets before supporting Schwann cells degenerate, and/or axons grow in the wrong direction and innervate inappropriate targets. To decipher the cellular and molecular mechanisms that guide regenerating axons toward their original targets, we study target selective regeneration of spinal motor nerves in larval zebrafish. Each nerve is composed of a ventral and a dorsal branch that diverge at a stereotyped choice-point. We have recently shown that following laser mediated nerve transection, axons of the dorsal branch select their original trajectory with high fidelity (>70%; Issacman-Beck et al, Neuron, 2015). Moreover, we find that *slit1a* mRNA is upregulated in denervated Schwann cells distal to the lesion site, suggesting a role for slit/robo signaling in directing regenerating dorsal axons. To directly probe the *in vivo* role of slit/robo signaling, we examined mutants for the slit receptor *robo2*. While *robo2* appears dispensable for motor nerve development, we find that regenerating dorsal axons in *robo2* mutants frequently (45%) fail to regenerate towards their original targets, and instead extend along ectopic trajectories. Interestingly, we find that exotosin-like 3 (*extl3*) mutants display a

similar phenotype with higher frequency (61%). *Extl3* modifies heparan sulfate proteoglycans, which are known to stabilize slit/robo binding. Finally, we find that *robo2* and *extl3* function specifically in directing axons of the dorsal nerve branch – regenerating axons of the ventral nerve branch select their original targets with high fidelity in *robo2* and *extl3* mutants. Thus, *extl3* and *robo2* mediate pathway specific axon guidance during peripheral nerve regeneration. We will present ongoing work on how *robo2* and *extl3* promote target specific regeneration.

Z624 MECP2-IGF1 signaling determines how neural circuits interpret sensory information. *Nicholas Santistevan*, Cole Gilsdorf, Marc Wolman. University of Wisconsin - Madison, Madison, WI.

Rett Syndrome (RTT) is an X-linked neurodevelopmental, autism spectrum disorder that affects 1 in 10,000 females and is characterized by motor and cognitive impairment. RTT is caused by dysfunction of the *methyl-CpG-binding protein 2 (mecp2)* gene; a transcriptional regulator known to promote neuron development and function. Despite *mecp2's* association with RTT, it remains poorly understood how MECP2 activity influences circuits comprised of various neuron types to control distinct aspects of behavior.

Larval stage behavioral analyses revealed that *mecp2* mutants exhibit reduced prepulse inhibition (PPI) of the acoustic startle response (ASR); and therefore, indicate a critical role for *mecp2* in the ASR circuits' ability to interpret sensory information. Stimulus interpretation is often impaired in autism spectrum disorders, but is typically attributed to sensory or motor defects. Notably, the *mecp2* mutants show normal acoustic sensitivity, execute a normal ASR, and habituate to repetitive acoustic stimulation; and therefore, indicate a specific, central role for *mecp2* in regulating PPI. Consistent with published reports, inhibition of NMDA-type glutamate receptors or GABA-A receptors reduces PPI in wild type and strengthens the *mecp2* mutants' PPI deficit. In contrast, stimulation of glutamatergic or GABAergic signaling in *mecp2* mutants reverses their PPI deficit. These results, coupled with our understanding of the ASR circuit's cellular makeup, suggest that *mecp2* is required for a feedforward inhibitory microcircuit that uses glutamate and GABA to suppress Mauthner neuron activity and hence, drive PPI. Imaging of this feedforward microcircuit revealed developmental defects in *mecp2* mutants.

As a transcriptional regulator, *mecp2* likely influences circuit development and function by activating or suppressing specific genes. For example, *mecp2* stimulates expression of *igf1*; a vital regulator of circuit formation and function. IGF1 recently became a promising therapeutic target for RTT when IGF1 stimulation was found to reverse motor defects in *MECP2* deficient mice. To determine whether *mecp2* acts through IGF1 signaling to control PPI, we combined genetic and pharmacological manipulations to attenuate and stimulate IGF1 signaling in wild type and *mecp2* mutants, respectively. Induction of a dominant negative *igfr1a* reduced PPI, suggesting that IGF1R activity is required for PPI. Stimulation of IG1R signaling in *mecp2* mutants was sufficient to improve PPI in *mecp2* mutants. Together, these results support the model that *mecp2* promotes IGF1 signaling to regulate how a neural circuit interprets sensory information.

Z625 Selenoprotein H is an essential regulator of redox homeostasis that cooperates with p53 in development and tumorigenesis. *A. Cox*[1], A. Kim[1], D. Saunders[1], A. Tsomides[1], K. Hwang[1], K. Evason[2], J. Heidel[3], K. Brown[4], M. Yuan[4], E. Lien[4], B. Lee[1,5], S. Nissim[1], B. Dickinson[6], S. Chhangawala[7], C. Chang[8,9], J. Asara[4], Y. Houvras[7], V. Gladyshev[1,12], W. Goessling[1,10,11,12]. 1) Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 2) University of Utah, Salt Lake City, UT; 3) Oregon State University, Corvallis, OR; 4) Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; 5) Korea University, Seoul, South Korea; 6) University of Chicago, Chicago, IL; 7) Weill Cornell Medical College and New York Presbyterian Hospital, NY; 8) Howard Hughes Medical Institute; 9) University of California, Berkeley, Berkeley, CA; 10) Harvard Stem Cell Institute, Cambridge, MA; 11) Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA; 12) Broad Institute of MIT and Harvard, Cambridge, MA.

Selenium is an essential micronutrient, known for its cancer prevention properties, that is incorporated into a class of selenocysteine-containing proteins (selenoproteins). The human genome encodes 25 selenoproteins which play diverse roles in redox homeostasis, thyroid hormone metabolism, endoplasmic reticulum function and selenium transport. Selenoprotein H (*seph*) is a recently identified nucleolar oxidoreductase with DNA-binding properties whose function is not well understood. Here, we discover that *seph* is an essential gene regulating organ development in zebrafish. Metabolite profiling by targeted LC-MS/MS demonstrated that SepH deficiency impaired redox balance by reducing the levels of ascorbate and methionine, whilst increasing methionine sulfoxide. Transcriptome analysis revealed that SepH deficiency induced an inflammatory response and activated the p53 pathway. Consequently, loss of *seph* rendered larvae susceptible to oxidative stress and DNA damage. Finally, we demonstrate that *seph* interacts with p53 deficiency in adulthood to accelerate gastrointestinal tumor development. Overall, these studies establish that *seph* regulates redox homeostasis and suppresses DNA damage. We hypothesize that SepH deficiency may contribute to the increased cancer risk observed in cohorts with low selenium levels.

Z626 Single-cell imaging of normal and malignant cell engraftment into optically clear immune deficient zebrafish. *Q. Tang*[1,2,3,4], J. C. Moore[1,2,3,4], N. Torres Yordán[4,5], F. E. Moore[1,2,3,4], E. G. Garcia[1,2,3,4], R. Lobbardi[1,2,3,4], A. Ramakrishnan[1,2,3,4], A. Anselmo[6,7], R. I. Sadreyev[6,7], D. M. Langenau[1,2,3,4]. 1) Molecular Pathology, Massachusetts General Hospital, Charlestown, MA 02129, USA; 2) Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA 02114, USA; 3) Cancer Center, Massachusetts General Hospital, Charlestown, MA 02129, USA; 4) Harvard Stem Cell Institute, Cambridge MA 02139, USA; 5) Harvard University, Cambridge, MA, 02138, USA; 6) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 002114, USA; 7) Department of Genetics, Harvard Medical School, Boston, MA 02115, USA.

Cell transplantation into immune deficient mice has revolutionized the fields of regenerative medicine and cancer biology. Yet, tools to facilitate direct visualization of the dynamic processes underlying carcinogenesis, tumor progression and metastasis *in vivo* remain limited. Here, we develop optically clear, immune deficient zebrafish that have mutations in *recombination activating gene 2* (*rag2)*, *DNA-dependent*

protein kinase (*prkdc*) and *janus kinase 3* (*jak3*). RNA sequencing and single-cell transcriptional profiling of the blood showed that *rag2* hypomorphic mutant zebrafish lack T cells, while *prkdc* deficiency resulted in lack of mature T and B cells and *jak3* in T and putative Natural Killer (NK) cells. While all three mutant lines engraft fluorescent-labeled normal and malignant cells, only the *prkdc* mutant fish reproduced as homozygotes and survived injury following tail fin clip and cell transplantation. Engraftment into optically clear, *prkdc*-mutant adult zebrafish facilitated dynamic live cell imaging of muscle regeneration, repopulation of muscle stem cells within their endogenous niche, and muscle fiber fusion at single-cell resolution. Serial imaging approaches also uncovered stochasticity in fluorescent-labeled leukemia regrowth following competitive cell transplantation into *prkdc*-mutant fish, providing refined models to assess clonal dominance and progression in the zebrafish. Our experiments provide an optimized and facile transplantation model, the *casper*-strain *prkdc* mutant fish, for efficient engraftment and direct visualization of fluorescent-labeled normal and malignant cells at single-cell resolution.

Z627 Dynamics of innate immunity guided tumor cell motility *in vivo*. *Minna Roh-Johnson*[1], Arish Shah[1], Susumu Antoku[2], John Condeelis[3], Cecilia Moens[1]. 1) Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 2) Columbia University, New York, NY, USA; 3) Albert Einstein College of Medicine, Bronx, NY, USA.

During cancer progression, interactions between tumor cells and immune cells play critical roles in the initiation of metastasis. In malignant melanoma, it is unclear how a melanocyte transitions from a premalignant nevus to an invasive tumor cell, and which components in the tumor microenvironment regulate this switch. A major limitation to understanding this switch *in vivo* is the lack of genetically tractable model systems that are amenable to high-resolution imaging of both the tumor and its microenvironment with cellular resolution. We have overcome this obstacle by directly visualizing tumor cells and their interactions with stromal cells in zebrafish. We found that injecting either human or zebrafish melanoma cells results in metastasis from the site of injection. Live imaging of the primary tumor revealed that tumor cells respond to contact with host macrophages by extending more actin-rich protrusions than those tumor cells not in contact with macrophages. Further, depleting host macrophages reduced melanoma metastasis. To reveal signaling pathways involved in macrophage-induced melanoma progression, we performed a reverse genetic *in vivo* screen for increased or decreased metastasis and identified *myd88*, a key regulator in the inflammatory response pathway, which functions downstream of Toll-Like Receptors. Depleting embryos of *myd88* decreased macrophage motility and recruitment of macrophages to the primary tumor, and inhibited melanoma metastasis. We further found that expression of constitutively active Myd88 in macrophages was sufficient to induce increased melanoma metastasis. Macrophages form long, thin, and dynamic protrusions to communicate with tumor cells *in vivo*, and our preliminary experiments suggest that these processes may be necessary for tumor cell metastasis. We are currently testing this hypothesis, and whether expression of Myd88 affects melanoma metastasis through the formation of these dynamic protrusions in macrophages, with high-resolution imaging approaches *in vivo*.

Z628 *tp53*-deficient zebrafish models of Malignant Nerve Sheath Tumor, Leukemia, Angiosarcoma, Rhabdomyosarcoma, and Germ cell tumors. *Myron Ignatius*[1], Finola Morre[1], Madeline Hayes[1], Qin Tang[1], Riadh Lobbardi[1], Sophia Reeder[1], Alexander Jin[1], Patrick Blackburn[2], Eleanor Chen[3], Petur Nielsen[1], Stephen Ekker[2], David Langenau[1]. 1) Harvard Medical School/Department of Pathology, Massachusetts General Hospital, Boston, MA; 2) Mayo Clinic, Rochester, MN; 3) Department of Pathology, University of Washington, Seattle, WA.

Individuals with Li-Fraumeni Syndrome inherit a mutant *TP53* allele and are predisposed to developing a wide range of cancers including sarcomas and leukemia. *TP53* is also somatically inactivated in more than half of all sporadic human cancers. Despite the well-studied role of p53 in cancer, mechanistic insights into how specific loss-of-function mutations regulate tumorigenesis are not clearly understood. Here, we have generated a new full-null tp53 deletion allele in syngeneic CG1 strain zebrafish. Two TALEN pairs were created to efficiently delete the entire 11kb locus. *tp53-/-* null zebrafish, lack p53 protein, are viable, survive at Mendelian ratios and can develop tumors as early as three months of age. In contrast to existing dominant-negative *tp53* alleles available in the zebrafish that develop MPNSTs with long latency, *tp53null* animals spontaneously develop lymphomas, MPNSTs, angiosarcomas, and germ cell tumors. 40% of null animals develop tumors within the first year of life (n=53 of 134). MPNSTs, lymphomas, and angiosarcomas could efficiently be transplanted into syngeneic recipient fish (2-4x10^4 cells/fish injected intraperitoneally). To visualize tumor growth *in vivo*, we next generated tp53null; Tg(Ubi-GFP) CG1 strain syngeneic zebrafish and transplanted tumors from fish with lymphoma into matched recipients. Transplanting either the kidney marrow or bulk tumor cells from diseased fish resulted in robust engraftment and seeding of leukemic cells to the kidney marrow, thymus, and spleen. Leukemia cells had atypical myeloid cell features, suggestive of myeloid leukemia. *tp53* is mutationally inactivated in 50% of human embryonal rhabdomyosarcomas (ERMS). To assess a role for *p53* in regulating ERMS growth, onset, tumor-propagating potential, and metastasis; we created zebrafish that were transgenic for kRASG12D and p53 loss. Self-renewal was unaffected in comparing kRASG12D tumors with and without p53 (n=3 tumors per genotype; p=0.66). By contrast, *tp53*null ERMS were more invasive and had elevated metastatic potential both in the primary and transplant settings (n=6 tumors per group). Taken together, we have developed a full null for the *tp53* allele in the zebrafish and show that null mutants develop a spectrum of tumor types with short latency and high penetrance. We are currently sequencing tumors generated in *tp53 null* mutants to perform comparative genomics with human tumors.

Z629 Investigating novel non-oncogene targets for cancer therapies. *Joan Heath*[1], Kimberly Morgan[1], Karen Doggett[1], Lachlan Whitehead[1], Stephen Mieruszynski[1], Zhiyuan Gong[2]. 1) Walter and Eliza Hall Institute of Medical Research, Parkville, Melbourne, Victoria, AU; 2) Department of Biological Science, National University of Singapore.

Somatic mutations in the *RAS* oncogenes are the most common activating lesions in human cancers. These mutations are frequently associated with poor responses to standard cancer therapies and over the last three decades intense effort has been directed towards the

identification of compounds that can bind to RAS and alter its activity. As yet this has been met with little success and mutant RAS is considered by many to be 'undruggable'. Thus identifying therapeutic vulnerabilities in cancer cells that harbour *RAS* mutations has the potential to greatly improve outcomes for patients with cancers of diverse tissue origin.

Chronic activation of RAS signalling places persistently high metabolic demands on cells to sustain robust cancer cell growth. This prompted us to investigate whether the genes underlying the endodermal phenotypes in a collection of zebrafish mutants we identified in the LiverPlus screen (1) might be required to sustain the rapid growth and proliferation of cells fuelled by oncogenic Ras expression. We have shown that these genes are required to maintain the high proliferative activity of intestinal epithelial cells and hepatocytes between 3 and 7 days of zebrafish development, and that they encode components of multi-subunit complexes that perform essential cellular functions, including transcription, RNA processing and nuclear pore assembly. Using a doxycycline inducible zebrafish hepatocellular carcinoma (HCC) model (2), we identified genetic interactions between oncogenic *kras*^{G12V} and three of our cloned zebrafish genes. Transgenic zebrafish, Tg(fabp10:rtTA2s-M2;TRE2:EGFP-Kras^{G12V}), hereafter TO(kras^{G12V}), were exposed to doxycycline (20µg/ml) at 2 dpf and again at 5 dpf, prior to analysis of liver volume at 7 dpf using two photon microscopy. This regime produced robust expression of EGFP-Kras^{G12V} and hepatocyte overgrowth, resulting in a 7.5 fold increase in the volume of the liver between 3-7 dpf. When this experiment was conducted with TO(kras^{G12V}) larvae carrying heterozygous mutations in genes important for U12-type splicing, ribosome biogenesis or nuclear pore formation, liver volume in this HCC model was reduced by up to 50%. Since these heterozygous mutations do not affect normal liver development, these experiments suggest that drugs designed to disrupt U12-type splicing, ribosome biogenesis and nuclear pore formation may provide a therapeutic window that could be exploited clinically to restrict the growth of cancer cells without affecting normal proliferative compartments.

1. Ober et al., Mech Dev (2003) 120:5-18
2. Chew et al., Oncogene (2014) 33:2717-27.

Z630 Chemical genetic approach identifies role of proton sensing GPR68 in modulation of migration in melanoma. *C. H. Williams*[1], C. C. Hong[1,2]. 1) Vanderbilt University, Nashville, TN; 2) Vanderbilt Univ. Med. Ctr., Nashville, TN.

Increased glycolysis resulting in local acidification is a hallmark of cancer. However, the mechanisms by which this acidification affects cellular behaviors such as migration are not understood. We report the discovery of Ogremorphin (OGM) a first in class inhibitor of GPR68 in a phenotypic zebrafish screen. The target of OGM was identified through a cheminformatics and receptor profiling, and validated genetically with knock down technology. GPR68 plays a critical role in neural crest development during zebrafish development. Furthermore, hiPSC derived Neural crest stem cell migration is inhibited by OGM. GPR68 is proton sensitive GPCR that is maximally active at ~pH6.6, and is upregulated in melanoma cell lines. We show that melanoma are more motile in acidic media. Furthermore, the increased migratory capacity is attenuated by OGM, which attenuates the formation of focal adhesions complexes. Taken together, the data suggests that pH mediated signaling is a critical component during embryonic development, and that GPR68 represents a possible novel pharmacological target for melanoma metastasis.

Z631 Zebrafish Pediatric Brain Tumor Modeling for Pre-clinical Drug Screening. *R. Stewart*[1], K. Modzelewska[1], E. Boer[1], R. Miles[1], J. Schiffman[1], A. Huang[2]. 1) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 2) Department of Pediatrics, Hospital for Sick Children, University of Toronto, ON, Canada.

Primitive Neuroectodermal Tumors (PNETs) represent the largest group of malignant brain tumors in children, with similar cellular histology but diverse clinical phenotypes/outcomes. The most common PNET is medulloblastoma located in the cerebellum, while more rare and aggressive PNET sub-groups located throughout the central nervous system (CNS) are collectively called CNS-PNETs. Due to the rarity of CNS-PNETs the cell of origin and oncogenic pathways driving their formation is not known, hindering development of targeted therapeutic options for these patients, who have an overall survival of ~ 20%. Recent genomic expression profiling has defined three CNS-PNETs subtypes: neural, oligoneural and mesenchymal. By modeling CNS-PNET gene signatures in zebrafish, we have generated the first animal model of oligoneural CNS-PNET. We show that activation of NRAS signaling in Sox10-expressing zebrafish embryonic cells with *p53*-deficiency generate oligoneural CNS-PNETs with conserved histological and molecular features to human CNS-PNETs, including activated MAPK/MEK signaling. We have also performed orthotopic brain tumor transplantation and drug-screening assays to show MEK activity is essential for oligoneural CNS-PNET tumor growth *in vivo*, and survival of human PNET cells *in vitro*. Thus, MEK inhibitors represent the first targeted therapy option for children with oligoneural CNS-PNETs.

Z632 Dissecting the mechanism of oncogenic glutamate receptor signaling in melanocytes and melanoma. *A. Neto*, C. Ceol. Program in Molecular Medicine and Department of Molecular, Cellular and Cancer Biology, UMass Medical School, Worcester, MA.

Glutamate signaling has been shown to have a role in melanoma progression, in part through activating mutations in the metabotropic glutamate receptor 3 (GRM3) gene. We hypothesize that altered glutamate signaling affects the development and function of melanocytes, endowing these cells with properties important for melanoma progression. By expressing mutant GRM3 variants in developing zebrafish melanocytes, we determined how oncogenic GRM3 variants affected these cells. In embryonic melanocytes oncogenic GRM3 mutants disrupted trafficking of melanosomes, causing their aggregation in the cell body. Wild-type GRM3 had no effect on melanosome distribution. Melanosomes are trafficked in a cAMP-dependent manner, and drugs that directly or indirectly increase cAMP levels were able to rescue the melanosome phenotype of oncogenic GRM3-expressing melanocytes. Our data indicate that oncogenic GRM3 variants dysregulate cyclic AMP (cAMP) signaling, a heretofore unknown role for these oncogenes. Preliminary data in cultured melanoma cells indicate that oncogenic GRM3

leads to a reduction of cAMP levels as compared to wild-type GRM3 or EGFP. cAMP signaling has been implicated in melanoma progression and drug resistance, and our data show that oncogenic properties of GRM3 could be mediated, at least in part, by alterations in cAMP signaling.

Z633 GDF6-induced BMP signaling promotes melanoma progression by reawakening a pro-survival neural crest identity. *Craig Ceol*[1], Arvind Venkatesan[1], Rajesh Vyas[1], Karen Dresser[1], Sharvari Gujja[1], Sanchita Bhatnagar[1], Sagar Chhangawala[2], Camilla Borges Ferreira Gomes[3], Haulin Simon Xi[1], Christine Lian[3], Yariv Houvras[2], Yvonne Edwards[1], April Deng[1], Michael Green[1]. 1) UMass Medical School, Worcester, MA; 2) Weill Cornell Medical College, New York, NY; 3) Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Oncogenomic studies have revealed genetic alterations that affect tumor progression. Included amongst these alterations are focal and broad recurrent copy number variations (CNVs). Identifying driver genes, which are responsible for disease progression, in broad CNVs is challenging as these regions harbor many bystander passenger genes that are altered due to their proximity to drivers. To enrich for driver genes in regions of CNV we performed comparative oncogenomics with human and zebrafish melanomas. Coupling this approach with transcriptome analyses, we identified the BMP factor *GDF6* as a novel melanoma oncogene. *GDF6* is specifically expressed in melanomas and not melanocytes, and its knockdown led to melanoma cell death. Enforced expression of *GDF6* promoted melanoma growth in an autochthonous zebrafish model as well as in mouse xenografts. Genetic epistasis analysis indicated that *GDF6* acts via *SMAD1* to aid in melanoma cell survival. Transcriptome analyses of melanoma cells with GDF6/BMP modulation found that a major role of *GDF6* is to regulate expression of genes important in neural crest development. Indeed, *GDF6* and its orthologs are expressed in the neural crest, where they regulate cell survival and fate specification. Mechanistic analyses determined that *GDF6* acts via suppression of the neural crest factor *SOX9,* which is an established pro-apoptotic factor in melanomas. To extend the relevance of these findings to the clinic we analyzed melanoma patient samples and found that nearly 80% of human melanomas express high levels of GDF6 and have an active BMP pathway. Furthermore, GDF6 expression correlated with poor overall survival of melanoma patients. We found that treatment with a BMP pathway small molecule inhibitor, DMH1, attenuated melanoma growth in mouse xenografts. Our findings have established that GDF6-dependent BMP signaling endows melanoma cells with a pro-survival, neural crest identity. Additionally, our results highlight GDF6 and BMP pathway components as novel targets for therapeutic intervention of melanomas.

Z634 Humanising the zebrafish liver shifts metabolic profiles, improves pharmacokinetics of CYP3A4 substrates and couples with development of fluorescent screening biomarkers. *T. J. Carney*[1,4], K. L. Poon[1], X. Wang[1], A. Ng[1], W. H. Goh[1], S. G. P. Lee[3], Z. Zhao[4], M. Al-Hadawwi[1], S. Fowler[2], H. Wang[1], P. Ingham[1,4], C. McGinnis[2]. 1) Institute of Molecular and Cell Biology (IMCB), A*STAR, Singapore; 2) Roche, Basel, Basel, Switzerland; 3) Genome Institute of Singapore, A*STAR, Singapore; 4) Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore.

Understanding and predicting whether new drug candidates will be safe in the clinic is a critical hurdle in pharmaceutical development, that relies in part on absorption, distribution, metabolism, excretion (ADME) and toxicology studies in vivo. Through toxicity and absorption analyses of a number of drugs, we find that zebrafish is generally predictive of drug toxicity although liver microsome assays reveal specific differences in metabolism of compounds between human and zebrafish livers, likely resulting from the divergence of the Cytochrome P450 superfamily between species. To reflect human metabolism more accurately, we generated a transgenic “humanized” zebrafish line that expresses the major human Phase I detoxifying enzyme, CYP3A4, in the liver. Here, we show that this humanized line shows an elevated metabolism of CYP3A4 specific substrates compared to wild-type zebrafish. The generation of this first described humanized zebrafish liver, suggests such approaches can enhance the accuracy of the zebrafish model for toxicity prediction. We subsequently utilise transcriptomics to identify highly upregulated genes as biomarkers of toxic responses in this model, finding candidate biomarkers which recurred in multiple treatments. Through promoter isolation and fosmid recombineering, eGFP reporter transgenic zebrafish lines were generated and showed a dose and time dependent induction in endodermal organs to reference drugs and an expanded drug set. Thus through integrated transcriptomics and transgenic approaches, we have developed a humanised zebrafish toxicity assay coupled with parallel independent zebrafish *in vivo* screening platforms able to predict organ toxicities of preclinical drugs.

Z635 Missing heritability for orofacial clefing identified through dissection of the gene regulatory network governing zebrafish periderm differentiation. *Robert A. Cornell*[1], Huan Liu[1], Elizabeth Leslie[2], Zhonglin Jia[1], Tiffany Smith[1], Mekonen Esthete[3], Azeez Butali[1], Martine Dunnwald[1], Jacquline Hecht[4], Ramat O. Braimah[3], Babatunde Aregbesola[3], Milliard Deribew[3], Mine Koruyucu[5], Figen Seyman[5], Lian Ma[6], Javier Enríquez de Salamanca[7], Seth M. Weinberg[2], Lina Moreno[1], Jeffrey Murray[1], Mary Marazita[2]. 1) University of Iowa, Iowa City, IA; 2) University of Pittsburgh, Pittsburgh, PA; 3) Addis Ababa University, Addis, Ethiopia; 4) University of Texas Health Science Center at Houston, Houston, TX; 5) Istanbul University, Istanbul, Turkey; 6) Peking University, Beijing, China; 7) Universitario Niño Jesús, Madrid, Spain.

Non-syndromic orofacial clefting, including cleft lip with or without cleft palate (CL/P), and cleft palate alone (CP), is a common disorder with a strong genetic underpinning. Genome-wide association studies (GWAS) have detected common variants associated with risk for this disorder. However, GWAS only explain a portion of the overall heritability for orofacial clefting; much of the risk is conferred by unidentified rare sequence variants. It is unclear what genes harbor such variants. In addition, the mechanistic basis of risk conferred by common, risk-associated variants (i.e., identified by GWAS) is unknown. Here we present our approach to both challenges using zebrafish. Most patients with Van der Woude orofacial clefting syndrome have mutations in *IRF6,* encoding Interferon Regulatory Factor 6, an essential member of the gene regulatory network (GRN) governing differentiation of periderm. Genes encoding other members of this GRN are candidates to harbor rare variants that confer risk for orofacial clefting. With epistasis experiments, we found that Krüppel-like factor 17 (Klf17) and Grainyhead-like 3

(Grhl3) both act downstream of Irf6 in the zebrafish periderm GRN. Klf17 expression is absent from mammalian oral epithelium, but a close homologue, Klf4, is expressed in this tissue and is required for the differentiation of epidermis. Chromosome configuration capture and reporter assays indicate that IRF6 directly regulates an oral-epithelium enhancer of *KLF4*. Next, we sequenced *KLF4* in approximately non-syndromic CL/ P cases and controls. Two patient-derived *KLF4* variants disrupted periderm differentiation upon forced expression in zebrafish embryos. Thus, rare variants of KLF4 account for some of the missing heritability for non-syndromic CL/P. Further, we detected rare coding variants of *GRHL3* in some patients with Van der Woude syndrome, and found they disrupted periderm differentiation in zebrafish, explaining some of the missing heritability for this disorder. Finally, a recent GWAS revealed that a common coding variant of *GRHL3* is associated with risk for non-syndromic CP. This variant, but not other common coding variants of *GRHL3*, has dominant negative activity in zebrafish. In sum, functional studies in zebrafish helped identify rare variants that contribute to the missing heritability for syndromic and non-syndromic forms of CL/P, and identified the mechanistic basis of a common variant associated with risk for non-syndromic CP.

Z636 Linking cilia motility and cerebrospinal fluid flow to the etiopathogenesis of adolescent idiopathic scoliosis. *D. T. Grimes*[1*], C. Boswell[2], N. F. C. Morante[1], R. M. Henkelman[2], R. D. Burdine[1], B. Ciruna[2]. 1) Princeton University, Princeton, NJ; 2) Hospital for Sick Children, University of Toronto, Canada.

Adolescent idiopathic scoliosis (AIS), a disease characterized by 3D spinal curvatures, afflicts 2-3% of children worldwide. However, the underlying biological basis for this disease has remained unknown. Recently, the teleost fish have emerged as robust models of AIS. Using zebrafish, we have discovered that spinal curves are caused by loss of motile cilia function, which in turn results in defective CSF flow. Human AIS can be caused by mutations in *PTK7*, and we find that cilia/CSF flow defects are also the basis of spinal curves in *ptk7* zebrafish mutants. Thus, we propose that AIS can be caused by disruptions to CSF flow, providing a novel mechanism for this prevalent disease. We also provide evidence that spinal curves can be partially rescued by restoring cilia motility after curve onset, opening potential therapeutic avenues.

Z637 Macrophage epithelial reprogramming underlies mycobacterial granuloma formation and promotes infection. *Mark Cronan*[1], Rebecca Beerman[1], Allison Rosenberg[1], Matthew Johnson[1], Joseph Saelens[1], Stefan Oehlers[1], Dana Sisk[1], Kristen Jurcic Smith[1], Le Trinh[2], Scott Fraser[2], John Madden[1], Joanne Turner[3], Jason Stout[1], Sunhee Lee[1], David Tobin[1]. 1) Duke University, Durham, NC; 2) University of Southern California, Los Angeles, CA; 3) The Ohio State University, Columbus, OH.

Mycobacterium tuberculosis infection in humans triggers formation of granulomas, tightly organized immune cell aggregates that are the central structure of tuberculosis. Infected and uninfected macrophages interdigitate, assuming an altered, flattened appearance. Although pathologists have described these changes for over a century, the molecular program that mediates this transition is unclear. We find that mycobacterial infection results in macrophage induction of canonical epithelial molecules, driving formation of granulomas. Using the zebrafish-*Mycobacterium marinum* model and intravital microscopy, we identify bona fide adherens junction formation between granuloma macrophages and nucleation of this process via interactions with host epithelium. Macrophage-specific disruption of E-cadherin function results in disordered granuloma formation, decreased bacterial burden, and enhanced long-term survival of infected animals, suggesting that a canonical mycobacterial granuloma may be fundamentally host-detrimental. In human clinical samples, granuloma macrophages are similarly transformed, paralleling E-cadherin-dependent mesenchymal-to-epithelial transitions in development and cancer, processes that profoundly regulate cell fate and function.

Z638 Metabolic stress induces Ripk3- and macrophage-dependent β-cell death in a zebrafish model of insulin resistance. *W. Chen*, L. Maddison. Vanderbilt University School of Medicine, Nashville, TN.

Insulin resistance can be compensated by increased insulin secretion and increased β-cell number. However, long-term insulin resistance can lead to β-cell failure, β-cell death and the development of type 2 diabetes. With its genetic, chemical, and anatomical tractability, zebrafish is poised to help elucidate the mechanisms that are involved in β-cell failure and death. To this end, we generated a transgenic model with skeletal muscle-specific insulin resistance, zMIR. When challenged with repeated exposure to overnutrition, the β-cell number in zMIR larvae initially increased as in control animals, but rapidly declined after the third exposure, indicating that β-cells of zMIR fish are more susceptible to metabolic stress. The β-cell loss can be prevented by anti-diabetic drugs and chemical chaperones. This β-cell loss was not through apoptosis or de-differentiation, but through necroptosis. Consistent with this, inhibitors of necroptosis protected the β-cells. Furthermore, genetic ablation of *ripk3*, a key regulator of necroptosis, also protected the β-cells. The β-cell loss coincided with islet macrophage infiltration in the zMIR animals. Macrophage depletion by clodronate liposomes or by genetic ablation of *irf8* protected β-cells. Interestingly, necroptosis inhibitors also suppressed macrophage infiltration. These data support a model in which metabolic stress triggers proinflammatory necroptosis of a small subset of β-cells in zMIR fish and the consequent macrophage infiltration causes death of additional β-cells.

Z639 Genetically encoded apolipoprotein reporters illuminate lipoprotein dynamics in the larval zebrafish. *Steven A. Farber*[1,2], James H. Thierer[2], Jessica P. Otis[1]. 1) Carnegie Institution for Science, Baltimore, MD; 2) Johns Hopkins U., Baltimore, MD.

Lipoproteins are central players in lipid transport and metabolism, and aberrations in the localization and metabolism of lipoproteins are major contributing factors to the growing incidence of metabolic disease. While it remains true that high serum high-density lipoprotein (HDL) cholesterol levels are associated with better clinical outcomes, recent data has called into question the cell biological and physiological functions attributed to HDL. The larval zebrafish has emerged as an excellent model for human metabolic disease, and has also proven a useful tool for monitoring lipid trafficking *in vivo* using fluorescent lipid analogs. Here we describe the development of optical reporters of lipoprotein

biology that provide an unprecedented opportunity to monitor lipoprotein dynamics at subcellular resolutions in a live vertebrate. Two distinct approaches were designed to address specific questions related to (1) HDL biology and (2) the diverse classes of non-HDL particles including low density lipoprotein (LDL) **Approach 1**: Zebrafish have two *apoa-I* genes that we determined are expressed in the yolk syncytial layer, intestine and liver. To study APOA-I (forms HDL) of hepatic vs. intestinal origin *in vivo*, we created transgenic zebrafish expressing fluorescently labeled human or zebrafish APOA-I driven by liver- or intestine-specific promoters. Confocal microscopy of live larvae reveals APOA-I-mCherry fluorescence in the circulation and in specific tissues and subcellular domains. Fluorescent puncta were observed in the apical late endosomal/lysosomal compartment of intestinal enterocytes of larvae expressing hepatic APOA-I-mCherry. APOA-I-mCherry of intestinal origin colocalizes with late endosomal markers in liver hepatocytes. These data suggest previously unappreciated roles for the intestine and liver in the recycling and/or degradation of ApoA-I of hepatic origin and intestinal origin, respectively. **Approach 2:** Apolipoprotein B (ApoB) is the obligate structural component of LDL and elevated levels are linked to metabolic diseases. Despite its heritability, many of the genetic factors governing LDL levels remain uncharacterized. We have developed zebrafish carrying a luciferase reporter fused to the ApoB gene locus to produce a tagged ApoB protein. We can now rapidly quantify ApoB levels in individual larvae, and have used the reporter to determine the size distribution of LDL particles using a novel native-PAGE approach. In summary, we have developed a fluorescent reporter for tracking the intracellular and inter-organ trafficking of HDL and a chemiluminescent reporter of LDL lipoprotein particles to support a chemical screen for regulators of LDL biology. To our knowledge, this is the first time tissue-specific apolipoprotein transport has been visualized *in vivo* in any vertebrate.

Z640 Estrogens Suppress a Behavioral Phenotype in Zebrafish Mutants of the Autism Risk Gene, CNTNAP2. *Ellen J. Hoffman*[1], Katherine J. Turner[2], Joseph M. Fernandez[1], Daniel Cifuentes[1,3], Marcus Ghosh[2], Sundas Ijaz[1], Roshan A. Jain[4,5], Fumi Kubo[6], Brent R. Bill[7,8], Herwig Baier[6], Michael Granato[4], Michael J. F. Barresi[9], Stephen W. Wilson[2], Jason Rihel[2], Matthew W. State[1,10], Antonio J. Giraldez[1]. 1) Yale University, New Haven, CT; 2) University College London, London, UK; 3) Boston University School of Medicine, Boston, MA; 4) University of Pennsylvania, Philadelphia, PA; 5) Haverford College, Haverford, PA; 6) Max Planck Institute of Neurobiology, Martinsried, Germany; 7) University of California, Los Angeles, CA; 8) The University of Texas at Tyler, Tyler, TX; 9) Smith College, Northampton, MA; 10) University of California, San Francisco, San Francisco, CA.

Autism spectrum disorders (ASD) are a group of devastating neurodevelopmental syndromes that affect up to 1 in 68 children. Despite advances in the identification of ASD risk genes, the mechanisms underlying ASD remain unknown. Homozygous loss-of-function mutations in *Contactin Associated Protein-like 2* (*CNTNAP2*) are strongly linked to ASD. We generated zebrafish mutants of *cntnap2* and conducted pharmacological screens to identify phenotypic suppressors. We found that zebrafish *cntnap2* mutants display GABAergic deficits particularly in the forebrain and sensitivity to drug-induced seizures. High-throughput behavioral profiling identified nighttime hyperactivity in *cntnap2* mutants, while pharmacological testing revealed dysregulation of GABAergic and glutamatergic systems. Finally, we found that estrogen receptor agonists elicit a behavioral fingerprint anti-correlative to that of *cntnap2* mutants and showed that the phytoestrogen biochanin A specifically reverses the mutant behavioral phenotype. These results identify estrogenic compounds as phenotypic suppressors and illuminate novel pharmacological pathways with relevance to autism. We are utilizing pharmaco-behavioral profiling of zebrafish mutants of multiple ASD risk genes to identify relevant biological pathways and potential pharmacological candidates for further evaluation.

Z641 Macrophage-mediated thrombus dissolution is rate limiting during vascular repair. *H. Clay*[1], I. Lam[1], J. Shavit[2], S. R. Coughlin[1]. 1) UCSF, San Francisco, CA; 2) University of Michigan, Ann Arbor, MI.

Vessel repair requires the coordinated actions of multiple cell types in order to effect coagulation, inflammation, repair of the injured endothelium, and resolution of inflammation. Using zebrafish embryos, we demonstrate that the dynamics of the thrombus, leukocytes, and the vascular endothelium can be tracked and quantified using real-time microscopy over the entire course of injury repair. This system affords a unique opportunity to study vascular repair *in vivo* using genetic and pharmacological manipulation. We find that macrophage influx to the wound site is coincident with thrombus dissolution, and that while initial macrophage recruitment is independent of thrombus formation, persistence at the wound site after re-establishment of blood flow requires local fibrin deposition, and macrophages are actively involved in clearance of the thrombus. Depletion of macrophages, but not neutrophils, leads to increased healing times secondary to the increased persistence of the thrombus at the wound site. Taken together, our data demonstrate that thrombus removal is rate-limiting during vascular repair and that macrophages are required during this process.

Z642 A story in translation: Phosphoinositide signaling and angiogenesis. *B. M. Weinstein*[1], A. N. Stratman[1], C. M. Mikelis[3], Z. Wang[3], O. M. Farrelly[1], M. F. Miller[1], S. A. Pezoa[1], V. N. Pham[1], D. Castranova[1], A. E. Davis[1], T. M. Kilts[4], G. E. Davis[5], J. S. Gutkind[3], W. Pan[1,2]. 1) Division of Developmental Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, 20892; 2) Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences & Shanghai Jiao Tong University School of Medicine, Shanghai, China, 200031; 3) Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892; 4) Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, 20892; 5) Department of Medical Pharmacology and Physiology, School of Medicine, Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO 65212.

Anti-angiogenic therapies have been regarded as one of the most promising new approaches for combating cancer, but they have yet to fulfill this promise. In large part, this is because of the ability of tumors to evade or overcome these therapies by up-regulating pro-angiogenic

cytokines. We report a new approach to inhibit angiogenesis - targeting recycling of the rate-limiting substrate used for transduction of VEGF-dependent angiogenic signaling. Beginning with zebrafish mutants identified in a genetic screen and using zebrafish, cell culture, and mouse tumor models we show that this new approach has the potential to result in a uniquely effective inhibition of tumor angiogenesis, since increased VEGF stimulation, rather than overcoming the inhibition, only results in faster consumption of the limiting substrate and more rapid and complete inhibition of angiogenesis.

Z643 LSD1-dependent shutdown of *etv2* promotes hematopoietic differentiation in hemangioblasts. *M. Kobayashi*, J. Tamaoki, Y. Fuse, M. Takeuchi. University of Tsukuba, Tsukuba, Ibaraki, JP.

The hemangioblast is a progenitor cell with the capacity to give rise to both hematopoietic and endothelial progenitors. Currently, the regulatory mechanisms underlying hemangioblast formation are being elucidated, whereas those controllers for the selection of hematopoietic or endothelial fates still remain a mystery. To answer these questions, we screened for zebrafish mutants that have defects in the hemangioblast expression of *gata1*, which is never expressed in endothelial progenitors. One of the isolated mutants, *it627*, showed not only down-regulation of hematopoietic genes but also up-regulation of endothelial genes. We identified the gene responsible for the *it627* mutant as the zebrafish homolog of Lys-specific demethylase 1 (LSD1/KDM1A). Surprisingly, the hematopoietic defects in *lsd1*^{it627} embryos, both erythropoiesis and ectopic myelopoiesis, were rescued by the gene knockdown of the Ets variant 2 gene (*etv2*), an essential regulator for hemangioblast development. Our results suggest that the LSD1-dependent shutdown of *etv2* expression may be a significant event required for hemangioblasts to initiate hematopoietic differentiation.

Z644 Growth Differentiation Factor 6 (GDF6) promotes vascular quiescence by maintaining stable endothelial cell adherens junctions. *S. Krispin*, A. N. Stratman, C. H. Melick, B. M. Weinstein. NICHD,NIH, Bethesda, MD.

The assembly of a functional vascular system requires coordinated signaling between various growth factors and receptors. The bone morphogenetic protein (BMP) receptors and their BMP ligands have important functions during embryonic vessel assembly and maturation. These crucial roles have been demonstrated in vascular disorders such as hereditary hemorrhagic telangiectasia (HHT). However, the identity of the responsible ligands and mechanism of action are still not clear. Growth Differentiation factor 6 (GDF6=BMP13), is a highly conserved BMP ligand. A previous report has suggested a role for gdf6a (one of the two zebrafish GDF6 homologues) in vascular integrity, however, the mechanism of gdf6a action was not clear. We find that the zebrafish gdf6a mutants demonstrate massive trunk hemorrhage in 20% of 48hpf mutants, with increased vascular permeability in non-hemorrhaging mutants. We also find that GDF6 is a negative regulator of VEGFA/VEGFR2 signaling. Loss of GDF6 in zebrafish leads to increased VEGFA, causing destabilization of VE-Cadherin and weakening of endothelial cell adherence junctions. The role of GDF6 is conserved in human endothelial cells; GDF6 siRNA mediated knockdown in human umbilical vein endothelial cells (HUVEC) resulted in increased monolayer permeability, VEGFA/VEGFR2 increase accompanied by an increased phosphorylation of VE-Cadherin and decrease in total levels of VE-Cad as seen in gdf6a^{s327} mutants. GDF6 activity is mediated preferentially through the BMPR1B-BMPR2 complex upstream of Smad2/3. Understanding how GDF6 affects vascular integrity will help provide insights into hemorrhage and associated pathologies in humans.

Z645 Embryonic hematopoiesis in vertebrate somites gives rise to definitive hematopoietic stem cells. *A. Meng*[1], J. Qiu[1], X. Fan[2], Y. Wang[1], H. Jin[1], Y. Song[1], Y. Han[3], S. Huang[1], Y. Meng[1], F. Tang[3]. 1) School of Life Sciences, Tsinghua University, Beijing, CN; 2) College of Life Sciences, Peking University, Beijing, CN; 3) College of Biological Sciences, China Agricultural University, Beijing, CN.

Hematopoietic stem cells (HSCs) replenish all types of blood cells. It is debating whether HSCs in adults solely originate from the aorta-gonad-mesonephros (AGM) region, more specifically, the dorsal aorta, during embryogenesis. Here we report that somite hematopoiesis, a previously unwitnessed hematopoiesis, can generate definitive HSCs (dHSCs) in zebrafish. We noticed that in several transgenic lines, in which GFP is expressed in somites during embryonic development, embryos and adults carry hematopoietic cells in the circulation. We established Tg(foxc1b:EOS) transgenic line, which expresses the photoconvertible fluorescence reporter EOS in somites. By photoactivating EOS in specific somite areas, we found that a subset of cells within the forming somites emigrate ventrally and mix with lateral plate mesoderm-derived primitive hematopoietic cells before the blood circulation starts. These somite-derived hematopoietic precursors and stem cells (sHPSCs) subsequently enter the circulation and colonize the kidney of larvae and adults. RNA seq analysis reveals that sHPSCs express hematopoietic genes with sustained expression of many muscle/skeletal genes, which is in contrast to absent or low expression of muscle/skeletal genes in gata1a-positive primitive hematopoietic precursors. Embryonic sHPSCs transplanted into wild-type embryos expand during growth and survive for life time with differentiation into various hematopoietic lineages, indicating self-renewal and multipotency features. In contrast, transplanted gata1a-positive primitive hematopoietic precursors cannot survive over 9 days in the recipients. Therefore, we propose that dHSCs can also originate from embryonic somites.

Z646 RUNX1-independent development of HSC and definitive hematopoiesis in zebrafish. *P. Liu*, Blake Carrington, B. Carrington, M. Jones, S. Wincovitch, R. Sood. NHGRI/NIH, Bethesda, MD.

The transcription factor RUNX1 is required for the emergence of definitive hematopoietic stem cells (HSCs) from the hemogenic endothelium during mouse embryonic development. Consequently, *Runx1* knockout mouse embryos lack all definitive blood lineages and cannot survive past embryonic day 13. Previously we found that mutating the only *runx1* homologue in zebrafish also resulted in deficiency of definitive blood cells during embryogenesis. However, the zebrafish *runx1* mutants, which carried an ENU-induced nonsense mutation in *runx1* (*runx1*$^{W84X/W84X}$),

were able to recover from a larval "bloodless" phase and develop to fertile adults with multi-lineage hematopoiesis (Sood et al., Blood 2010), suggesting that definitive hematopoiesis can develop without RUNX1. To confirm this finding and to further study the underlying mechanism, we generated three new *runx1* mutants: two mutations in exon 4 of *runx1* (a deletion of 8 bp, *runx1*$^{del8/del8}$, and a deletion of 25 bp, *runx1*$^{del25/del25}$) that truncate the runt-homologous domain; and a large deletion of exons 3 through 8 (*runx1*$^{del(e3-8)/del(e3-8)}$), which removes most coding region of *runx1,* using the TALEN and CRISPR-Cas9 technologies. All three new *runx1* mutants failed to develop definitive hematopoiesis during early embryonic development. Time-lapse recordings with confocal microscopy revealed no emergence of *c-myb*$^{+}$ HSCs from the ventral wall of dorsal aorta in the runx1 mutant embryos at 48 hours post fertilization. However, a second wave of HSCs emerged that led to circulating blood cells between 15 and 20 days post fertilization. Eventually, about 40% of the *runx1* mutants developed to fertile adults with circulating blood cells of multi-lineages. Taken together, our data strongly suggests for a RUNX1-independent mechanism for HSC formation and definitive hematopoiesis.

Z647 TopBP1 Governs Hematopoietic Stem/Progenitor Cells Survival in Zebrafish Definitive Hematopoiesis. *Weijun Pan*[1*], Lei Gao[1], Yi Zhou[2], Leonard I. Zon[2]. 1) Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences & Shanghai Jiao Tong University School of Medicine, Shanghai, China; 2) Stem Cell Program, Hematology/Oncology Program at Children's Hospital Boston and Dana Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, United States of America.

In vertebrate definitive hematopoiesis, nascent hematopoietic stem/progenitor cells (HSPCs) migrate to and reside in proliferative hematopoietic microenvironment for transitory expansion. In this process, well-established DNA damage response pathways are vital to resolve the replication stress, which is deleterious for genome stability and cell survival. However, the detailed mechanism on the response and repair of the replication stress-induced DNA damage during hematopoietic progenitor expansion remains elusive. Here we report that a novel zebrafish mutantcas003 with nonsense mutation in **topbp1** gene encoding topoisomerase II β binding protein 1 (**TopBP1**) exhibits severe definitive hematopoiesis failure. Homozygous topbp1cas003 mutants manifest reduced number of HSPCs during definitive hematopoietic cell expansion, without affecting the formation and migration of HSPCs. Moreover, HSPCs in the caudal hematopoietic tissue (an equivalent of the fetal liver in mammals) in topbp1cas003 mutant embryos are more sensitive to hydroxyurea (HU) treatment. Mechanistically, subcellular mislocalization of TopBP1cas003 protein results in ATR/Chk1 activation failure and DNA damage accumulation in HSPCs, and eventually induces the p53-dependent apoptosis of HSPCs. Collectively, this study demonstrates a novel and vital role of **TopBP1** in the maintenance of HSPCs genome integrity and survival during hematopoietic progenitor expansion.

Z648 Structural basis of endothelial Adgra2/Reck complex activity during Wnt7-dependent brain angiogenesis and blood-brain barrier formation in zebrafish. *N. Bostaille*, B. Vanhollebeke. Neurovascular Signaling Laboratory, ULB Neuroscience Institute, Université Libre de Bruxelles, Brussels, Belgium.

Distinct Wnt/β-catenin signaling cascades operate within endothelial cells (ECs) to control central nervous system vascular invasion and blood-brain barrier formation. In the mouse forebrain and zebrafish brain, Wnt7 ligands prime perineural ECs for invasion. In order to recognize these ligands, and hence to be competent for brain invasion, endothelial cells assemble unique receptor complexes at the plasma membrane that, in addition to classical receptor components like Fzd/Lrp, contain Adgra2 (previously known as Gpr124), an orphan member of the adhesion class of G protein-coupled receptors and Reck, a GPI-anchored glycoprotein. Adgra2 and Reck physically interact to assemble a potent synergistic Wnt7-specific co-activator complex that controls tip cell function during brain vascularization. How the partners interact and whether Adgra2 transmits a signal inside the receiving EC remains to be determined. In light of their function as key brain angiogenic regulators, determining the molecular modalities of Adgra2/Reck activity is of both clinical and fundamental importance. Adhesion class G protein-coupled receptors (aGPCRs) form the second largest class of GPCRs that context-dependently function as adhesion molecules, signal transducing GPCRs, or both concurrently. Adgra2 is a typical aGPCR characterized by a long extracellular domain containing multiple protein adhesion motifs, followed by a membrane-proximal GPCR-autoproteolysis autoinduced (GAIN) domain, seven membrane-spanning domains and a C-terminal intracellular domain. Through a combination of mutagenesis and *in vivo* brain angiogenic assays, we have molecularly dissected the Adgra2 protein motifs and determined their contribution to protein trafficking, interaction with Reck and angiogenic function. These analyses reveal key determinants of Adgra2/Reck function, uncover a novel mode of action for adhesion GPCRs and provide insights into the pharmacological potential of the Adgra2/Reck complex in cerebrovascular disorders.

Z649 Microtubule-actin crosslinking factor (Macf1) Function in Oocyte Polarity and Nuclear Positioning. *M. Escobar-Aguirre*, H. Zhang, R. Fuentes, A. Jamieson-Lucy, M. C. Mullins. University of Pennsylvania, Cell and Developmental Biology, Philadelphia, PA.

Cell polarity allows cells to regionalize their intracellular environment to perform a variety of specialized functions. In most vertebrates, polarity of the egg defines the anterior-posterior and dorsal-ventral axes of the embryo. Egg animal-vegetal (AV) polarity is derived from oocyte polarity, which is established early in oogenesis . AV polarity is established through the formation, translocation and disassembly of the Balbiani Body (Bb) at the vegetal pole. The Bb is conserved from insects to humans and is composed of organelles, RNAs and proteins. The Bb components, which include germ cell determinants, anchor to the vegetal cortex upon Bb disassembly in late stage I oocytes. Our lab discovered in zebrafish the only genes known to function in AV polarity formation in vertebrates: *bucky ball* and *macf1*. While Bucky ball is required for Bb formation, Macf1 is crucial for its disassembly. Macf1 is a conserved multi-domain cytoskeletal linker protein that can interact with microtubules (MTs), actin filaments (AF) and intermediate filaments (IF). Zebrafish *macf1* mutant oocytes display an: 1) enlarged Bb, 2)

acentric nucleus, and 3) a failure of Bb disassembly. Our aim is to elucidate the Macf1 dependent cytoskeletal linking mechanism that regulates AV polarity. First, we determined that Macf1 protein localizes perinuclearly, to the Bb, and upon Bb disassembly it distributes to the vegetal cortex. Second, we examined cytoskeleton function. Interestingly, disruption of AF phenocopies the *macf1* mutant phenotype. We found that cytokeratins (CK)- a type of intermediate filament- localize around the nucleus, are enriched in the Bb and exhibit a peripheral distribution in the cytoplasm in late stage I oocytes. These findings suggest that Macf1 Actin binding domain (ABD) and Plectin repeat domain (PRD) integrate cortical actin and CK to mediate Bb disassembly. To specifically test if the Macf1 actin binding domain (ABD) and Plectin repeat domain (PRD) function in Bb disassembly and nuclear positioning, we deleted the exons encoding these domains from the *macf1* endogenous locus by using Crispr/Cas9 technology. We found that Macf1Del-ABD mutant oocytes display the *macf1* null mutant phenotype whereby the nucleus is acentric and the Bb fails to disassemble. Importantly, Macf1Del-ABD protein is localized to the Bb like in wt. Surprisingly, deleting the PRD domain (Macf1Del-PRD) does not affect Bb disassembly or nuclear positioning. In summary, we determined that Macf1 functions via its ABD and actin filaments to mediate Bb disassembly and nuclear positioning, while the PRD is dispensable. To our knowledge, this is the first study to use genome editing to unravel the module-dependent function of a cytoskeletal linker in the context of cell polarity establishment.

Z650 Actr10, a component of the dynactin complex, regulates retrograde mitochondrial transport in axons. *C. Drerup*, A. Nechiporuk. Oregon Health & Science University, Portland, OR.

Mitochondrial transport in axons is imperative to mitochondrial health as well as the formation, stability and function of neural circuits. How mitochondria attach uniquely to the motor protein complexes necessary for their unidirectional movement is largely unknown. Using forward genetics, *in vivo* transport analyses, and biochemistry, we identified Actr10, a member of the dynactin complex, as a critical regulator of mitochondrial retrograde transport. Loss of Actr10 leads to mitochondrial accumulation in axon terminals due to failed attachment of this organelle to the dynein-dynactin complex. Anterograde mitochondrial transport and the localization and transport of dynein and other cargos in *actr10* mutants is unaffected. Biochemical and genetic interaction studies revealed the dynamin-like protein Drp1 as a partner for Actr10 in the retrograde transport of mitochondria in axons. Together, this work identifies Actr10 as the first specific regulator of mitochondrial attachment to the retrograde motor complex and enhances our understanding of the intricate cellular mechanisms required for the proper localization of this organelle in axons.

Z651 Apoptotic cartilage remodelng requires Kinesin I. *A. Santos*, P. D. Campbell, M. Gronska, F. L. Marlow. Albert Einstein College of Medicine, Bronx, NY.

Intramembranous ossification (bone formation within connective tissue), endochondral ossification (bone formation via mineralization and reabsorption of cartilage anlagen) and apoptotic remodeling of unmineralized cartilage (chondroptosis) are mechanisms underlying skeletal tissue morphogenesis. Chondroptosis, a chondrocyte specific form of apoptosis, is important for cartilage morphogenesis and is altered in some diseases, such as osteoarthritis. Nevertheless, the molecular basis remains elusive because chondrocytes undergo classical apoptosis in culture and *in vivo* models are lacking.

Here we describe a conserved role for the motor protein Kinesin I, *kif5B*, in maintaining muscle fiber integrity, and a novel role in apoptosis-mediated remodeling of cartilage. In addition to the hallmark features of chondroptosis (expansion of rER and Golgi, increased autophagocytic vacuoles, and patchy chromatin), extrusion and engulfment of mutant chondrocytes by neighboring cells occurs in *kif5B* loss of function (*kif5Blof*) mutants and chimeric contexts. Genetic interactions with Wnt planar cell polarity (PCP) mutants indicate distinct roles for PCP and *kif5B* in cartilage morphogenesis. Initial establishment of the cartilage is unaffected as early chondrocytic markers (*sox9* and *col2α1*) are properly expressed. Later, between 60 and 72hpf, when cartilage remodeling occurs, *kif5Blof* mutants undergo prolonged chondroptosis. Chimeric analyses, using cell transplantation, indicate a cell-autonomous requirement for Kif5B in regulating secretion, nuclear position, cell elongation and survival. Surprisingly, in these assays large groups of wild-type cells could support elongation of neighboring mutant cells. Mosaic expression of *kif5Ba* but not *kif5Aa,* in cartilage using Gal4/UAS similarly rescues the chondroptosis phenotype.

Metalloproteinases are crucial for cartilage remodeling before mineralization, and the cartilage phenotypes of MT1-MMP mutant mice resemble those of *kif5Blof* zebrafish. Moreover, Kif5B mediates membrane localization of MT1-MMP in other contexts; therfore, Kif5B may promote MT1-MMP functions in cartilage remodeling. To circumvent the earlier phenotypes reported for MT1-MMPa knockdown in zebrafish we utilized a pharmacological approach. However, general inhibition of metalloproteinase activity (EDTA) or more specific inhibition of MMP activity (GM6001) has failed to phenocopy *kif5Blof* mutants, and thus MT1-MMP contribution remains ambiguous. Cumulatively, our study reveals an essential role for Kif5B in cell polarization, secretion, and chondroptosis. *kif5Blof* mutants provide a new *in vivo* model to study a poorly understood form of programmed cell death that contributes to cartilage remodeling and osteochondrodysplasia in humans.

Z652 Protocadherin18a organizes notochord formation by regulating E-cadherin-mediated cell migration. *B. Bösze*[1], K. Stricker[1], S. Weber[1], V. Gourain[1], T. Thumberger[2], J. Wittbrodt[2], U. Strähle[1], S. Scholpp[1]. 1) Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, DE; 2) Centre for Organismal Studies, Heidelberg, DE.

Collective cell migration is a fundamental process required for tissue development, wound repair and cancer invasion. Gastrulation, the formation of the three germ layers is characterized by collectively migrating cells that anteriorly form the prechordal plate and posteriorly shape the notochord, the major skeletal element of the developing embryo. The cellular process leading to the formation of the rod-shaped notochord during collective cell migration of the mesodermal plate is unknown.

We found Protocadherin18a (Pcdh18a), a member of the cadherin superfamily as a novel regulator of cohesive cell migration during zebrafish

gastrulation. We show that Pcdh18a is expressed in the notochord tip cells (NTCs) and controls the adhesive and migratory behavior of this cell group. Enhanced adhesiveness and fast migration of the NTCs are required for formation of the 3D structure of the posteriorly-located notochord. Migrating cells rapidly adhere and de-adhere in order to translocate over their substrate. We indeed find that Pcdh18a regulates endocytosis of the E-cad/Fzd7 adhesion complexes in E-cad positive mouse fibroblasts, in human cervical cancer cells and during zebrafish gastrulation. Consistently, endocytic blockade in Pcdh18a positive cells reduces cell migration *in vitro* and *in vivo*. In summary, we conclude that during gastrulation, Pcdh18a positive NTCs organize the shape of the notochord, independently of cell migration events in the lateral plate mesoderm.

Z653 RGMa/Neogenin signaling promotes neural convergence by enhancing cell polarity and organizing microtubules. *Sharlene P. Brown*, Pradeepa Jayachandran, Valerie Olmo, Rebecca MacFarland, Rachel Brewster. UMBC, Baltimore, MD.

Neural convergence (NC) is the evolutionary conserved mechanism that narrows the neural plate (NP) enabling neural tube (NT) closure. Failure of this critical developmental process contributes to neural tube defects (NTDs), which occur in 1/1000 live births. Among the many candidate genes identified in model organisms that mediate NC, only those implicated in the planar cell polarity (PCP) pathway have so far been implicated in the etiology of human NTDs.

This study investigates the role of the Repulsive Guidance Molecule A (RGMa) and Neogenin signaling as regulators of NC in the zebrafish NP. RGMa is chemorepulsive to emerging retinal ganglion cell axons and prohibitive to the regeneration of damaged nerves. However, evidence from mouse, frog, and zebrafish suggest that RGMa/Neogenin signaling is required for NT formation. Although this latter function is poorly understood, the Cooper lab determined that RGMa signaling directly affected NT closure, the latest stage of tube formation. Absent from the literature is an understanding of the role of RGMa/Neogenin signaling in the earliest, most conserved stage of anterior NT development, NC, without which adequate NT closure is improbable due increased NP tissue width. We found that insufficient ligand or receptor similarly disrupted NC. While control embryos underwent adequate NP narrowing, RGMa and Neogenin-deficient embryos had widened NPs at the same somitic stage. To determine the cellular behaviors contributing to this NC defect, we examined movies of hindbrain NP cells in vivo from control and loss-of-function (LOF) embryos. Our quantitative data show that control embryos extend membranous protrusions toward the midline and lateral surface of the tissue. However, both RGMa and Neogenin LOF randomized the protrusiveness of NP cell membranes. Cells deficient in this pathway were also rounded instead of polarized perpendicular to the midline. The lack of polarization in these embryos is reminiscent of defects observed when microtubule (MT) stability/organization is disrupted. Therefore, we investigated the requirement of RGMa and Neogenin for effective MT organization. As we have reported previously, NC in the zebrafish involves rearrangement of the MT cytoskeleton into a progressively linear array perpendicular or oblique to the midline. In this study, control NP cells were found to have archetypical MT organization. However, RGMa and Neogenin LOF severely shortened the length of MT arrays. These observations suggest that MTs may be downstream targets of RGMa/Neogenin signaling to promote convergence of the NP and thus proper NT formation. Ongoing work investigates the connection between RGMa/Neogenin and cadherin-based cell adhesion to mediate cell behavior essential to NC. .

Z654 Hippo signaling regulates ventricle morphogenesis via Taz-dependent activation of Wnt and Notch signaling. *L. G. Selland*, A. J. Waskiewicz. University of Alberta, Edmonton, Alberta, CA.

Specialized boundaries form between the rhombomeres in the hindbrain and provide signaling cues to the surrounding tissues. We are investigating the role of rhombomere boundaries in the morphogenesis of the hindbrain ventricle in zebrafish. The ventricular system forms a specialized neural circulatory system and aberrant ventricle development correlates with hydrocephalus and neurodevelopmental disorders such as schizophrenia and autism. Initial patterning of the neural tube enables the correct positioning of the ventricles. Characteristic morphogenesis and cell proliferation then shapes the ventricles, while the initial secretion of embryonic cerebrospinal fluid inflates the ventricles. The Grinblat and Wilkinson laboratories have provided evidence that Zic1 and Zic4 from the roof plate activate Notch signaling via *rfng,* which subsequently drives Wnt signaling at rhombomere boundaries. Our research goal is to identify novel signaling pathways that regulate hindbrain ventricle formation. By screening for pathway components with the correct spatio-temporal expression, we determined that the Hippo signaling pathway transcriptional co-activator *taz,* is expressed in the hindbrain roof plate. Using TALEN mutagenesis, we created mutants with a 29 bp deletion, resulting in a loss of all functional domains of the Taz protein. *Taz*$^{-/-}$ mutants display midline separation defects in the hindbrain ventricle. This phenotype is apparent by 22hpf and persists through 36hpf indicating defects in the initial shaping of the brain and ventricle inflation. Examination of genes identified by the Sive lab to be required for ventricle morphogenesis (*ppp1r12a, mpp5a* and *crb2a*) showed no alterations in expression. Therefore, we hypothesized that *taz* may regulate rhombomere boundary signals independent of other known roof plate derived cues in order to drive ventricle morphogenesis. Examination of roof plate signals, including the *zic* family of genes, revealed no alterations in expression, however *taz*$^{-/-}$ mutants fail to express both *wnt1* and *rfng* at rhombomere boundaries. Treatment with a pharmacological inhibitor of Wnt signaling (XAV939) results in defective ventricle morphogenesis. To determine if *rfng* is responsible for the expression of *wnt1* at rhombomere boundaries, we used a pharmacological inhibitor of Notch signaling (DAPT) and assayed for *wnt1* expression. DAPT treatment did not affect *wnt1* expression at rhombomere boundaries, although it did perturb ventricle morphogenesis. This is the first evidence of Hippo signaling involvement in ventricle development, and supports a model in which Taz-dependent activation of Wnt and Notch signaling pathways at rhombomere boundaries regulates hindbrain ventricle formation.

Z655 The Joubert syndrome protein INPP5E controls ciliogenesis by regulating phosphoinositides at the apical memebrane. *Y. Cao*, W. Xu, M. Jin, R. Hu, F. Zhang, H. Wang. Tongji University, Shanghai, Shanghai, CN.

Phosphoinositides, a family of phosphorylated derivatives of phosphatidylinositol (PtdIns), are tightly regulated both temporally and spatially by PtdIns phosphatases and kinases. Mutations in *Inositol polyphosphate 5-phosphatase E* (*INPP5E*) cause Joubert syndrome, a human disorder associated with numerous ciliopathic defects including renal cyst formation, linking phosphoinositides to ciliopathies. However, the molecular mechanism by which INPP5E-mediated phosphatidylinositol signaling regulates ciliogenesis and cystogenesis is unclear. Here, we utilize an *in vivo* vertebrate model of renal cystogenesis to show that Inpp5e functions in both by hydrolyzing PtdIns(3,4,5)P3 and stabilizing PtdIns(4,5)P2 at the apical membrane, which in turn directs apical docking of basal bodies in renal epithelia. Knockdown or knockout of *inpp5e* leads to ciliogenesis defects and cystic kidney in zebrafish. Furthermore, knockdown of *inpp5e* in embryos leads to defects in cell polarity, cortical organization of F-actin, apical docking of basal bodies, and apical segregation of PtdIns(4,5)P2 and PtdIns(3,4,5)P3. Knockdown of *ezrin*, which encodes an ERM (Ezrin, Radixin and Moesin) protein that crosslinks PtdIns(4,5)P2 and F-actin, phenocopied *inpp5e* knockdowns. Strikingly, overexpression of *ezrin* rescued *inpp5e* morphants, suggesting that *ezrin* functions downstream of *inpp5e*. Finally, treatment with the PtdIns(3,4,5)P3 inhibitor, LY294002, rescues the cellular, phenotypic and renal function defects in *inpp5e* knockdown embryos. Together our data indicate that Inpp5e is a key regulator of cell polarity in the renal epithelia by inhibiting PtdIns(3,4,5)P3 and subsequently recruiting PtdIns(4,5)P2, Ezrin, F-actin and basal bodies to the apical membrane, and suggest a possible novel approach for treating human ciliopathies.

Z656 An unexpected influence of the extracellular matrix on cilia function in zebrafish. *E. K. LeMosy*, H. Neiswender, S. Navarre, D. J. Kozlowski. Augusta Univ., Augusta, GA.

Tinagl1 is a basement membrane protein conserved across animal species having three germ layers; mammals have an additional family member, Tinag. Genetic studies by others in fly and mouse models have not yielded a clear *in vivo* requirement for Tinagl1. Principally using MO knockdown in zebrafish, we observed defects consistent with a 'ciliopathy' spectrum, including small eye, body curvature, L/R randomization of heart looping, hydrocephalus, renal cysts, and craniofacial defects. New results with transient Crispr/Cas9 mosaic knockout and an ENU truncation mutant support heart looping, renal, and craniofacial defects, and mutant analysis is our current focus. With the MOs, we documented shorter and fewer cilia in Kupffer's vesicle and pronephric duct. Tinagl1 morphant pronephros and craniofacial defects were enhanced by co-injection with a Wnt MO but not control MO, and partially rescued by *tinagl1* mRNA; *ctnnb2* but not *egfp* mRNA was also demonstrated to partially rescue the pronephric cilia defect. These results are consistent with a Nusse lab demonstration that fruitfly Tinagl1 binds to Wg and is required for its function at a distance from secreting cells *in vitro*. Overall, our results support a hypothesis that, in zebrafish, Tinagl1 is broadly required for cilia function and may act via Wnt/beta-catenin signaling shown by others to function upstream of the motile cilia regulator *foxj1a* in pronephros and KV. We plan to test whether Tinagl1 can be linked to cilia regulation in mammalian cells, and whether it physically interacts with zebrafish Wnt(s) and activates downstream target genes or regulates PCP components in either model. Zebrafish may be a particularly favorable model for studying Tinagl1: it has only the *tinagl1* gene, so compensatory expression of Tinag is removed as an issue, yet it has good conservation of the organ systems in which Tinagl1 is highly expressed in mammals. In contrast, flies express modified cilia only in sperm and sensory neurons, and have more divergent organs, offering less scope for comparative studies and detection of context-specific effects. Interestingly, a zebrafish laminin mutant is reported to show short KV cilia able to initially establish L/R pattern, but later signal diffusion across the midline results in L/R randomization of abdominal organs. Mechanisms for basement membrane Tinagl1 and laminin roles in cilia regulation may involve signaling at focal adhesions in addition to promoting signaling of growth factors such as Wnts.

Z657 A High-Throughput workflow for CRISPR/Cas9 mediated targeted mutagenesis to model human disease genes in zebrafish . *Gaurav Varshney*[1], Wuhong Pei[1], Blake Carrington[1], Kevin Bishop[1], Erin Jimenez[1], Johan Ledin[2], Raman Sood[1], Shawn Burgess[1]. 1) National Human Genome Research Institute, NIH, Bethesda, MD; 2) Uppsala University, Uppsala, Sweden.

Advances in sequencing technologies have enabled the rapid identification of human disease genes by GWAS or whole exome sequencing techniques. There is a large-gap between identification of human disease genes and their functional validation. Numerous publications have demonstrated the efficacy of gene targeting in zebrafish using CRISPR/Cas9 including a variety of tools and methods for guide RNA synthesis and mutant identification. While all the published techniques work, not all approaches are readily scalable to increase throughput. In addition, zebrafish have been shown to effectively recapitulate disease phenotypes, however models that fail to generate relevant phenotypes are rarely reported. We recently described a CRISPR/Cas9 based high-throughput mutagenesis and phenotyping pipeline in zebrafish. Here we present a complete workflow including target selection, cloning-free single guide RNA (sgRNA) synthesis, microinjection, validation of the target-specific activity of the sgRNAs, founder screening to identify germline transmitting mutations, determination of the exact lesion by PCR and next generation sequencing (including software for analysis), and genotyping in the F_1 or subsequent generations. We used this high throughput pipeline to target all published human genes linked to non-syndromic deafness in zebrafish. We will present phenotyping data from homozygous mutants, and determine a rate of successful modeling in zebrafish.

Z658 Resource Construction at the China Zebrafish Resource Center. *Y. Sun*, L. Pan, K. Li, F. Xiong, X. Xie, Y. Zhang. China Zebrafish Resource Center, Institute of Hydrobiology, CAS, Wuhan, China.

In China, zebrafish was adopted in lab research in early 1990s, and the zebrafish research community has been rapidly growing since 2000. To meet the growing needs of zebrafish strains and technical support, in October 2012, the China Zebrafish Resource Center (CZRC, website: http://zfish.cn) was established at the Institute of Hydrobiology (IHB), Chinese Academy of Sciences (CAS) in Wuhan. After three years'

development, CZRC has maintained more than 1000 mutant and transgenic zebrafish lines, and a number of DNA plasmids and cell lines tillnow. All the fish lines provided by CZRC are tightly linked to the web information of Zebrafish Information Network (ZFIN). In addition to providing the regular fish resources, CZRC also provides transgenic service, knock-out service and technical training. In 2013, 40 labs in China started the Zebrafish All Gene Knock-out Project (ZKO Project) for Chromosome 1. The final goal of ZKO project is to knock-out all the 1333 genes on zebrafish chromosome 1 using CRISPR/Cas9 technology. Up to now, more than 900 genes have been successfully knocked out. CZRC is the project coordinator, and all mutant lines built in the project will be verified and preserved by CZRC and published as open resources to the whole research field. With the progress of ZKO project, CZRC will expand its fish stocks to 2000 lines by the end of 2016, and it is becoming one of the major resource centers in the global zebrafish community.

Z659 Phenome-scale screen defines post-embryonic gene function during the zebrafish larval-to-adult transition. *R. Fuentes*[1], A. Kugath[1], C. Nwaezeapu[1], G. Hu[1], T. Cooper[2], J. Fried[2], C. Scahill[4], J. Copper[2], K. Helde[3], C. Moens[3], K. Cheng[2], E. Busch-Nentwich[4], D. Stemple[4], S. Fisher[1], M. Mullins[1]. 1) University of Pennsylvania, Philadelphia, PA; 2) Penn State College of Medicine, Hershey, PA USA; 3) Fred Hutchinson Cancer Research Center, WA, USA; 4) Wellcome Trust Sanger Institute, Cambridge, UK.

With the dawn of the post-genomic era and the impact of the genomic revolution, knowledge of most of the encoded genes and their molecular nature has been revealed. However, knowledge of the *in vivo* functions for most of the genes remains incomplete. Thus, the study of phenotypes, or phenomics, has emerged as a genome-wide discipline to link gene sequence and function. Zebrafish has become an attractive and accessible vertebrate model system for phenomics, providing new insights into genotype-phenotype relationships and for modeling human disease. The Sanger Center has developed a high-throughput mutation identification approach to generate a large set of molecularly-defined mutant genes in the zebrafish. It has focused its phenotypic analysis on the embryonic to early larval period and has found that most null mutant alleles do not exhibit an apparent function during these early developmental stages. We have established a new phenotyping screen strategy to identify genes regulating later developmental periods and that act in the adult, as well as to identify human disease genes that typically manifest in the adult. We have analyzed more than 500 molecularly-defined gene knockouts in the zebrafish during late larval to adult stages. We have isolated numerous factors regulating post-embryonic stages, including male sterile, maternal-effect, late larval lethal and adult morphology genes, corresponding to several human disease genes. Our screen serves as the first step towards gaining mechanistic insights into the general structure of complex traits during post-embryonic developmental stages in vertebrates. Importantly, it will contribute to determining the zebrafish phenome during late larval, juvenile, and adult stages, including a collection of traits dispensable for reproduction, survival and body formation. Our studies provide a resource of phenomics data for future in-depth analysis of gene function by the scientific community.

Z660 Precise editing of the zebrafish genome by homologous recombination made simple and efficient. *K. Hoshijima*, M. Jurynec, D. Grunwald. University of Utah, Salt Lake City, UT.

Studies in zebrafish have made substantial contributions to our understanding of gene function in vertebrates. Large-scale genetic screens have led to discoveries of components and pathways underlying essential biological processes. More recently the imprecise repair of targeted DSBs induced by programmable nucleases has expanded use of the zebrafish further by providing a means for creating loss of function mutations affecting selected genes of interest. Importantly, the DSB lesions induced by the programmable nucleases stimulate both repair and recombination pathways, dramatically increasing the chance of integrating donor DNA sequences at the targeted locus. Indeed, a handful of reports have described successful integration of donor sequences at the targeted locus in zebrafish using different molecular configurations, *i.e.*, single-stranded oligodeoxynucleotides or double-stranded DNA (dsDNA), with or without homologous arms.

We have focused on sequence replacement or integration via homologous recombination between the host genome and exogenously supplied dsDNA as donor molecules. We found that digestion of donor ends with I-SceI enzyme in defined orientation enhances homologous recombination efficiency in zebrafish embryos. Under these conditions, several kilobase long stretches of sequence can be replaced efficiently. We used this approach to accomplish a variety of precise editing events, including: a single base substitution, in-frame integration of sequences encoding an epitope tag, and in-frame integration of reporter sequences accompanied by disruption of the targeted host gene. Taking advantage of the ability to integrate long donor dsDNA molecules, we also developed a method for efficiently isolating silent genome modifications, by incorporating a linked reporter within the donor sequences. Presence of the linked reporter, which can be excised subsequently by FLP/FRT-mediated recombination, makes it easy to recover and identify edited alleles. With these improvements, we created a conditional mutant in zebrafish in which a pair of loxP sequences flanked a target exon. The floxed allele maintains wild-type function but can be converted to a null mutation in the presence of Cre protein. Thus, genome editing mediated by programmable nucleases continues to advance our ability to perform genetic analysis in zebrafish.

C. elegans DEVELOPMENT, CELL BIOLOGY AND GENE EXPRESSION MEETING

Poster Session Abstracts

W4001A Unearthing Aneuploidy: A Study of the Influence of Double Strand Breaks on Oocytes in *Caenorhabditis elegans*. *F. Balmir*, J. Yanowitz. Magee Womens Research Institute, Pittsburgh, PA.

Chromosomal abnormalities in oocytes are a leading cause of miscarriage and genetic disorders of the fetus. A major underlying cause of these abnormalities is defects in meiotic crossover (CO) formation. To achieve COs, the topoisomerase-like enzyme, SPO-11, creates DNA double strand breaks (DSBs), allowing for repair of those breaks by homologous recombination. COs can be seen as chiasmata that help to correctly orient chromosomes on the spindle during metaphase I for proper segregation. Therefore, without DSBs and subsequent CO events, chromosomes risk missegregation. By understanding the components of DSB formation, we gain insight into events in recombination and the creation of COs with their associated chiasmata. Nevertheless, our understanding of the protein complexes that regulate DSB formation is lacking. *Caenorhabditis elegans* is ideal for the study of meiosis, DSBs, and aneuploidy given its ease of genetic manipulation and shared meiotic characteristics with women. By analyzing double mutants of genes involved in DSBs, we can build networks of the genetic interactions required for DSB formation. We have analyzed a matrix of interactions between partial loss-of-function alleles of genes involved in DSB formation in C. elegans. These include mutations in *lin-35, cep-1, dsb-2, rec-1, him-17, him-5, parg-1,* and *mre-11.* We then analyzed diakinesis oocytes by whole mount staining followed by confocal microscopy and 3D visualization. Assessment of achiasmate chromosomes has allowed us to determine synthetic interactions for CO formation in these sets of mutants. We also analyzed the effects of the mutations on brood size and frequency of males. Preliminary analysis suggests that the DSB genes fall into multiple groups that interact synergistically to impact break formation.

W4002B New complexities in ATM/ATR regulation in meiosis. *W. Li*[1,2], J. Yanowitz[2]. 1) School of Medicine of Tsinghua University, Beijing, China; 2) Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Ataxia-telangiectasia mutated (ATM) and RAD3-related (ATR) are key players in the mitotic DNA damage response (DDR) functioning to both recognize double-strand breaks (DSBs) and signal their presence to the cell. ATM and ATR have also more recently been shown to have a role in meiosis where they can influence the number of programmed DSBs that are made by the topoisomerase-like enzyme Spo11. In yeast and mammals, ATM acts as a negative regulator of Spo11-dependent DSB formation. By contrast, yeast ATR appears to function in a positive feedback loop for the formation of DSBs. We are interested in understanding how ATM and ATR work antagonistically to achieve a balance of meiotic DSBs. Efforts to further elucidate the *in vivo* roles of ATM and ATR in meiosis in mammals have been hampered by the fact that *atm-1* mutant mice are infertile, deficient in making mature gametes. Therefore we exploit the facile genetics and superb cytological of the nematode, *C. elegans* to probe the function of these genes in meiotic.

I have been investigating the roles of *C. elegans* ATM and ATR orthologs, ATM-1 and ATL-1, respectively, by analyzing the phenotypic consequences of *atm-1* and *atl-1* mutations and double mutants with components of the DSB machinery. I have been analyzing the effects of these mutations on COs formation through analysis of diakinesis-stage oocytes when chromosomes with COs condense into bivalent, cruciform structures. I also monitor how many DSBs are channeled into homologous recombination by assaying recruitment of RAD-51 to the chromatin by immunolocalization.

In my studies to date, we have found the surprising result that while *atm-1* mutants produce more DSBs during meiosis, as in other systems, fewer crossovers (COs) are formed. This finding suggests that ATM-1 not only regulates DSB formation but also influences downstream processing of these breaks. Two exciting possibilities are being explored: *atm-1* could modulate repair pathway choice, directing DSBs to CO outcomes; alternatively *atm-1* may function in a crossover checkpoint, preventing apoptosis if the correct level of DSBs is achieved. Further study will focus on determining the functions of *atm-1* and *atl-1* in formation of DSBs and their downstream processing.

W4003C NuRD Chromatin Remodelers Block Checkpoint Activation in the *C. elegans* Germ Line. *Sol Sloat*, Julia Rigothi, Carolyn Turcotte, Nicolas Andrews, Paula Checchi. Marist College, Poughkeepsie, MY.

In order to form viable gametes, double stranded breaks (DSBs) must be induced and correctly repaired during meiotic prophase I. Failure to correctly repair DSBs can result in defective gametes, which in humans, results in infertility, miscarriage, or chromosome disorders. We have found that in *C. elegans*, timely DSB repair is mediated by the Nucleosome Remodeling Deacetylase (NuRD) complex. Mutations in the genes encoding Chromodomain helicase DNA binding protein CHD-3 and its paralog LET-418 result in persisting DSBs and chromosome fragmentation that are coincident with increased germline apoptosis. Here, we investigate the genetic, cellular and molecular role of the NuRD chromatin remodeling complex and its relationship with meiotic checkpoint signaling in the *C. elegans* germ line. As in other organisms, checkpoint kinase 1 (CHK-1) is responsible for coordination of the DNA damage response (DDR) that leads to either cell cycle arrest, DNA repair, or apoptosis. We found that *let-418* mutants had higher levels of phosphorylated CHK-1 protein (pCHK-1) than wild type controls. These data are corroborated by cellular evidence in which pCHK-1 foci are detected in *let-418* germ cells using antibody staining, as well as genetic evidence demonstrating that knockdown of *chk-1* as well as its downstream effector *cep-1*(p53) suppress apoptosis in *let-418* mutants. Taken together, these results support a model wherein the NuRD complex machinery ensures error-free gamete formation by inhibiting meiotic checkpoints.

W4004A NuRD paralogs CHD-3/LET-418 promote meiotic double-stranded break repair In *C. elegans* . *Carolyn Turcotte*, Julia Rigothi, Erika Rosenkranse, Paula Checchi. Marist College, Poughkeepsie, NY.

Normal meiotic progression requires that programmed double stranded breaks (DSBs) are induced on all chromosomes. This is significant, as unrepaired DSBs lead to defective gametes, which manifest in severe problems such as miscarriages and chromosomal disorders such as Down syndrome. Accordingly, DSBs must be faithfully repaired. This is accomplished by a series of conserved mechanisms which include ATP-dependent chromatin modifiers, proteins that epigenetically alter DNA. We have demonstrated a role for the nucleosome remodeling (NuRD) complex in DSB repair, wherein the Chromodomain helicase DNA binding protein CHD-3 and its paralog LET-418 promote normal meiotic

progression and DSB repair in *C. elegans*. Loss of *chd-3* alone results in a significantly decreased brood size, and these defects are exacerbated when combined with a loss-of-function *let-418* allele. We discovered that the disruption of both paralogs also leads to chromosome fragmentation, which is indicative of faulty repair mechanisms. Using an antibody against the DSB marker RAD-51, we also found that DSBs abnormally persist in meiotic nuclei of *let-418* mutant germ lines. We are currently generating several strains which will help us to further understand the molecular nature of these defects. Taken together, these experiments will enable us to determine the role of the NuRD complex in the systematic repair of DSBs, which can be applied to other organisms such as humans.

W4005B SMRC-1, a putative annealing helicase, links chromatin regulation and DNA repair in the *C. elegans* germ line. *Bing Yang*, Xia Xu, Eleanor Maine. Syracuse University, Syracuse, NY.

Histones can receive many different modifications, and the presence/absence of certain modifications is implicated in transcriptional control and chromosomal events. One modification, histone H3 lysine 9 dimethylation (H3K9me2), is associated with unsynapsed chromatin during meiosis in many organisms, including *C. elegans* [1-3]. For example, in the male *C. elegans* germ line, the single X chromosome does not synapse and, during pachytene, is detected as a strong focus of H3K9me2 by immuno-labeling. In contrast, a much lower level of H3K9me2 staining is detected on synapsed autosomes. This interesting phenomenon led to the question of how H3K9me2 marks are specifically targeted to their genomic sites.

In order to address this question, we are identifying H3K9me2 methyltransferase (MET-2) interactors. To do so, we generated epitope-tagged *met-2* transgenes and antibody against MET-2. Using these reagents, we performed immunoprecipitation and tandem mass spec analysis to recover potential interactors. One candidate protein that we recovered is the *smrc-1* (C16A3.1) gene product. SMRC-1 is the *C. elegans* ortholog of mammalian SMARCAL1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein). Mammalian and *Drosophila* SMARCAL1 family members are annealing helicases that provide protection from DNA replication stress. Little is known about SMARCAL1 function in the germ line.

Using CRISPR-Cas9 methods, we generated *smrc-1* nonsense mutations and *flag*-tagged the endogenous *smrc-1* gene. We immunoprecipitated 3xFLAG::SMRC-1 and confirmed the interaction with MET-2. We evaluated 3xFLAG::SMRC-1 expression and observed that it localizes to mitotic and meiotic nuclei in both male and hermaphrodite germ lines. *smrc-1* nonsense mutations cause germline defects, including meiotic defects consistent with impaired recombination. Meiotic H3K9me2 is elevated and abnormally distributed in *smrc-1* males. *smrc-1* hermaphrodites exhibit maternal-effect embryonic lethality and, under conditions of temperature stress, exhibit a progressive reduction in brood size leading to complete infertility within several generations. We are investigating the relationships among SMRC-1 function, MET-2 function, and DNA repair.

References: (1) Shiu *et al.* (2001) Cell 107:905-916. (2) Kelly *et al.* (2002) Development 129:479-492. (3) Turner *et al.* (2005) Nat Genet 37:41-47.

W4006C Sex pheromones of *C. elegans* males potentiate the female reproductive system. *E. Zucker Aprison*, I. Ruvinsky. University of Chicago, Chicago, IL.

The *Caenorhabditis elegans* metabolome contains over a hundred ascaroside molecules. Most of them have no known function, or no function at all, but some act as pheromones. Two of these molecules, ascr#10 and ascr#3, are produced in different proportions by males and hermaphrodites. We report that when a hermaphrodite senses a male-specific mixture of these molecules, it changes several aspects of its reproductive physiology, including signaling that guides sperm toward oocytes. During evolution from an ancestor that had both males and females, *C. elegans* hermaphrodites lost several female-specific traits, but their reproductive system retained the ability to respond to male pheromones. This greatly aids them during recovery from heat stress. We suggest that serendipitous side benefits of female-specific traits could be a general cause of their retention during evolution.

W4007A Identifying a role for ETR-1 in *C. elegans* reproduction and germ line apoptosis. *Ruby Boateng*[1], Andy Golden[2], David Hall[3], Anna Allen[4]. 1) Howard University, Washington, DC; 2) NIDDK/NIH, Bethesda, MD; 3) Albert Einstein College of Medicine, Bronx, NY; 4) Howard University, Washington, DC.

In the *C. elegans* hermaphrodite germline, over half of the developing germ cells are eliminated through "physiological" germ cell apoptosis. This elimination is hypothesized to provide the surviving germ cells both the cytoplasmic nutrients and space required to undergo the growth necessitated to form a healthy, fertilization-competent oocyte. While this elimination involves the core apoptotic machinery of CED-9, CED-4 and CED-3, how this machinery is activated in "physiological" germ cell apoptosis remains unknown. Recently, we identified an unknown role for the ELAV-type RNA binding protein ETR-1 in germline apoptosis. This study aims to investigate the role of ETR-1 in both reproduction and "physiological" germ cell apoptosis.

ETR-1 is known to play a role in muscle development, as *etr-1(RNAi)* animals arrest at the two-fold stage of embryogenesis. Our fertility assays conducted in wild-type, a somatic defective RNAi mutant *(rrf-1(pk1417))* and a germline defective RNAi mutants *(ppw-1(pk1425))* implicate a reproductive role of ETR-1. *etr-1(RNAi)* animals have a reduced brood size compared to control animals in all three mutant lines tested. Additionally, the ability of ETR-1 depletion to suppress the published WEE-1.3-depletion infertility phenotype is dependent on ETR-1 being depleted in the soma. While investigating the cause of the reduced brood size of *etr-1(RNAi)* animals, we observed an increase in the number of germ cells undergoing apoptosis.

We utilized Transmission Electron Microscopy (TEM) to investigate the germlines of ETR-1-depleted animals. TEM confirms the increase in apoptotic germ cells and reveals significant defects in the structure of the somatic gonadal sheath cells encasing the germline compared to controls. Since the gonadal sheath cells are involved in the engulfment of apoptotic germ cells, and we had previously localized ETR-1 to the

sheath, this implies a defect in the engulfment of dying germ cells. We will demonstrate that ETR-1 depletion in *ced-1(e1735)* engulfment mutants rescues the *etr-1(RNAi)* reduced brood size, and co-depletion of *ced-1(RNAi)* and *etr-1(RNAi)* suppresses the increase in the number of apoptotic bodies observed in *etr-1(RNAi)* animals.

Combined this data reveals a novel role for ETR-1 in reproduction and "physiological" germ cell apoptosis, potentially in the process of engulfment. These studies will increase our understanding of the apoptotic pathway and how ETR-1, a highly conserved CELF/BRUNOL protein, influences an organism's reproductive capability.

W4008B Developmental consequences of the inappropriate transgenerational inheritance of histone methylation in *spr-5;met-2* mutant worms. *B. Carpenter*, D. Katz. Emory University, Atlanta, GA.

Reestablishing the epigenetic ground state of the *Caenorhabditis elegans* (*C. elegans*) zygote is required for normal development and ensures that information is transferred properly from one generation to the next. Two epigenetic enzymes, the H3K4me2 demethylase, SPR-5, and the H3K9 methyltransferase, MET-2, are maternally deposited into the oocyte and cooperate to reestablish the epigenetic ground state by modifying histone methylation. Progeny of worms lacking *spr-5* and *met-2* rapidly accumulate H3K4me2 resulting in somatic expression of spermatogenesis genes and sterility, while worms that lack both SPR-5 and MET-2 exhibit a maternal effect sterile phenotype. Compared to Wildtype, these double mutants display multiple developmental defects including, developmental delay, perturbed vulva formation, and defects in oogenesis. Despite this, the mechanisms by which SPR-5 and MET-2 transgenerationally regulate development is unclear. Here, we show that *spr-5;met-2* mutant worm progeny exhibit a severe L2 developmental delay in both the germline and soma. This phenotype hints that there could be feedback between germline and soma. In addition, we discuss experimental approaches to examine the mechanisms by which SPR-5 and MET-2 regulate cell fate decisions during *C. elegans* development.

W4009C SAMP-1 Regulates Chromosome Segregation in *C. elegans* Spermatogenesis. *A. J. Deshong*, A. Kuzmanov, J. Engebrecht. University of California Davis, Davis, CA.

Meiosis is the specialized form of cell division in which a single DNA replication event is followed by two successive rounds of chromosome segregation to produce haploid gametes. Errors in meiosis can cause aneuploidy, a major cause of both miscarriage and genetic developmental disorders in humans. The meiotic program must be differentially regulated between males and females to generate sperm and eggs. The highly conserved protein, SAMP-1, is required for regulating chromosome segregation during *C. elegans* spermatogenesis, but not oogenesis. *samp-1(tm2710)* mutant hermaphrodites show complete sterility. However, sterility of the *samp-1(tm2710)* mutants was rescued by providing wild-type sperm, suggesting the sterility phenotype is largely sperm-dependent. SAMP-1 is a 588 amino acid predicted transmembrane protein embedded in the inner nuclear membrane. By immunofluorescence, SAMP-1 is detected at the nuclear periphery in the germ lines of both males and hermaphrodites using an antibody generated against amino acids 338-555. Interestingly, SAMP-1 undergoes a dynamic shift in localization during male meiosis. At the end of prophase I in the male germ line, SAMP-1 begins to localize in the nucleoplasm and by metaphase I becomes concentrated on bivalent chromosomes at the interface between homologs. Preliminary evidence suggests that this change in localization is regulated by proteolytic cleavage. We are using CRISPR gene editing techniques to fluorescently tag SAMP-1 at the N-terminus, C-terminus, and an internal site to determine the mechanisms underlying dynamic localization in the male germ line. Lastly *samp-1(tm2710)* mutant males exhibit defects in meiotic spindle formation. To monitor these highly dynamic processes, we are using live-cell imaging to capture meiotic cell division in real time to characterize the dynamics of the defects that are observed in *samp-1(tm2710)* mutant males. *samp-1(tm2710)* mutant males show a hypomorphic phenotype during anaphase I of meiosis in which severe segregation defects are present in 66% of observed divisions. Defects include shortened metaphase spindles, multiple lagging chromosomes, delayed anaphase, and inappropriate centrosome separation. Interestingly, in the observed "normal" divisions in *samp-1(tm2710)* mutant males which lack the above defects, anaphase I occurred faster than in wild type. Severe anaphase defects suggest that SAMP-1 is playing a role in regulating cohesion release and/or kinetochore attachments.

W4010A Sex specific differences in *C. elegans* meiosis. *S. Fielder*, W. G. Kelly. Emory University, Atlanta, GA.

Meiotic synapsis is a conserved process that is required for proper segregation of homologous chromosomes in both oogenesis and spermatogenesis. In early prophase I, homolog sets are connected together by a bridge-like complex of synapsis proteins that keep them in close proximity to facilitate crossing over. If a chromosome is unable to pair and synapse, such as the lone X in *C. elegans* males, it is targeted for heterochromatin assembly in a process called meiotic silencing. This process has been observed in numerous organisms, including in mammals. I have observed in *C. elegans*, meiotic silencing, measured by histone H3 lysine 9 dimethylation accumulation (H3K9me2), occurs in both male and hermaphrodite mutants that are unable to pair and synapse one or two sets of homologs (e.g., *zim-2* mutants, which are unable to pair chromosome V). Consistent with this trend, male mutants that completely lack synapsis show accumulation of H3K9me2 on all chromosomes. However, this pattern is strikingly different in hermaphrodites that lack synapsis: they do not show any increased accumulation of H3K9me2. This indicates that there may be a previously undescribed checkpoint that recognizes a failure to initiate synapsis and bypasses meiotic silencing in hermaphrodites. The global H3K9me2 enrichment in males indicates that this proposed checkpoint is sex specific or regulated differently in males. Analysis of this sex specific difference will be presented.

W4011B Identification of Genes that Regulate the Activation of *C. elegans* Sperm. *A. Greer*, D. Chavez, T. Shimko, B. Philpot, A. Duffy, K. Mylroie, G. Stanfield. University of Utah, Salt Lake City, UT.

Nematode sperm become competent for motility and fertilization during a process termed sperm activation, which is regulated by specific extracellular signals in males and hermaphrodites. While hermaphrodites create their own sperm during their last larval stage and store it for

future use, adult males continually create sperm, which activate when the sperm is transferred to a hermaphrodite. Furthermore, hermaphrodites and male sperm activation pathways differ from each other. Hermaphrodite sperm activation requires the activity of a set of sperm genes termed the *spe-8* group, which mediate the response to extracellular zinc. The pathway by which male sperm become active involves a signaling pathway including *swm-1*, *try-5*, and *snf-10*. SWM-1 is a serine protease inhibitor. When it is absent, sperm is prematurely activated within the male gonad and is rarely transferred to hermaphrodites. TRY-5 is the next protein in the sperm activation cascade and is a seminal fluid serine protease. When TRY-5 is absent, males do not transfer activator, though they are still fertile due to activation by hermaphrodite signals. The protein SNF-10, an SLC6 transporter, is found in the plasma membrane of sperm and is required for activation of male sperm by TRY-5. To identify additional genes within the sperm activation cascade, the Swm-1 activated-sperm phenotype was used as the basis of a genetic suppressor screen.

Analysis of mutants from the *swm-1* suppressor screen suggests that many genes are involved in sperm activation. One goal of this project is to characterize the phenotype of suppressor mutants. First, we are using hermaphrodite brood counts to measure the fertility of each mutant. We find that multiple male pathway mutants have decreased fertility, unlike the previous characterized mutants in this class. We are also performing experiments to distinguish between potential roles in sperm vs. upstream of the TRY-5 protease. We are using in vitro activation assays to test if any of these genes functions in sperm or is needed for response to the protease signaling pathway. We are also examining the effect of these mutations on localization or transfer of TRY-5::GFP. A second current goal of this project is to identify two of these genes, which we have shown to represent two novel regulators in the male (*jn19*) and hermaphrodite (*jn15*) pathways. We mapped and sequenced these mutants. Prioritizing candidate genes according to preexisting data for sperm-enriched gene expression, we are using CRISPR to generate candidate gene deletions that may mimic the *jn19* and *jn15* phenotypes. These experiments will provide insight into protease signaling, which is important for cell motility as well as other processes.

W4012C Maternal diet influences intergenerational phenotypic plasticity affecting progeny size and starvation resistance. *Jonathan Hibshman*, Anthony Hung, Ryan Baugh. Duke University, Durham, NC.

Dietary restriction (DR) delays aging but reduces fertility. We demonstrate maternal effects of DR in the roundworm *C. elegans*, suggesting physiological regulation of maternal provisioning. Compared to being fed *ad libitum* (AL), worms cultured under DR are smaller, delay reproduction, and produce fewer but larger progeny. Furthermore, progeny of DR worms are buffered from advearse consequences of early-larval starvation: They grow faster and are more fertile than control progeny following extended starvation, suggesting a fitness advantage. Nutrient availability is assessed during oogenesis, in late larvae and young adults, rather than affecting a set point in young larvae. Insulin-like signaling mediates effects of nutrient availability on progeny size. Reduced signaling through the insulin-like receptor *daf-2*/InsR in the soma causes constitutively large progeny, and its effector *daf-16/*FoxO is required for this effect. Additional regulators of energy homeostasis including the nuclear hormone receptor *nhr-49/*Hnf4*, pha-4/*FoxA, and *skn-1/*Nrf are also required for progeny size plasticity in response to diet. Disruption of *daf-2*/InsR does not require *nhr-49*/Hnf4, *pha-4*/FoxA, or *skn-1*/Nrf for increased embryo size, suggesting they act upstream or in parallel to insulin-like signaling. This work presents an invertebrate model for maternal effects of DR, demonstrates potentially adaptive intergenerational phenotypic plasticity, and identifies conserved pathways mediating these effects.

W4013A TGFβ and prostaglandin synthesis in *C. elegans*: linking environmental cues to sperm motility function. *Muhan Hu*, Michael Miller. University of Alabama at Birmingham, Birmingham, AL.

Sexual reproduction is critical for maintaining population diversity and species survival. Environmental exposures are thought to influence animal fertility, but the underlying mechanisms are not well understood. Fertilization depends on sperm guidance mechanisms for locating maturing oocytes within the oviduct. The signaling pathways that mediate sperm guidance have been well studied in marine species, but less is known in internally fertilizing animals, such as *C. elegans*. We have shown that *C. elegans* oocytes synthesize specific classes of F-series prostaglandins (PGFs) that guide migrating sperm toward them. These PGFs are synthesized via an unknown and possibly evolutionarily conserved metabolic pathway. The DAF-7 TGFβ homologue acts in ASI sensory neurons, downstream of ascaroside pheromones, to modulate oocyte PGF synthesis, linking environmental perception to sperm performance. Loss of the DAF-1 type I TGFβ receptor causes sperm guidance defects and decreased PGF synthesis. Here we show that the PGF synthesis phenotype can be suppressed by *daf-3* co-SMAD loss. DAF-3 activity is required in part within the germ line, suggesting DAF-3 transcriptional targets in oocyte precursors modulate PGF metabolism. To identify these TGFβ targets, we conducted RNA sequencing analyses of wild type, *daf-1* mutant, and *daf-1;daf-3* mutant hermaphrodites. Currently, RNAi screens are being used to test whether the TGFβ target (direct or indirect) genes play roles in PGF metabolism. Genome-editing strategies are ongoing to investigate how TGFβ signals are transduced to the germ line. Mass spectrometry approaches are being developed to help identify biochemical steps mediated by TGFβ. These efforts are providing insight into the molecular mechanisms of fertilization and the link between environmental factors and sperm function.

W4014B Characterization and identification of new genes required for sperm activation in *C. elegans*. *A. R. Krauchunas*[1], E. Mendez[1], D. Chen[1], J. Parry[1], J. Ni[1], S. Gu[1], G. Stanfield[2], A. Singson[1]. 1) Rutgers, The State University of New Jersey, Piscataway, NJ; 2) University of Utah, Salt Lake City, UT.

Successful fertilization requires that sperm are activated prior to contacting an egg. In *C. elegans*, this activation process, called spermiogenesis, transforms round immobile spermatids into motile, fertilization-competent spermatozoa. Spermiogenesis in males uses the TRY-5 protease pathway, whereas in hermaphrodites it is controlled by the SPE-8 pathway. Here we describe the characterization of two new spermiogenesis-defective mutants. We show that *spe-43* is a new component of the SPE-8 pathway; *spe-43* hermaphrodites are self-sterile, while *spe-43* males show wild-type fertility. Consistent with other members of the *spe-8* class, *spe-43* hermaphrodite sperm can be trans-

activated by male seminal fluid.When *spe-43* spermatids are exposed to Pronaseto activate the sperm *in vitro*, they form long rigid spikes radiating outward from the cell periphery instead of forming a motile pseudopod confirming a defect in spermiogenesis.Using a combination of recombinant and deletion mapping and whole genome sequencing we identified a C>T mutation in F09E8.1 as the molecular lesion in *spe-43(eb63)*. A second allele *spe-43(jn5)* allows us to conclude that although the *spe-43* transcript exists in two isoforms, only the isoform predicted to encode a transmembrane domain and a large extracellular domain is necessary for its function in spermiogenesis.Unlike *spe-43*, our second mutant, *as41,* affects spermiogenesis in both sexes, suggesting that the mutated gene may be downstream of both the TRY-5 and SPE-8 pathways. Spermatids from *as41*mutants show no signs of *in vitro* activation in the presence of Pronase, failing to even form spikes. We are currently in the process of using a similar whole genome sequencing strategy to identify the molecular nature of *as41.*

W4015C A calcineurin-interacting protein regulates ovulation and male mating in C.elegans. *Sun-Kyung Lee*, Hana Jung, Joohong Ahnn. Hanyang University, Seoul, KR.

Substrate candidates for calcineurin, a serine/threonine phosphatase, have been screened by yeast two-hybrid assays. We found that a 85 kDa basic protein interacted with the catalytic domain of TAX-6. The calcineurin interacting protein, cnp-2, is expressed in head neurons, posterior intestine, spermatheca-uterus valve in hermaphrodites, and in spicule retractor neurons, rays and at the end of fan, which are mating behavior-mediated organs, in males. cnp-2 is required in the ovulation, arrangement of eggs, ray structure and mating behaviors, and is critical in the mating efficiency and sperm activation. Expression of IP3 signaling molecules was significantly decreased in hermaphrodite reproductory organs of *cnp-2* mutants, which suggest that IP3 signaling is suppressed when cnp-2 was absent. We are currently further investigating functions of cnp-2 on reproductive process.

W4016A Identification and Characterization of Genes Essential for *C. Elegans* Sperm Guidance. *S. B. Legg*, M. A. Miller. University of Alabama at Birmingham, Birmingham, Alabama.

Fertilization is the fusion of sperm and oocyte to initiate embryonic development. It can occur internally or externally, depending on the animal species. In internally fertilizing animals, sperm must locate freshly ovulated oocytes within the convoluted architecture of the female reproductive tract. These sperm guidance mechanisms are not well understood. Our lab has shown that *C. elegans* oocytes secrete F-series prostaglandins (PGFs) to guide sperm to the spermatheca. These PGFs are synthesized independent of the canonical cyclooxygenase cascade, which catalyzes PGF synthesis in mammals. We have recently shown that cyclooxygenase null mice and worms produce similar PGF isomers, suggesting that a second PGF enzymatic pathway exists. Moreover, we have identified these PGF isomers in human ovarian follicular fluid. The goal of my project is to identify genes critical for *C. elegans* sperm guidance and in particular, PGF metabolism.

In order to identify genes essential for sperm guidance in *C. elegans*, we have devised an *in vivo* screening method using RNAi and a one hour sperm guidance assay. *C. elegans* oocytes synthesize sperm-guiding PGFs from PUFA precursors provided in yolk, so the screen will focus on genes expressed in the adult germ line (Reinke et al., 2004). N2 hermaphrodites are administered RNAi by the feeding method. L4 stage worms are added to the plates and kept at 25ºC for 36-48 hours. RNAi hermaphrodites are anesthetized and incubated with wild-type males stained with MitoTracker Red CMXRos. Mated hermaphrodites are removed from males and allowed to rest for an hour. Fluorescent sperm distribution within the uterus is viewed on plates using a stereomicroscope. In controls, sperm are observed in two foci roughly equidistant to the vulva, corresponding to the two spermathecae. Deficiency in PGF synthesis causes more uniform sperm distribution throughout the uterus. This strategy permits screening of about 50-100 RNAi clones per month. My project focuses on screening 266 germline genes on Chromosome II. Out of 75 RNAi clones screened to date, I have identified 10 clones that caused strong sperm guidance defects, and 11 clones that caused moderate defects. These RNAi clones correspond to genes implicated in diverse functions, including receptor-mediated endocytosis, protein secretion, calcium ion binding, and lipid storage. For selected positive clones, null mutations will be generated using CRISPR/Cas9. Transgenic and genetic mosaic experiments will be used to determine where these genes are expressed and function. Liquid chromatography tandem mass spectrometry methods will be used to determine if the genes modulate PGF metabolism.

W4017B PP1α phosphatase GSP-2 regulates meiotic chromosome segregation during spermatogenesis in *C. elegans*. *Yi-Hsiu Lin*[1], Jui-Ching Wu[1,2]. 1) Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei, Taiwan; 2) Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan.

During spermatogenesis, it is crucial to properly partition duplicated chromosomes into four haploid cells with two consecutive divisions. It is unclear if the two chromosome segregation events are regulated by the same mechanism. Previously it is shown that two male-specific PP1 phosphatases are specifically required for chromosome segregation in meiosis II, suggesting the two chromosome segregation events, which separate homologs and sisters respectively, are regulated by different mechanisms. Here we investigate the roles of GSP-1 and GSP-2, the *C. elegans* homologs of the ubiquitously expressed PP1β and PP1α, in male meiotic divisions. Interestingly, despite high sequence identity, *gsp-1* and *gsp-2* mutant male worms show very different effects in spermatogenesis. Although *gsp-1* mutant hermaphrodites are sterile, males lacking *gsp-1* are completely fertile and show normal progression of sperm meiotic divisions in our analyses, indicating GSP-1 primarily functions in oocyte production. Contrarily, male worms lacking *gsp-2* show high penetrance of male infertility. Examination of mature sperm revealed high percentage of sperm with abnormal nuclei and variable sizes produced by *gsp-2* mutant males. By immunofluorescence staining, *gsp-2* mutant male gonads exhibited a variety of abnormal chromosome morphology at division zones, indicating chromosome segregation defects during male meiosis. To further investigate the defects, we examined the progression of male meiotic chromosome segregation in live *gsp-2* mutant males. In the absence of GSP-2, the chromosomes fail to be organized at the center of the cell, causing a delay of anaphase I onset. Furthermore, during anaphase I, the chromosomes are either separated rapidly without typical dumbbell-shape arrangement, or at a reduced speed with lagging chromosomes. During the second division, onset of anaphase II is also delayed and chromosome bridges were

detected during separation. These results suggest GSP-2 is required for proper orientation of chromosomes during both meiotic divisions. In addition to defects in chromosome separation, we found paired chromosomes are often disengaged into multiple chromosomal bodies in *gsp-2* mutant spermatocytes during meiosis I, indicating premature cohesion removal during first meiotic division. We reasoned GSP-2 might be required for protecting sister chromatid cohesin by restricting the localization of Aurora B kinase AIR-2 as described in oocytes. Consistent with this, we found AIR-2 is spread to the sister chromatids in the absence of GSP-2. These results suggest GSP-2 antagonizes AIR-2 function to regulate timely cohesin removal during male meiosis as in female meiosis. Taken together, we conclude that GSP-2 PP1 phosphatase is required for both chromosome orientation as well as stepwise chromosomal cohesion removal during male meiotic divisions.

W4018C PAM-1, the C. elegans ortholog of the puromycin sensitive aminopeptidase, and autophagy pathways genetically collaborate to regulate gametogenesis. *A. Munie*, A. Cude, C. Trzepacz. Murray State University, Murray, KY.

Autophagy, the cell's recycling system, is a highly conserved and regulated biological process. Autophagy normally functions as a housekeeping role and is utilized to remove any damaged, malfunctioning, or unnecessary organelles, but can be stimulated in times of cellular stress. Starvation, hypoxia, and the accumulation of cytotoxic aggregates, such as those linked to neurodegenerative disorders such as Huntington's and Alzheimer's disease, are all remediated by autophagic mechanisms. Studies in a number of model organisms, including mice, fruit flies, and nematodes have identified numerous genes involved in mediating autophagy, including the Puromycin-sensitive aminopeptidase (*Psa*). The *Caenorhabditis elegans* orthologue of *Psa, pam-1*, governs fertility in the nematode. The gonads of adult worms harboring mutant *pam-1* alleles display several pheotypes indicating compromised worm gametogenesis, including a decreased brood size, increased embryonic lethality, and an expansion of the population of immature pachytene stage germinal nuclei toward the proximal end of the gonad. Because PAM-1 also functions in autophagic pathways, we examined the reciprocal involvement of autophagy in gametogenesis.

RNAi was employed to independently inhibit the expression of five conserved *C. elegans* autophagy genes: *ATG5/bec-1, ATG8/lgg-1. ATG7/atg-7, ATG18/atg-18,* and *VPS-34/vps-34*, in both wild-type N2 and *pam-1* strains, and adult animals were examined for changes in *pam-1*-influenced phenotype metrics. Inhibition of *vps-34* in N2 animals had a statistically significant effect on pachytene expansion; RNAi of the remaining autophagy genes had no discernible impact on the N2 aniamls. However, the independent inhibition of each of the autophagy genes in mutant *pam-1* animals results in a synergistic exacerbation of multiple fertility metrics, most consistently an expansion of the pachytene population. We are currently employing fluorescently-labelled transgenes to examine the colocalization of PAM-1 and the autophagy machinery in the gonad. Our data suggests a novel collaboration of PAM-1. and autophagy functions in mediating meiotic transitions and fertility in *C. elegans*.

W4019A Screening of compounds that can dissect the *C. elegans* spermiogenesis pathway. *H. Nishimura*[1], S. Nakamura[1], T. Tajima[1], K. Nakanishi[1], F. Ogawa[2], M. Omote[2]. 1) Department of Life Science, Setsunan University, Neyagawa, Osaka, JP; 2) Department of Pharmaceutical Sciences, Setsunan University, Hirakata, Osaka, JP.

For sexual reproduction in most animal species, spermiogenesis is an essential, post-meiotic event in which spermatids transform into spermatozoa. During *C. elegans* spermiogenesis, pseudopods extend from spermatids, and membranous organelles (MOs) are fused with the spermatid plasma membrane. *C. elegans* is so useful for studying spermiogenesis; in addition to easy availability of various genetic tools, we can use *in vitro* spermiogenesis system in a simple, chemically defined medium. This advantage allows us to use compounds that can directly control spermatid activation into spermatozoa to dissect the *C. elegans* spermiogenesis pathway.

Based on microscopic observation of Pronase-induced *in vitro* spermiogenesis, we screened a library of compounds with diverse structures. One compound, named DDI-1, blocked pseudopod extension, but not MO fusion with the spermatid plasma membrane (IC_{50} = ~30 µM). Since DDI-1 did not affect the proteolytic activity of Pronase towards FITC-casein, the compound seemed to target a spermatid protein(s) that is involved in pseudopod extension.

Moreover, we examined brood sizes after incubation of L4 hermaphrodites with DDI-1 at 20ºC for 48 h in liquid culture. The compound-treated worms, unexpectedly, produced F1 progeny ~50% more than control worms. As spermatids were released from DDI-1-treated males and then activated with Pronase, spermiogenesis normally occurred. These data suggest that, in liquid culture, DDI-1 probably does not reach spermatids present within the male gonads and that this compound has an additional effect(s) on reproduction besides blocking spermiogenesis.

Currently we are producing a series of DDI-1 derivatives to identify its target(s) and keeping to screen the same library to obtain more compounds that affect spermiogenesis.

W4020B A role for sperm-gonad signaling in competition for reproductive success. *G. M. Stanfield*, J. M. Hansen. University of Utah, Salt Lake City, UT.

In *C. elegans*, fertilization occurs only within the spermathecae, specialized regions of the hermaphrodite gonad in which newly ovulated oocytes interact with stored sperm. Thus, a key determinant of male reproductive success is the ability of sperm cells to be present within the spermathecae at the time of ovulation. We have found that the divergent kinase COMP-1 functions in sperm to promote their residence within the spermathecae. Surprisingly, we detect only subtle defects in the directional migration of mutant sperm. Instead, *comp-1* sperm accumulate just outside the spermathecae, and largely fail to gain entry until self sperm are depleted. Both sperm usage and localization defects in *comp-1* mutants are limited to competitive contexts, raising the question of how sperm signal to other sperm to influence their function. Our data suggest that rather than interacting directly, sperm resident within the sperm storage organ may signal through the female gonad. Bidirectional communication between sperm and the female gonad is known to be used to recruit sperm to migrate toward oocytes and to couple ovulation to the presence of sperm. We find that a subset of these signaling pathways also influence sperm competition, and that wild-type and *comp-1*

sperm respond differentially to perturbations of the signaling environment. For example, wild-type sperm, unlike those of *comp-1*, show defects within older hermaphrodites that have depleted their sperm. While oocyte-derived prostaglandins promote migration of wild-type sperm, prostaglandins appear to inhibit *comp-1* mutant sperm. Mutants for *spe-9* generate sperm that localize correctly but fail to fertilize oocytes; when *spe-9* sperm is present in the spermathecae, *comp-1* sperm migration is rescued, but wild-type sperm migration is perturbed. Thus, it seems that sperm signal their presence using a mechanism distinct from release of MSP, but potentially involving *spe-9*. In addition to analyzing known signaling pathways, we are testing whether different substrates for migration, such as fertilized eggs vs. unfertilized oocytes, are differentially permissive for sperm migration or affect the outcome of competition.

W4021C Are all hermaphroditic nematodes like *C. elegans*? Lessons from *Rhabditis* hermaphroditic nematodes that produce sperm and oocytes simultaneously. *XIAOXUE LIN*, Caitlin McCaig. College of William and Mary, Williamsburg, VA.

On multiple separate occasions, ancestral male/female nematode species have convergently evolved self-fertile hermaphroditism. In *C. elegans,* hermaphrodites achieve self-fertility by producing sperm as larval L4s, storing their sperm, and then making a one-time switch to oogenesis. Here we report that hermaphrodites of the same family but in the *Rhabditis* clade, specifically *Rhabditis* sp. SB347, JU1782, and JU1783, achieve self-fertility by continuously producing both sperm and oocytes from L4 and throughout the adult lifespan.

In the hermaphrodite germlines of these male/female/hermaphrodite *Rhabditis* species, we find distinct clusters of cells alongside the developing oocytes. In contrast, these cell clusters are not present in females, which implies their sperm-related function. The number of cells per cluster increases in a distal to proximal fashion most often in a pattern of 1, 2, 4, or 8 cells. In addition, many clusters contain dividing cells with tubulin spindles, suggesting that they proliferate along the germline. Immunocytochemistry shows that the expression of major sperm protein (MSP) is found only in the late clusters (often in the vicinity of the -1 to -3 oocyte). Our analysis suggests that the early cell clusters are mitotic progenitors of meiotic spermatocytes in the late clusters. The mitotic, undifferentiated character of the progenitor cells leads us to conclude that they are analogous to the recently clarified transit amplifying (TA) cells in the *C. elegans* mitotic region. However, unlike in *C. elegans*, the presumed TA cells in *Rhabditis* hermaphrodites exist in isolated clusters, physically distant from both the stem cell niche and other cell clusters. Such mitotic cluster formation presents a unique opportunity to study TA cell development. Ongoing studies of this divergent mechanism of spermatogenesis are characterizing both protein and RNA expression of conserved regulators of the cell cycle and the germ cell differentiation. By understanding the cellular and genetic pathways in non-*C. elegans* nematode species, we hope to elucidate germ cell development and differentiation in a novel evolutionary context.

W4022A Characterization of the germline stem cell niche in *C. elegans* males. *S. L. Crittenden*[1,2], C. H. Lee[1,2], I. Mohanty[1], J. Kimble[1,2]. 1) University of Wisconsin-Madison, Madison, WI; 2) HHMI.

In *C. elegans*, the distal tip cell (DTC) niche maintains germline stem cells (GSCs) in both sexes. In addition, the same GSC regulators are used in both sexes (GLP-1/Notch signaling, the LST-1and SYGL-1 downstream effectors and FBF RNA-binding proteins). Yet, there are differences between the sexes. Hermaphrodites have a single DTC while males have two, and DTC expression of GLP-1/Notch ligands differs (1). Male germ cells have a faster cell cycle than hermaphrodites, and the male progenitor zone is longer (2).

We used a GFP marker to examine adult male DTC architecture. We find that each DTC has a cap region extending over 3-4 cell rows, similar to the extent of the cap in the single hermaphrodite DTC (3). The male DTCs also form a plexus of processes that intercalate between germ cells. Yet the male plexus is longer than the hermaphrodite plexus.

We examined the transcriptional response to GLP-1/Notch signaling in male germ cells with single molecule FISH to *sygl-1* (4). As in hermaphrodites (4,5), *sygl-1* transcripts are spatially restricted to the distal progenitor zone. Preliminary results indicate that *sygl-1* transcripts extend further proximally in males than in hermaphrodites. Using the *emb-30* assay that revealed a distal pool of undifferentiated germ cells in hermaphrodites (6), we find that males have an expanded distal pool, which correlates with the expanded *sygl-1* transcriptional response.

(1) Chesney et al., Developmental Biology 331 (2009).
(2) Morgan et al., Developmental Biology 346 (2010).
(3) Byrd et al., PLoS ONE 9 (2014).
(4) Kershner et al., PNAS 111 (2014).
(5) Kimble et al. abstract this meeting.
(6) Cinquin et al., PNAS 107 (2010).

W4023B Molecular and genetic analysis of the *ego-3* gene reveals a critical role for HSP90 in GLP-1/Notch signaling in the *C. elegans* germline. *J. Lissemore*[1], M. Edgley[2], S. Flibotte[2], J. Taylor[2], V. Au[2], E. Connors[3], Y. Liu[3], L. Qiao[3], B. Yang[3], O. Zucaro[1], J. Krach[1], M. Lee[1], D. Moerman[2], E. Maine[3]. 1) John Carroll University, University Heights, OH; 2) Dept. of Zoology, University of British Columbia; 3) Biology Dept., Syracuse University.

glp-1 encodes a Notch family transmembrane receptor required for mitotic proliferation of the *C. elegans* germline. Mutations in *glp-1* lead to reduced germline proliferation, with strong loss-of-function mutations rendering hermaphrodites sterile. *ego-3* was identified in a screen for genetic enhancers of the mild proliferation defect in *glp-1(bn18ts)* mutants raised at 20°C. The screen was designed to identify mutations that decrease GLP-1/NOTCH signaling in the germline. The best studied allele of *ego-3, ego-3(om40)*, has a complex recessive phenotype that includes reduced and delayed larval germline proliferation, slow development characterized by extended larval period, proximal germline proliferation in adults, delayed and abnormal gamete formation, and a severe mobility defect that improves to nearly wildtype mobility in adults. Another allele obtained by non-complementation, *ego-3(om118)*, has a recessive embryonic lethal phenotype. To identify *ego-3(om40)*, we first carried out three-factor and SNP mapping, which placed *ego-3* on chromosome VR, to the left of *unc-61* and close to *daf-21*, in a region

containing 14 known protein-coding genes. We next conducted whole genome sequencing (WGS) on homozygous *ego-3(om40)* mutants. WGS identified one sequence variant within the protein coding region of one gene in the mapped region, *daf-21*, which encodes the *C. elegans* ortholog of the molecular chaperone HSP90. The sequence variant is a T>A transition causing an I>N non-conservative missense mutation. Sanger sequencing confirmed this mutation; subsequently, we identified the *ego-3(om118)* mutation, a 20 bp non-tandem duplication within the same exon containing the *ego-3(om40)* mutation. *ego-3(om118)* is predicted to cause a frameshift and introduce a premature termination codon. *ego-3(om40)* fails to complement *daf-21(ok1333)*, a putative null allele carrying a large deletion. *daf-21* RNAi partially phenocopies *ego-3* in a wildtype background, causing sterility, embryonic lethality, reduced germline proliferation in the larva, and, in some adults, proximal germline proliferation. Thus, by various genetic and molecular criteria, we have confirmed the identity of *ego-3* as *daf-21*. While HSP90 has long been known to participate in a variety of oncogenic, proliferative signaling pathways in metazoans, our results provide the first evidence that HSP90 plays a role in GLP-1/NOTCH signaling and in mitotic proliferation in the *C. elegans* germline.

W4024C Investigating the remodeling of *C. elegans* primordial germ cells into germline stem cells. *C. Maniscalco*, J. Nance. NYU School of Medicine, New York, NY.

Germ cells allow us to transmit our genome to future generations. The embryonic precursors of germ cells, called primordial germ cells (PGCs), are set aside early during development and are subjected to unique regulation, such as transcriptional repression, so that differentiation does not occur. In order to produce gametes, quiescent PGCs must activate and transition into dividing germline stem cells (GSCs). The mechanism of this transition, however, is poorly understood. The two embryonic PGCs in *C. elegans* extend large cytoplasm-filled lobes into the surrounding endoderm; lobes and their contents are subsequently removed and digested by endodermal cells (our unpublished observations), suggesting that they may function as a means for PGCs to discard unwanted components before they transition to GSCs. In support of this hypothesis, live imaging results show that PGC lobes concentrate certain cellular components, such as most of the mitochondria, before the lobes and their contents are pinched off and eaten by adjacent endodermal cells. In search of a mechanism for lobe formation, I observed that non-muscle myosin (NMY-2), a component of the contractile ring in dividing cells, forms a ring at the neck between PGCs and their lobes. This led us to hypothesize that lobes form when a contractile ring-like structure constricts the cell body into two halves. I am currently testing this hypothesis using temperature-sensitive mutations in components required for contractile ring function. By blocking formation of PGC lobes, I hope to determine if they function in the transition of PGCs to GSCs in the developing embryo.

W4025A Regulation of germline stem cell maintenance by S6-Kinase in *C. elegans*. *D. Roy*, E. Jane Hubbard. New York University School of Medicine, New York, NY.

Accumulation and maintenance of stem cells is key to proper organogenesis during development and to tissue homeostasis in adulthood. However, our understanding of signals regulating these cells remains incomplete. The *C. elegans* germ line provides a simple model for studying cellular and molecular regulation of the accumulation and maintenance of germline stem/progenitor cells. Previously, our lab established that the Target of Rapamycin (TOR) pathway: *let*-363/TOR, *daf*-15/RAPTOR, *rsks*-1/p-70-S6-Kinase (S6K) and *ife*-1/eukaryotic initiation factor-4E (eIF4E), and ample food, promote the accumulation of proliferative germ cells during larval development.

Additionally, and unexpectedly, we found that S6K acts in concert with GLP-1/Notch to maintain undifferentiated germ cells. GLP-1/Notch signaling prevents differentiation and/or maintains proliferative germ cells. We found that loss of S6K enhances and suppresses specific phenotypes associated with reduced and elevated *glp-1* activity, respectively. More recently we found that, similar to its role in promoting germ cell accumulation, S6K acts germline-autonomously and requires a conserved TOR phosphorylation site to prevent germ cell differentiation. However, neither TOR nor eIF4E shared the same genetic interaction with *glp-1*. These results suggest that the role of S6K in preventing differentiation may be a special feature of the S6K branch of the TOR pathway.

S6K is highly conserved. While its role as translational regulator is well characterized, examples of S6K-mediated cell fate regulation are limited. Therefore, we are using our genetics-based functional assays to undertake both candidate and unbiased approaches to determine the molecular mechanism by which S6K prevents differentiation. Others have shown that, like loss of S6K, reduced *cye-1*/Cyclin-E or MAPK activity can enhance *glp-1*. However, we found that neither acts in a simple linear fashion with S6K to regulate cell fate. We are currently testing additional candidates, including translational regulators, for their roles in germ cell fate maintenance and functional interaction with S6K. In parallel, we are conducting genome-wide genetic and proteomic screens to identify candidates that will be tested for their role in cell fate regulation.

W4026B GLD-1, FOG-2, and the Emergence of Self-fertility in *C. elegans*. *E. Haag*[1], S. Hu[1], L. Ryan[2], T. W. Lo[2]. 1) Univ. of Maryland, College Park, MD; 2) Ithaca College, Ithaca NY.

Germ cell sex determination in *Caenorhabditis* nematodes is regulated by a network of translational repressors [1]. In species where the XX female has evolved self-fertility, this network is reconfigured to allow limited spermatogenesis. This is achieved by changes to both properties of repressor proteins and in the *cis*-regulatory sites of their client mRNAs [e.g. 2]. Here, we focus on a highly conserved mRNA-binding protein, GLD-1, and its *C. elegans*-specific cofactor, FOG-2. Previous work has implicated both proteins in the translational regulation of *C. elegans tra*-2, a key promoter of female fate [3-5]. The STAR family protein GLD-1 targets hundreds of mRNAs in addition to *tra-2* [6]. Consistent with this, GLD-1 is not only required for XX spermatogenesis, but for other aspects of meiosis and oocyte differentiation. These oocyte-related roles are conserved across the *Caenorhabditis* genus. In contrast to this pleiotropy, the function of FOG-2 is restricted to specification of XX spermatogenesis [7]. *fog-2* is the product of a recent gene duplication, and is found only in *C. elegans*, consistent with it representing an essential player in the emergence of the novel self-fertile phenotype [8,9]. We seek to understand the molecular mechanism of how FOG-2 affects sex determination, and in particular how it modifies the activity of GLD-1 and/or its target mRNAs. We used CRISPR/Cas9 to introduce

small epitope tags into the endogenous TRA-2 and FOG-2 proteins, and are currently using these to examine expression in wild-type and mutant contexts. We are also working to identify proteins that bind to FOG-2, and to compare mRNAs associated with FOG-2 to those already known for GLD-1.

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W4027C Novel regulation of *C. briggsae* spermatogenesis. *Katharine Pelletier*[1], Keith Reidy[1], Jill Dewar[1], Carlos Carvalho[2], Dave Pilgrim[1]. 1) University of Alberta, Edmonton, Alberta, CA; 2) University of Saskatchewan Saskatoon, Saskatchewan, CA.

The evolution of novel traits relies on heritable changes in gene content or gene expression, but the processes by which these occur is not always clear. Sex determination is a particularly interesting trait with which to model these processes because its regulation seems to be subject to rapid evolution. Androdioecy, or a species ability to make an ovotestis in an otherwise female animal, has independently evolved three times in the *Caenorhabditis* nematode clade from a dioecious ancestor. By comparing the genetic regulatory network allowing for androdioecy in two species, *C. elegans* and *C. briggsae*, to their dioecious close relatives, we hope to understand how the novelty arose. Sex determination is well understood in *C. elegans*, and we have been using similar molecular and genetic tools to understand hermaphroditism in *C. briggsae*.

Forward genetic screens allowed genetic identification of the *C. briggsae* orthologs of *C. elegans* genes (*tra, fem*) required for somatic and gonadal sex determination, but unlike in *C. elegans*, none of the feminizing mutants blocked spermatogenesis in the female ovotestis. A second screen, looking for suppressors of masculinizing mutants identified several phenotypes not seen in *C. elegans*. Many of these suppressors permit the development of XX hermaphrodites and XO males in a masculinized *tra-2* background, in contrast to *C. elegans* feminizing mutants where XO animals are feminized. Mutant alleles with this phenotype have been identified both within know sex determination genes, such as *fem-3*, and in novel regulatory loci. Through examination of these alleles, we will gain key insights into differences in *C. briggsae* sex determination in both the soma and the germ line. We will present our current understanding of somatic and germ line sex determination in *C. briggsae* and compare that to what is known in *C. elegans.* This illustrates how we can use comparative genomics within the *Caenorhabditis* clade to better understand the evolution of genetic regulatory network.

W4028A TGFß and insulin-like signaling mediate the feeding state-dependent expression of the food chemoreceptor ODR-10 in *C. elegans* males. *Emily R. Wexler*, Deborah A. Ryan, Douglas S. Portman. University of Rochester, Rochester, NY.

The biological mechanisms by which neural circuits are modulated by an animal's sex ("gender") is poorly understood. We have previously found that *C. elegans* males and hermaphrodites differ in their response to several odorants detected by shared circuitry. In particular, hermaphrodites are more attracted than males to the odorant diacetyl, a food cue sensed by the chemoreceptor *odr-10*, expressed in the AWA sensory neuron. Recently, we have shown that regulated *odr-10* expression underlies sex differences in diacetyl attraction. In adult males, *odr-10* expression is significantly reduced compared to hermaphrodites, promoting the prioritization of exploratory behavior over feeding. Moreover, transient starvation upregulates *odr-10* in males to promote feeding. We have identified the TGFβ and the insulin-like signaling pathways as regulators of *odr-10* expression in males. Males with mutations in the TGFβ ligand *daf-7* or the downstream SMAD *daf-8* have increased expression of *odr-10.* In contrast, these mutations have no apparent effect on *odr-10* expression in hermaphrodites. Animals with mutations in *daf-5*, a transcription factor inhibited by DAF-7 signaling, show decreased expression of *odr-10*; moreover, *daf-5* males show no increase in *odr-10* expression in response to starvation. Likewise, loss of the insulin receptor *daf-2* results in increased *odr-10* expression in males and *daf-16* mutant males do not upregulate *odr-10* upon starvation. These findings suggest the TGFβ and insulin-like signaling pathways regulate feeding-state-mediated changes in *odr-10* expression in a sex-dependent manner. The apparent sensitivity of males to these signals suggests that the sex determination pathway intersects with TGFβ and insulin-like signaling to bring about state-dependent changes in odr-10 expression and behavior.

W4029B PUP-1/CDE-1 and PUP-2 poly(U) polymerases function redundantly in germline development in *C. elegans*. *Y. Li*, M. Snyder, E. Maine. Syracuse University, Syracuse, NY.

Poly(U) polymerases (PUPs) add uridine to the 3'end of RNAs, a modification that correlates with reduced RNA stability in many organisms. Targets of uridylation include mRNAs and small RNAs, including small-interfering (si) RNAs and unmethylated micro (mi) RNAs. Despite the prevalence of uridylation, our knowledge of its developmental importance is limited. Three members of the PUP family have been identified in *Caenorhabditis elegans* [1]. Two have been studied in some detail. PUP-1/CDE-1 (co-suppression defective) targets a subset of siRNAs and is expressed in the germ line [2]; PUP-2 activity regulates a subset of miRNAs that act in the soma [3, 4]. Despite these distinct functions, we find that PUP-1/CDE-1 and PUP-2 are required redundantly in meiotic germ cells. We used the CRISPR-Cas9 system to generate a double knockout of *pup-1/cde-1* and *pup-2* [*pup-1(0) pup-2(0)*] and then compared its phenotype to those of *pup-1(0)* and *pup-2(0)* single mutants. The single mutants have numerous defects that are present additively in the double mutant. In addition, we observe unique

phenotypes in the double mutant. Under conditions of temperature stress, *pup-1(0) pup-2(0)* hermaphrodites produce progressively smaller numbers of progeny and become sterile within three generations. The majority (~93%) of those sterile adults contain no germ cells. In addition, meiotic chromatin regulation is abnormal: in *pup-1(0) pup-2(0)* males, the histone H3 lysine 9 dimethylation (H3K9me2) modification is elevated from pachytene stage of first meiotic prophase until late spermiogenesis compared with controls. In keeping with delayed H3K9me2 removal, developing *pup-1(0) pup-2(0)* sperm progress through spermiogenesis relatively slowly compared with controls. Small RNAs are known to impact both germline survival and H3K9me2 regulation. We observe PUP-2 protein expression in germ line cytoplasm, particularly in developing oocytes. Taken together, our findings suggest that PUP-1 and PUP-2 function redundantly to ensure germline survival and chromatin regulation, perhaps by modulating small RNA levels. We are working to distinguish between alternative hypotheses for how PUP-1 and PUP-2 activities promote germline survival and chromatin regulation.

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W4030C Analysis of germ cell proliferation and germline histone dynamics in *C.elegans*. *Simona Rosu*, Orna Cohen-Fix. NIH-NIDDK, Bethesda, MD.

The *C.elegans* adult contains a renewable pool of germ cells that make up the proliferative zone (PZ), which contains mitotically dividing cells. From this pool, cells enter meiosis and differentiate, ensuring continued production of oocytes. To date, studies on the PZ have utilized mainly fixed samples. Consequently, many questions remain about the operation of the PZ: where and how large is the stem cell pool? Is there a transit-amplifying population of germ cells? How is the number and rate of cell divisions regulated during germline proliferation? To investigate these questions, I have constructed a strain containing germline-expressed histone H2B::Dendra. Dendra is a green-fluorescing protein, which, when stimulated by laser light at 405 nm, photo-converts to red fluorescence. I have worked out a protocol to individually immobilize live worms and photo-convert selected germ cell nuclei, recover the worms to plates, and image the same worms many hours later. This allows me to determine the fate of the converted cells and their daughters arising from cell division. In the worm strain used, the entire PZ contains ~200 cells. Preliminary results indicate that a pool of 40-70 cells at the distal tip of the PZ expands an average of 2.6 times in about 8 hours, filling the majority of the PZ in this timeframe. A pool of cells in the middle of the PZ expands an average of 1.6 times in the same timeframe, indicating a subset of cells in this region divide, and some do not. This entire labeled pool originally in the mid-PZ moves proximally, with ~60% of the cells already entering meiosis. Thus the mid-PZ is unlikely to contain stem cells. Finally, a pool in the proximal zone of the PZ has little to no expansion (1.1 times average), and the entire pool moves proximally and enters meiosis. This is consistent with a model in which stem cells reside in the distal pool, and divide once in about 8 hours, giving rise to both daughters that self renew to remain in the stem cell pool, and daughters that will divide one more time as they move to the middle of the PZ, after which they cease divisions and enter meiosis. I am currently refining the analysis by converting smaller pools of cells. This work establishes the parameters that govern germ cell proliferation, which in future studies can be investigated under altered conditions or in mutant situations to determine regulatory mechanisms.

In addition, I am also using this tool to analyze histone dynamics during oogenesis.

W4031A Epigenetic Contributions to Homolog Recognition in Meiosis. *C. Doronio*, W. Kelly. Emory University , Atlanta, GA.

During meiosis, homologous chromosomes must correctly identify one another in order for proper alignment, synapsis, and recombination to occur. Improper pairing between chromosomes can lead to aneuploidies causing defective gamete formation and embryonic lethality. Currently, very little is known about homologous chromosome recognition and discrimination. There has been evidence supporting the role of DNA Double Strand Breaks (DSB) and homologous recombination in homolog pairing. However, mutants lacking the ability to form DSBs in *Drosophila* and *C. elegans* can still properly align homologous chromosomes, showing that DNA DSB independent mechanisms exist. *C. elegans* chromosomes have pairing centers composed of DNA sequences at ends of chromosomes that are required for efficient pairing, but the sequences are not all unique to single chromsomes. Furthermore, many organisms have highly repetitive genome content, making DNA sequence risky for homology searching. What forms the basis of homolog recognition?

The specific patterns of active transcription are distinct for each chromosome during meiosis. Peter Cook has previously proposed that aspects of such transcription patterns may provide a “bar-code” for homolog recognition, possibly through shared “transcription factories”(Xu and Cook, 2008). This hypothesis, while intriguing, is fundamentally difficult to test. However, transcription through a gene also “marks” that gene with specific epigenetic modifications, such as the methylation of Lysines 4, 36, and 79 on Histone H3 (H3K4me, H3K36me, and H3K79me, respectively). Thus regions of transcription and repression create alternating patterns of chromatin modifications, unique to each chromosome, that could be recognized by specific proteins and contribute to homology recognition. Indeed, the Nabeshima lab recently showed that pairing center-independent pairing defects were observed in the germline of *C.elegans mrg-1* mutants (Dombecki et al., 2011). *mrg-1* encodes a chromodomain protein whose mammalian homolog, MRG15 recognizes and binds to H3K36me. We are currently examining the role of histone modifications, and their cognate binding proteins such as MRG-1, in homology recognition and discrimination during meiosis.

W4032B Cortical microtubule dynamics in C. elegans oocytes. *C.-H. Chuang*, B. Bowerman. University of Oregon, Eugene, OR.

In many animal oocytes, meiotic spindles assemble in the absence of centrosomes to segregate chromosomes; yet, little is known about the origin of the spindle microtubules and the proteins that regulate spindle microtubule dynamics. We recently showed that in the absence of the microtubule depolymerizing kinesin KLP-7, extra microtubules accumulated around the oocyte chromosomes[1]. As others have reported[2], we also have observed a dramatic increase in microtubule levels throughout the oocyte cortex during meiosis I in *klp-7(-)* mutant oocytes. To further investigate microtubule dynamics, we are using spinning disc confocal microscopy in live oocytes to image GFP::β-tubulin during meiosis I in both wild-type and *klp-7(-)* mutants. In *klp-7(-)* oocytes, we found that microtubule foci were formed at the cortex during

prometaphase; some foci moved towards each other and then merged as larger microtubule organizing centers, while other foci moved into the assembling meiotic spindles. In wild-type oocytes, we observed that smaller and less dense of microtubule foci assembled in the cortex area; some foci still merged together, but fewer foci were recruited to the spindles relative to *klp-7(-)* mutants. We then used a GFP fusion to a spindle pole protein ASPM-1 to further investigate the dynamics of cortical microtubules in wild-type oocytes, and found that GFP::ASPM-1 also was present as multiple foci at the cortex. We are currently making an mKate2 fusion to ASPM-1 to further investigate the interplay of cortical microtubules and ASPM-1. In addition, we have found that the microtubule motor protein complex dynein influences oocyte cortical microtubule dynamics and is essential for proper organization of the oocyte meiotic spindle. In *dhc-1(-)* oocytes, cortical microtubules were present but did not appear to aggregate as microtubule foci. Moreover, in contrast to the barrel-shaped wild-type spindle, *dhc-1(-)* spindles collapsed soon after they started to assemble. After collapsing, the spindle microtubules spread around the cortex. Similarly, chromosomes distributed around the cortex, rather than aligning at the equator of the spindle barrel, based on analysis of an mCherry::Histone 2B fusion. We are currently further investigating how cortical microtubules and dynein contribute to oocyte meiotic spindle assembly.

W4033C Investigating Oocyte Meiotic Spindle Assembly and Bipolarity in *C. elegans*. *A. Schlientz*, B. Bowerman. University of Oregon, Eugene, OR.

Unlike mitotic spindles, oocyte meiotic spindles assemble in the absence of centrosomes. This phenomena sparks two interesting questions: 1) how do these acentrosomal spindles arise and 2) what dictates their bipolarity? To better understand acentrosomal oocyte meiotic spindle assembly in *C. elegans*, we are investigating the requirements for *cls-2*, a member of the CLASP family of microtubule binding proteins, and the kinesin-14 family members *klp-15/16*.

Using feeding RNAi mediated knockdown of *cls-2* and spinning disk confocal microscopy, we have observed a requirement for CLS-2 during oocyte meiotic spindle assembly, consistent with a previous study[1]. Interestingly, when using a GFP fusion to the spindle pole marker ASPM-1, we often see only a single focus of GFP::ASPM-1 signal, implying that CLS-2 may play an important role in either establishing or maintaining spindle bipolarity.

The *Drosophila* orthologue of *klp-15/16*, *non-claret disjunctional* (*ncd*), has been shown to play a role in oocyte meiotic spindle pole assembly, with split or extra poles forming in *ncd(-)* mutants. However, a role for this kinesin family in *C. elegans* has not been reported. Using feeding RNAi mediated knockdown of *klp-15/16* and spinning disk confocal microscopy, with our GFP::ASPM-1 pole marker, we observed what appears to be diffuse early spindle poles and a late establishment of spindle bipolarity, indicating that a role for this kinesin family in oocyte meiotic spindle pole assembly may be conserved across species.

1. Dumont J, Oegema K, Desai A. *Nat Cell Biol*. 2010;12(9):894-901.

W4034A Analysis of DLC-1 mediated regulation of the tumor suppressor protein GLD-1. *M. Ellenbecker*, E. Voronina. University of Montana, Missoula, MT.

Dynein light chain (DLC-1) was originally characterized as a cargo-binding component of the dynein motor complex that is involved in retrograde transport of mRNA and protein particles. More recent studies have revealed that DLC-1 interacts with a diversity of cellular proteins and likely also functions as an allosteric regulator in ribonucleoprotein complexes (mRNPs). Data from the Voronina lab suggests that DLC-1 regulates developmentally important mRNA expression by functioning as a cofactor to RNA binding proteins such as FBF-2 and GLD-1. GLD-1 is an RNA-binding protein that promotes germ cell differentiation during animal development by binding to and repressing translation of target mRNAs. This RNA regulatory function is essential for preventing ectopic proliferation in germ cells and *gld-1* mutant worms form germline tumors. Mammalian Quaking proteins are GLD-1 orthologs and function as potent glioblastoma multiforme tumor suppressors regulating the TGFβ signaling network that controls cell growth, proliferation and differentiation. RNA immunoprecipitation and microarrays or transcriptome wide sequencing experiments have identified hundreds of mRNAs as potential GLD-1 targets, however, the mechanism(s) of GLD-1 regulation of this diverse set of mRNAs is not well understood. We discovered that mutation in *dlc-1* gene predisposes nematode to tumor formation, GLD-1 target mRNAs are enriched in DLC-1 ribonucleoprotein complexes and that DLC-1 regulates translation of the GLD-1 target mRNA *mex-3*. Depletion of *dlc-1* using RNA interference disrupts the GLD-1-dependent control of germ cell proliferation and differentiation and biochemical assays show a direct DLC-1/GLD-1 binding interaction. Together, these data support the hypothesis that DLC-1 mediates the RNA regulatory and tumor suppressor functions of GLD-1 through a direct protein interaction. Some mechanistic possibilities for DLC-1 promoting the function(s) of GLD-1 are: regulating GLD-1 levels in germ cells, enhancing formation of GLD-1 mRNP complexes, cooperating in translational repression of GLD-1 mRNA targets and/or by mediating GLD-1 sub-cellular localization. We are currently using genetic and molecular biology techniques to determine how DLC-1 contributes to GLD-1 function *in vivo* and biochemical studies to elucidate the molecular details of the DLC-1/GLD-1 binding interaction. Since GLD-1 and mammalian Quaking proteins are closely related and their mutation causes cancer, molecular insights into germline tumor formation identified in this study using *C. elegans* as a model organism will be broadly relevant and significantly advance our understanding of human cancers.

W4035B Ubiquitin Conjugating Enzymes required for Ubiquitination of Paternal Organelles during post fertilization events. *Paola Molina*, Lynn Boyd. Middle Tennessee State University, Murfreesboro, TN.

The elimination of paternal organelles following fertilization is well conserved amongst metazoans, but the mechanism of organelle selection and process execution is not well understood. Previous studies conducted on *Caenorhabditis elegans* reported that the ubiquitin and autophagy pathways are involved in the elimination of paternal organelles. The current working model for the removal of paternal organelles in *C. elegans* is that membranous organelles (MOs) are ubiquitinated and cluster together with paternal mitochondria and together they are discarded through autophagy. Two different types of ubiquitin chains are found on the MOs in newly fertilized embryos. Lysine 48 chains are

found only during meiosis I and lysine 63 chains persist through all stages of meiosis and mitosis. These findings indicate that two different ubiquitination events are taking place during the tagging of paternal organelles. We have used transgenic nematodes with a GFP::Ub tag in the germline and RNAi to individually knockdown ubiquitin conjugating enzymes (UBC) to establish which UBC(s) is responsible for ubiquitinating the paternal organelles. The two different types of ubiquitin chains present on MOs suggest multiple ubiquitin events. Therefore, UBC combination knockdowns were also screened. The screen revealed *ubc-18/ubc-16* and *ubc-18/ubc-13/ubc-2* as the combinations that reduced the presence of GFP:Ub on MOs. Interestingly, *ubc-18* appears in both of our hits. Therefore, we took a closer look at WY34 (ku354) *ubc-18* mutants and stained for K48 and K63 ubiquitin chains. K63 chains were not affected by the absence of *ubc-18*. However, K48 chains were absent in the WY34 embryos. These results indicate that *ubc-18* is a key player in initial ubiquitination of paternal organelles.

W4036C Elucidating the Role of Securin in Regulating Separase during Cortical Granule Exocytosis. *C. Turpin*, M. LaForest, L. Uehlein-Klebanow, Q. Caylor, D. Mitchell, J. Bembenek. University of Tennessee, Knoxville, TN.

Meiosis is a tightly regulated series of events leading to the production of genetically distinct haploid gametes. A key player in this process is the cysteine protease separase (SEP-1). Known for its canonical role in chromosome segregation, recent studies suggest that SEP-1 has an additional function in vesicular trafficking during cell division. We hypothesize that cell cycle machinery known to control SEP-1 protease activity for chromosome segregation also controls its localization to the cortex and subsequent exocytic activity. Following spindle attachment and chromosome alignment during the meiotic M phase, the anaphase promoting complex/cyclosome (APC/C) is activated resulting in the degradation of SEP-1 inhibitory chaperone securin (IFY-1) and entry into anaphase I. In recent studies, we have observed that SEP-1 localizes to specialized vesicles called cortical granules and regulates their exocytosis during anaphase I. Cortical granule exocytosis is necessary for the process of eggshell formation. Before it appears on cortical granules, SEP-1 localizes to cytosolic filaments near the plasma membrane. We have shown that SEP-1 colocalizes with its inhibitor, IFY-1, on filaments during prometaphase, and both disassociate from these structures after the onset of anaphase I. Inhibition of APC/C activity prevents SEP-1 and IFY-1 from leaving the filaments, These data suggest the hypothesis that degradation of IFY-1 may regulate SEP-1 localization to vesicles. IFY-1 depletion does not prevent SEP-1 localization to cortical granules although exocytosis is impaired. To address whether IFY-1 degradation is required to allow SEP-1 translocation onto vesicles, we generated a non-degradeable IFY-1 (IFY-1^{DM}::GFP). Consistent with enhanced IFY-1 stability, IFY-1^{DM}::GFP is not completely degraded following anaphase I onset, remaining on chromosomes and in the cytoplasm into anaphase II. As expected, expression of IFY-1^{DM}::GFP causes embryonic lethality. Additionally, IFY-1^{DM}::GFP localizes to filaments normally and persists longer on these structures and chromosomes than wild type IFY-1 following the resumption of meiosis I. Interestingly, IFY-1^{DM}::GFP causes polar body extrusion failure, which could be related to defects in cortical granule exocytosis. In the future we will investigate how IFY-1^{DM}::GFP affects SEP-1 localization to cortical granules. This will provide insight into how key regulatory components of the cell cycle control SEP-1 localization to promote timely cortical granule exocytosis during anaphase I.

W4037A The Protease Activity of Separase Is Required for Both Chromosome Segregation and Membrane Trafficking During Anaphase. *Xiaofei Bai*, Diana Mitchell, Lindsey Klebanow, Joshua Bembenek. University of Tennessee, Knoxville, TN.

Chromosomal segregation and cytokinesis are tightly regulated processes involved in cell division. The cysteine protease separase is required for proper chromosomal segregation. Separase cleaves a subunit of the protein complex called cohesin, which keeps the sister chromatids together until the end of metaphase. In addition, separase is a key player in meiotic cortical granule exocytosis and vesicle trafficking during mitotic cytokinesis. RAB-11 is known to regulate both cortical granule exocytosis and exocytosis during cytokinesis. RNAi knockdown of separase impairs RAB-11 vesicular trafficking during both events. This raises the question of whether or not separase's protease activity regulates RAB-11 vesicle exocytosis. To address this question, we generated a catalytically inactive separase, SEP-1^{PD}::GFP. We found that the expression of SEP-1^{PD}::GFP causes chromosomal nondisjunction. Depletion of cohesin rescues this defect, indicating that cohesin cleavage is prevented by the inactive protease, possibly by a substrate trapping mechanism. To test whether SEP-1^{PD}::GFP also impairs RAB-11 vesicular trafficking during cytokinesis, we imaged the embryos that express RAB-11::mCherry and SEP-1^{PD}::GFP. Interestingly, expression of SEP-1^{PD}::GFP causes an abnormal accumulation of RAB-11 vesicles at the cleavage furrow site, similar to depletion of separase by RNAi. Moreover, we found that RAB-11 co-localized with both wild type separase and our protease dead mutant on cortical granules during meiotic anaphase I. We filmed SEP-1^{PD}::GFP expressing embryos during meiosis I to observe cortical granule exocytosis. Our results indicated that SEP-1^{PD}::GFP expression delayed the completion of cortical granule exocytosis. We tested possible genetic interactions between SEP-1^{PD}::GFP and core exocytic proteins. Our results indicate that the depletion of the t-SNARE syntaxin-4 (*syx-4*) further enhanced RAB-11::mCherry and SEP-1^{PD}::GFP accumulation on the cleavage furrow and the midbody. Moreover, we found an additive increase in embryonic cytokinesis failure relative to *syx-4* depletion or SEP-1^{PD}::GFP expression alone. These findings suggest that the protease activity of separase is required for the exocytosis of RAB-11 vesicles during mitotic cytokinesis and cortical granule exocytosis during meiosis I.

W4038B A Potential Role for Midbodies in Developing Tissues of *C. elegans*. *J. N. Bembenek*[1], X. Bai[1], B. C. Chen[2], R. Simmons[1], C. Turpin[1], L. Uehlein[1], D. Mitchell[1], E. Betzig[2]. 1) University of Tennessee, Knoxville, Knoxville, TN; 2) Janelia Research Campus, HHMI, Ashburn, VA.

The midbody forms at the end of cytokinesis and facilitates abscission, the final separation of the daughter cells. Midbodies have been implicated in several cellular processes in addition to abscission including cell fate, dorsoventral axis formation, neurite growth, and apical polarity during epithelial lumen formation. To investigate midbody function we examined midbody fate in the invariant lineage of the *C. elegans* embryo. Live-cell imaging has revealed unique and tissue-specific patterns of the fate of the midbody and different midbody components. In early embryo cell divisions, midbody flank proteins are lost from the midbody soon after cytokinesis while proteins associated with the midbody ring persists for much longer. A dramatic shift in midbody behavior is observed in several tissues at around the 300-cell stage

when the embryo undergoes global morphogenetic changes. In two lumen-forming tissues, the gut and pharynx, midbodies form and migrate to the apical midline. This midbody migration event coincides with previously characterized polarization events in gut epethelia. Upon reaching the intestinal midline the midbody ring is internalized and disappears; however, the Aurora B kinase, AIR-2, remains on the apical surface for over an hour after polarization. A similar apical localization pattern is observed for AIR-2 in the pharyngeal primordium. AIR-2 also localizes to midbodies in the sensilla primordium, which aggregate in foci over multiple successive divisions. AIR-2 persists along the leading edge of developing dendrites as they migrate towards the anterior end of the embryo and extend as dendritic processes. Other midbody markers are either internalized and degraded or maintained with AIR-2 in a tissue-specific manner. Inactivation of the central spindle organizer, *spd-1*, disrupted Aurora B kinase localization to the midbody as expected. However, Aurora B kinase was still capable of accumulating on the apical surface possibly by localizing to centrosomes. Inactivating several fast-inactivating temperature sensitive cytokinesis mutants late in embryogenesis causes severe morphogenesis defects. We hypothesize that the midbody and specific cytokinesis regulators such as AIR-2 may regulate the final interphase architecture of a terminally dividing cell.

W4039C TPXL-1 mediates aster-based clearing of contractile ring proteins from the cell poles during cytokinesis. *E. Zanin*[1], K. Oegema[2]. 1) Biozentrum der LMU München, München, DE; 2) Ludwig Cancer Research and Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92037.

In the last step of cell division, a process called cytokinesis, the mother cell splits into two daughter cells. During cytokinesis a contractile actin-myosin ring forms beneath the plasma membrane around the cell equator. To coordinate cytokinesis with chromosome segregation, the contractile ring assembles in response to signals from the mitotic spindle. A stimulatory signal promotes cortical contractility by activating RhoA at the cell equator. At the same time, an inhibitory signal emanating from the centrosomal microtubule asters suppresses contractility at the cell poles. Attempts to identify the molecular basis of the inhibitory signal have been hindered by the absence of a robust assay. To address this problem, we established an assay for aster-based suppression of contractility in the *C. elegans* embryo. Using this assay, we identified TPXL-1, the homologue of TPX2, to be essential for aster-based clearing of contractile ring proteins from the cell poles. TPXL-1 is an aurora A kinase activator that localizes to the centrosomal microtubule asters. In *tpxl-1* mutant embryos the kinetochore microtubules are extremely short, which results in a short mitotic spindle at anaphase onset. To determine whether the short mitotic spindle or *tpxl-1* depletion itself causes defects in aster-based suppression, we increased spindle length in *tpxl-1* mutants by depleting the kinetochore component *hcp-4*. Rescuing spindle length in *tpxl-1* mutants did not rescue the defects in aster-based suppression, suggesting that TPXL-1 has a direct role in this process. We are currently testing whether aster-based clearing of contractile ring proteins depends on the ability of TPXL-1 to activate aurora A kinase. In summary, we identified the first molecular component of the aster-based mechanism that supports contractile ring assembly by inhibiting the accumulation of contractile ring proteins at the cell poles.

W4040A *t3421,* a novel mutation required for bipolar spindle assembly in the one-cell stage *C. elegans* embryo. *T. Mikeladze-Dvali*[1], A. C. Erpf[1], N. Memar[1], R. Schnabel[2]. 1) Biocenter, Ludwig-Maximilians-University, Munich, Germany; 2) Technical University Braunschweig, Braunschweig, Germany.

Centrosomes, the primary microtubule organizing centers (MTOC) of animal cells, consist of a pair of centrioles surrounded by pericentriolar-material (PCM). As in many metazoans, in the nematode *C. elegans*, the sperm contributes the sole centriole pair to the zygote at fertilization, whereas the centrioles of the oocyte are eliminated during oogenesis. Upon fertilization, paternally derived centrioles disengage, duplicate and mature to centrosomes. During the process of maturation centrioles recruit maternal PCM proteins and assemble the centrosomes, which in turn are required for the formation of a bipolar spindle and faithful chromosome segregation. Our overall objective is to uncover molecular mechanisms that regulate maternal centrioles during oogenesis and to understand how paternally derived centrioles orchestrate bipolar spindle formation.

Among a set of temperature-sensitive embryonic lethal mutations, we identified the mutant allele *t3421,* which causes defective spindle assembly in early embryos. Live imaging of embryos at a restrictive temperature, revealed the formation of monopolar spindles during the first cell cycle, followed by an abnormal cytokinesis, as well as tri- or tetrapolar divisions in the second cell cycle. Our preliminary results suggest that one of the two paternally contributed centrioles is defective in the process of PCM assembly during the first mitosis, indicating a failure in centrosome maturation. Since only one of the centrioles fails to assemble a functional aster, we speculate that the affected centriole is the younger one of two paternally contributed centrioles.

Currently we are conducting experiments to further explore the molecular nature of the mutation and to gain a better understanding of the phenotype. So far the mutation has been mapped to a region on Chromosome I that among others includes a nonsense mutation in an unknown coiled-coiled domain protein.

We expect that the detailed analysis of *t3421*will provide key insights into the mechanisms of maturation of the paternally contributed centrioles.

W4041B Polo-like kinase 1 is required for nuclear envelope breakdown and parental chromosome mixing during Caenorhabditis elegans early embryonic divisions. *M. M. Rahman*, O. Cohen-Fix. NIDDK at the National Institutes of Health, Bethesda, MD.

Mitosis is a highly regulated process that facilitates faithful segregation of genetic material into daughter cells. During mitosis in animal cells, nuclear envelope breakdown (NEBD) allows microtubules emanating from cytoplasmic centrosomes to connect to the duplicated chromosomes. The nuclear envelope (NE) reforms at the end of mitosis, generating a single nucleus in each daughter cell. NEBD following fertilization in a 1-cell embryo is poorly understood, especially in mammals where visualizing this process is challenging. It is assumed that once the maternal and paternal nuclei are in close apposition NEBD is initiated. The signals that promote timely NEBD are unknown. To visualize

mitotic events following fertilization we utilized early-stage embryos from *Caenorhabditis elegans.* Polo-like kinase 1 (Plk1) is a conserved kinase involved in multiple steps of mitosis. While Plk1 function is studied extensively in somatic cells, little is known about its function during embryonic development. We observed that in *C. elegans* embryo, growth of a temperature sensitive *plk-1* mutant (*plk-1*^{ts}) at the semi-permissive temperature led to the formation of two nuclei per cell, containing either the maternal or paternal DNA. This paired-nuclei phenotype was caused by a defect in NEBD, and specifically the formation of a gap in the NE at the interface between the maternal and paternal pronuclei during the first mitosis. In the *plk-1*^{ts} mutant we also observed a defect in chromosome alignment of the maternal and paternal metaphase plates relative to each other. Since PLK-1 is known to associate with chromosomes, the NEBD defect in a *plk-1*^{ts} mutant could be due to a direct requirement for PLK-1 at the NE region where the membrane gap is formed. We hypothesize that chromosome alignment may be necessary to achieve a critical concentration of chromosome-bound PLK-1 at the site of the future membrane gap. Alternatively, the NEBD in the *plk-1*^{ts} mutant could be due to an indirect requirement for PLK-1 in chromosome alignment, which in turn leads to a signal of an unknown nature that is necessary for gap formation in NE. Either way, PLK-1- dependent gap formation in the NE is essential for parental chromosome mixing. To further understand this process, we utilized data from a RNAi screen where we down regulated ~2000 genes critical for embryogenesis, and recorded changes in nuclear morphology in embryonic cells. To date, we have identified 17 candidates that, when down regulated result in paired nuclei formation. We are now investigating how inactivation of these gene functions leads to paired nuclei formation, and whether there's any functional relationship between these genes and the PLK-1 mediated NEBD regulation pathway.

W4042C Microtubule glutamylation is dispensable for *C. elegans* viability. *Katherine Badecker*, Jessica Lee, Ruchi Shah, Daniel Chawla, Megan Brewster, Zachary Barth, Nina Peel. The College of New Jersey, Ewing, NJ.

Glutamylation, the covalent attachment of glutamic acid to tubulin in the polymerized microtubule, is enriched on long-lived microtubules, and is proposed to contribute to centriole stability, cilia motility and axon function. Glutamylation of the microtubules is catalysed by a family of tubulin tyrosine ligase like (TTLL) enzymes. Comprehensive in vivo analyses of the function of tubulin glutamylation have proved challenging because of the existence of a large family of TTLL enzymes in many species. To investigate the function of tubulin glutamylation we have generated a *ttll-4(tm3310)*; *ttll-11(tm4059); ttll-15(tm3871) ttll-5(tm3360) ttll-9(tm3889)* quint mutant that lacks all five *C. elegans* glutamylating TTLL enzymes. The quint mutant shows normal embryonic viability and brood size indicating that the centrioles are functional and the microtubules of the spindle are competent for cell division. Recent evidence suggests that in human cells glutamylation modulates microtubule severing by spastin and katanin. To investigating whether a similar regulatory mechanism also exists in worms we have combined our quint mutant with mei-1(GOF) and mei-1(LOF) alleles of katanin. We do not however observe any suppression of the mei-1-associated lethality when microtubule glutamylation is lost. Our data therefore suggest that, contrary to expectations, microtubule glutamylation is not essential for *C. elegans* viability, although we do observe a male mating defect indicative of ciliary dysfunction. We are currently undertaking a detailed analysis of microtubule dynamics to determine whether minor perturbations of microtubule function occur in the absence of glutamylation.

W4043A The Power of One: A single wild type chromosome pair promotes chromosome partition in the first spermatocyte division of meiotic mutants. *Katherine A. Rivera Gomez*[1], Gunar Fabig[2], Thomas Muller-Reichert[2], Anne Villeneuve[3], Mara Schvarzstein[1]. 1) Brooklyn College, Brooklyn, NY; 2) Dresden University of Technology, Dresden, Germany; 3) Stanford University, Stanford, CA.

Errors in chromosome partitioning during meiosis result in aneuploid gametes that form embryos that die, arrest in development or have developmental abnormalities. Most of meiosis is devoted to temporarily connecting each maternally-derived homologous chromosome to its paternally-derived counterpart. These connections are generated by crossover recombination and are key in enabling the ordered partitioning the genome in the two meiotic divisions. In the first division, the connected homologous chromosomes are partitioned while keeping sister chromatids together in each homolog until the second nuclear division. The REC-8 cohesin is required for sister chromatids to segregate together to the same spindle pole in this division. Mutants that fail to form crossovers result in separate maternally- and paternally-derived homologous chromosomes. Observations by Severson *et al.* (2009) suggest that meiotic mutants that do not undergo crossover recombination segregate the homologous chromosomes in one of two ways in the first meiotic division of oocytes. In meiotic mutants that have REC-8, sister chromatids co-orient and each homologous chromosome segregates randomly. In meiotic mutants that lack REC-8, sister chromatids bi-orient and segregate away from each other in the first meiotic division. We will report our observations of chromosome partitioning defects in spermatocytes of meiotic mutants. Unlike mutants lacking REC-8, meiotic mutants defective in different steps leading to crossover recombination including; *him-3, syp-1, syp-2* and *spo-11*, fail to partition their chromosomes in the first division. These mutants still progress into the second meiotic division and form multipolar or connected spindles with bi-oriented sister chromatids that segregate to form four mostly aneuploid spermatids. Interestingly, analysis of *spo-11* mutant spermatocytes suggests that a single bi-oriented homologous chromosome pair is sufficient to suppress the formation of abnormal spindle organization in these mutants. The SPO-11 protein makes the double strand DNA breaks required for the initiation of crossover recombination. *spo-11* mutants can be rescued by inducing exogenously the formation of double strand DNA breaks. Induction of a single double strand break per nucleus generates one homologous chromosome pair. The formation of a single homologous chromosomes pair is sufficient to suppress the abnormal spindle organization seen in these mutant spermatocytes. We are currently investigating the cellular mechanism by which a single homologous chromosome pair might promote normal bi-polar spindle organization and segregation of meiotic mutant chromosomes that otherwise would not be partitioned in the first division.

W4044B Asymmetric positioning of organelles during epithelial cell polarization. *James Brandt*, Greg Hermann. Lewis & Clark College, Portland, OR.

The intestinal cells of C. *elegans* display asymmetrical positioning of multiple organelles soon after they become polarized at the E16 stage. At

this point in intestinal development, early and late endosomes, nuclei, and lysosomes are positioned near the nascent apical membrane while yolk granules are positioned basally. Our group studies the formation and behavior of an intestinal cell-specific lysosome related organelle (LRO) called the gut granule. The asymmetric positioning of LROs can be essential for their function, for example in movement of lytic granules to the immunological synapse in cytotoxic T-cells. In wild-type embryos, gut granules are positioned basally and excluded from the apical domain, which is where the nucleus is located. In *unc-83(-)* mutants, which lack polarized nuclei, gut granules are located both apically and basally, indicating that the positioning of the nucleus excludes gut granules from the apical domain. We have found that defects in two genes encoding ATP binding cassette (ABC) transporters lead to the accumulation of gut granules near the apical membrane. In these mutants, nuclei are still positioned apically. It is possible that gut granules are mispolarized in the mutants due to taking on the identity of an apical organelle. Our colocalization analyses between gut granule markers and markers for apically polarized organelles, including early endosomes (RAB-5, FYVE::GFP), late endosomes (RAB-7), and lysosomes (LMP-1), suggest the identity of gut granules are unaltered in the mutants. The positioning of endolysosomes, nuclei, and yolk were not altered in the mutants indicating that the overall cytoplasmic polarity of intestinal cells is not reversed. Interestingly, mutations in *mrp-4*, another ABC transporter, suppress the asymmetrical polarization of gut granules in the mutants. We will present models for the function of these ABC transporters in gut granule positioning.

W4045C SLO BK K^+ channels couple gap junctions to inhibition of Ca^{2+} signaling in olfactory neuron diversification. *A. Alqadah*[1,4], Y. W. Hsieh[1,4], J. Schumacher[2], X. Wang[2], S. Merrill[3], G. Millington[2], B. Bayne[2], E. Jorgensen[3], C. F. Chuang[1]. 1) Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL; 2) Cincinnati Children's Hospital Research Foundation, Cincinnati, OH; 3) HHMI, University of Utah, Salt Lake City, UT; 4) Equal Contribution.

The *C. elegans* AWC olfactory neuron pair communicates to specify asymmetric subtypes, AWC^{OFF} and AWC^{ON} across the left-right axis. Intercellular communication between AWC and other neurons in a transient NSY-5 gap junction network antagonizes voltage-activated Ca^{2+} channels, UNC-2 (CaV2) and EGL-19 (CaV1), in the AWC^{ON} cell, but how Ca^{2+} signaling is downregulated by NSY-5 is only partly understood. We show that voltage- and Ca^{2+} -activated SLO BK potassium channels mediate gap junction signaling to inhibit Ca^{2+} pathways for asymmetric AWC differentiation. Activation of vertebrate SLO-1 channels causes transient membrane hyperpolarization, making it an important negative feedback system for Ca^{2+} entry through voltage-activated Ca^{2+} channels. Consistent with the physiological roles of SLO-1, our genetic results suggest that *slo-1* acts downstream of *nsy-5* to inhibit Ca^{2+} channel-mediated signaling to specify AWC^{ON}. We also show for the first time that SLO-2 BK channels are important for AWC asymmetry and act redundantly with SLO-1 to inhibit Ca^{2+} signaling. *nsy-5*-dependent asymmetric expression of *slo-1* and *slo-2* in the AWC^{ON} neuron is necessary and sufficient for AWC asymmetry. SLO-1 and SLO-2 localize close to UNC-2 and EGL-19 in AWC, consistent with functional coupling between these channels in AWC asymmetry. Furthermore, *slo-1* and *slo-2* regulate the localization of synaptic markers in AWC neurons to control AWC asymmetry. We also identify the requirement of *bkip-1*, which encodes a previously identified auxiliary subunit of SLO-1, for *slo-1* and *slo-2* function in AWC asymmetry. Together, these results provide an unprecedented molecular link between gap junctions and Ca^{2+} pathways for terminal differentiation of olfactory neurons. To identify the genes required for *slo-1* function in inhibiting Ca^{2+} signaling for promoting AWC^{ON}, we performed a non-biased forward genetic screen to isolate *mok* (modifier of K^+ channel) mutants that suppress the *slo-1(gf)* $2AWC^{ON}$ phenotype. From about 6000 genomes screened, we identified 16 new *mok* mutants that define genes required for *slo-1* function in promoting AWC^{ON}. We have identified the mutations responsible for these *mok* phenotypes using whole genome sequencing (kindly performed by Oliver Hobert's group). Molecular characterization of these *mok* genes will provide insight into how K^+ channels and Ca^{2+} channels may interact to coordinate stochastic AWC asymmetry.

W4046A Identifying factors that interact with PAX-3, a Paired-box protein involved in hypodermal cell fate specification in C. elegans. *M. CORREA-MENDEZ*[1], K. W. Thompson[1], P. Joshi[1], C. Y. Kang[1], J. Dymond[1], H. Smith[2], M. Krause[2], D. M. Eisenmann[1]. 1) University of Maryland Baltimore County, Baltimore, MD; 2) National Institutes of Health, Bethesda, MD.

The development of the *C. elegans* hypodermis is an excellent model for understanding cell fate specification and differentiation. Early events in *C.elegans* embryogenesis induce hypodermal precursor cells to adopt one of several fates among them, becoming dorsal, lateral or ventral hypodermal cells. The regulatory network that drives hypodermal cell fate specification is an intriguing process that still has much to be understood. While several transcription factors that function in specifying the major hypodermal cell fates have been identified, little is known about how the ventral and the lateral hypodermal cells are specified to adopt different fates and undergo distinct morphogenetic processes. Our findings begin to address how the ventral hypodermis is prevented from adopting a seam cell fate. Previously, we showed by mapping experiments and whole genome sequencing that *pvl-4* is the Paired-box gene *pax-3*, which encodes the sole PAX-3 transcription factor homolog in *C. elegans*. We (and others) report that *pax-3* is expressed in the ventral P cells during embryogenesis and early larval stages. Mutants for *pax-3*show embryonic and larval lethality as well as several body morphology defects that indicate abnormal cell fate specification in the hypodermis. Using several reporter genes we observed that in *pax-3* reduction-of-function animals the ventral P cells appear to undergo a cell fate transformation and adopt a lateral seam cell-like fate. Furthermore, forced expression of *pax-3* in the seam cells caused them to lose expression of seam cell markers. Based on these findings, we propose that *pax-3* functions in the embryonic ventral hypodermal cells to repress the lateral seam cell fate. *pax-3* promoter deletion analysis identified a *cis*-regulatory element that is necessary for *pax-3*expression in the embryonic P cells. Our current aim is to identify factors that regulate *pax-3* expression and that may function with *pax-3* in ventral cell fate specification. To do so, we used the Yeast One-Hybrid (Y1H) method to screen for factors that bind the *pax-3 cis*-regulatory element. Potential candidates are currently being analyzed.

W4047B Forward genetic screens for TLD mutants with defective localization of the TIR-1 Ca^{2+} signaling scaffold protein in left-right neuronal asymmetry. *Y.-W. Hsieh*, C.-F. Chuang. Department of Biological Sciences, University of Illinois Chicago, Chicago, IL.

The *C. elegans* left and right AWC olfactory neurons display stochastic asymmetry: one AWC subtype adopts the default AWC^{OFF} identity and the contralateral neuron is specified as the induced AWC^{ON} subtype. We previously showed that the TIR-1 (Sarm1) scaffold protein assembles a Ca^{2+}-regulated signaling complex at synaptic regions in the AWC axons, in a microtubule-dependent mechanism, to specify AWC^{OFF}. We also showed that proper localization of the TIR-1 Ca^{2+} signaling complex at the AWC synapses is important for precise AWC asymmetry.

To identify additional molecules required for the localization of TIR-1 in AWC subtype choice, we performed unbiased forward genetic screens to identify mutants with defective TIR-1 localization and AWC diversity. We screened through 8,560 genomes and identified six TIR-1 localization defective (*tld*) mutants with reduced localization of TIR-1 at synapses in the AWC axons, and/or accumulation of TIR-1 in the AWC cell body. Most of the *tld* mutants alone do not show a defect in AWC asymmetry. However, some *tld* mutants enhance the *tir-1(ky388)* reduction of function $2AWC^{ON}$ phenotype, supporting an important role of these *tld* genes in regulating TIR-1 trafficking and /or stability to specify AWC^{OFF}. We have identified the mutations responsible for the *tld* mutant phenotypes using whole genome sequencing (kindly performed by Alexander Boyanov in Oliver Hober's lab). Our study of the *tld* genes will provide insights into how cell-specific Ca^{2+} signaling proteins such as TIR-1 are linked to the conserved synaptic assembly in neuronal diversification. In addition to neuronal diversification, TIR-1 (Sarm1) is also implicated in innate immunity and axon degeneration. Identification and characterization of the genes, such as *tld*, that interact with *tir-1* in AWC asymmetry may advance our understanding of how TIR-1 (Sarm1) functions in other biological contexts including innate immunity and axon degeneration.

W4048C Investigation into the Regulatory Dynamics of LIN-1 and LIN-31, Transcription Factors Involved in *C. elegans* Cell Fate Specification. *R. Kousnetsov*, A. Dewey, L. Miller. Santa Clara University, Santa Clara, CA.

The Hox gene *lin-39* may play a role in initiating the 1° cell fate during vulval development in *C. elegans*. This cell fate specification process is triggered by a Ras/MAPK signal transduction cascade, wherein the transcription factors LIN-1 and LIN-31 are activated in order to upregulate expression of *lin-39*. However, the mechanistic details behind LIN-1 and LIN-31 regulation are not fully understood. Prior to activation, LIN-1 is SUMOlyated and serves as a transcriptional repressor, while the post-translational and transcriptional states of LIN-31 are unknown. Phosphorylation appears to convert LIN-1 and LIN-31 into transcriptional activators. Preliminary evidence from an electrophoretic mobility shift assay (EMSA) may indicate that SUMOlyation represses LIN-31 DNA-binding activity. Moreover, structural analysis of LIN-31 may suggest that SUMO serves as a linker that mediates the formation of a heterodimer between LIN-1 and LIN-31. Altogether, these findings may imply that LIN-1 and LIN-31 are concurrently regulated in similar manners, such that SUMOlyation acts as an OFF switch and phosphorylation acts as an ON switch for transcriptional activity. A variety of molecular techniques will be used to elucidate the regulatory dynamics of LIN-1 and LIN-31. EMSA and ChIP-seq will be used to determine the precise binding site of LIN-1 and LIN-31 on the *lin-39* promoter as well as to test the heterodimerization between LIN-1 and LIN-31. Further experiments with the CRISPR/Cas9 genome editing system will query whether particular structural elements on each transcription factor are necessary for proper regulation of vulval development.

W4049A The histone chaperone RBA-1 is critical for *C. elegans* postembryonic mesoderm development. *Jun Liu*, Stephen Sammons, Kevin Sun, Herong Shi, Vikas Ghai, Alice Hamilton, (Jun Liu is presenting the work primarily carried out by Stephen Sammons, who has left the lab). Cornell University, Ithaca, NY.

We use the *C. elegans* postembryonic mesodermal lineage, the M lineage, as a model system to study mesodermal patterning and cell fate specification. The M lineage arises from a single pluripotent cell, the M mesoblast, during embryogenesis. During postembryonic development of a hermaphrodite animal, the M cell undergoes a series of cell divisions to first produce 18 cells: 14 striated bodywall muscles (BWMs), 2 non-muscle coelomocytes (CCs), and 2 sex myoblasts (SMs), which further divide to produce the egg-laying vulval and uterine muscles (VMs and UMs). We and others have previously identified a number of transcription factors important for the proper patterning and fate specification in this lineage. In order to identify additional transcription factors that play a role in the M lineage, we used RNAi feeding and screened through over 500 putative chromatin-associated factors for their role in the M lineage using cell-type specific GFP reporters. One of the factors identified from the screen is the histone chaperone RBA-1. Both *rba-1(RNAi)* and *rba-1(0)* null mutants exhibit an extra VM phenotype. These extra VMs appear to arise from the fate transformation of M-derived CCs and BWMs to the VM precursors, the SMs. We have found that this M lineage defect is specific to *rba-1* loss-of-function, because a null mutation in the *rba-1* paralog *lin-53* did not cause any M lineage defects, and forced expression of *lin-53* failed to rescue the M lineage phenotype of *rba-1(0)* mutants. We are currently determining whether RBA-1 exerts its function by working with specific transcription factors important in CC and BWM fate specification in the M lineage.

W4050B SWI/SNF chromatin remodeling complexes interact with *hnd-1* and *let-381* to regulate the SGP/hmc cell fate decision. *L. D. Mathies*[1,2], D. M. Klompstra[2,3], E. E. Large[2,4]. 1) Virginia Commonwealth University, Richmond, VA; 2) North Carolina State University, Raleigh, NC; 3) NYU School of Medicine, New York, NY; 4) University of Missouri, Columbia, MO.

The two somatic gonadal progenitors (SGPs) of *Caenorhabditis elegans* are multipotent progenitor cells that give rise to all somatic tissues of the reproductive system, including uterus, sheath, spermatheca, distal tip cells and the anchor cell. Their sister cells are head mesodermal cells (hmcs), one undergoes programmed cell death and the other terminally differentiates shortly after it is born. We use this cell fate decision – SGP versus hmc – as a simple and genetically tractable model to understand the determinants of multipotency. Forward and reverse genetic approaches have identified *hnd-1/*dHand, *let-381/*FoxF and three different subunits of the SWI/SNF chromatin remodeling complex (*swsn-1, swsn-4,* and *pbrm-1*) as regulators of the SGP/hmc cell fate decision. Loss-of-function mutants in any of these genes have fewer than the normal number of SGPs and when the SGPs are present, they often express a marker that is characteristic of the hmc, suggesting that mutant

SGPs are partially transformed into hmcs. We examined *hnd-1; pbrm-1* double mutants and found that they have a synergistic phenotype, indicating that *hnd-1* and SWI/SNF act in parallel. We also found that *let-381* is co-expressed with *hnd-1* in the SGPs and *hnd-1* promoter deletions that remove three predicted LET-381 binding sites abolish expression of a rescuing *hnd-1::GFP* reporter in SGPs. Taken together, our data support a model in which *let-381* acts upstream of *hnd-1* and the SWI/SNF complex acts in parallel to *hnd-1* to regulate gene expression in SGPs. HND-1 is a transcription factor and SWI/SNF chromatin remodeling complexes influence gene expression by altering chromatin structure. Therefore, a simple model is that SWI/SNF acts by facilitating the binding of HND-1 to its target genes to regulate gene expression in SGPs. SWI/SNF chromatin remodeling complexes are important for the proliferation of mammalian multipotent progenitors. The identification of downstream targets of SWI/SNF in the SGP/hmc cell fate decision may therefore uncover conserved molecular mechanisms regulating multipotency in diverse species.

W4051C Genetic Control of the Maintenance of the AIA Cell Fate. *Josh Saul*, Takashi Hirose, Bob Horvitz. HHMI, MIT, Cambridge, MA.

Cell fate can be considered to involve two steps: establishment, in which an undifferentiated cell commits to a final, differentiated fate; and maintenance, in which a differentiated cell preserves the expression of its fate while precluding others. Cell-fate establishment has been well studied and is characterized by changes in cell morphology, gene expression and cellular function. Cell-fate maintenance has received comparatively little attention, and the mechanisms by which cell fate is maintained are poorly understood.

From a screen for *C. elegans* genes that specify cells for programmed cell death, we identified a gene, *ctbp-1*, that instead appears to be involved in maintaining one or more cell fates. *ctbp-1* encodes the worm homolog of the CtBP family of proteins, which in mice have been shown to act as transcriptional co-repressors that regulate gene expression during embryonic development. *ctbp-1* loss-of-function adult animals show ectopic gene expression in the AIA neurons, whereas L1 *ctbp-1* animals do not display this abnormality. Furthermore, adult but not L1 *ctbp-1* animals are defective in a turning behavior mediated by the AIAs. These observations indicate that AIA is normal in L1 but abnormal in adult animals, consistent with the hypothesis that *ctbp-1* mutants properly establish the AIA cell-fate establishment but fail to maintain this fate later.

We are currently attempting to test this hypothesis and to determine the mechanism by which *ctbp-1* might regulate cell-fate maintenance. We have performed a mutagenesis screen for suppressors of the misexpression phenotype of *ctbp-1* mutants to identify genetic interacting partners of *ctbp-1* and have identified 21 independent isolates that we are currently mapping. We have also performed RNA-seq to identify other genes misexpressed in *ctbp-1* mutants.

We hope to develop an understanding of the genetic control of cell-fate maintenance by specifically investigating how CTBP-1 promotes the maintenance of the AIA fate. This work might provide insight into diseases, such as cancer, in which perturbation of the maintenance of tumor cell fate might intervene with the oncogenic process.

W4052A Dissecting the roles of the zinc finger transcription factor SEM-4/SALL in distinct cell fate specification programs in the *C. elegans* postembryonic mesoderm. *Qinfang Shen*, Vikas Ghai, Chenxi Tian, Herong Shi, Jun Liu. Cornell University, Ithaca, NY.

We are interested in dissecting the regulatory network underlying the generation of multiple distinct cell types from a single pluripotent precursor cell. We are using the *C. elegans* postembryonic mesodermal lineage, the M lineage, as a model system. The M lineage is derived from a single pluripotent cell, the M mesoblast. During hermaphrodite postembryonic development, the M cell divides to produce fourteen striated body wall muscles (BWMs), two non-muscle coelomocytes (CCs), and two sex myoblasts (SMs) that are precursors of the sixteen non-striated egg-laying muscles. Mutations in the zinc finger transcription factor SEM-4/SALL lead to the transformation of M-derived CCs and SMs to BWMs (Basson and Hortitz, 1996). We have found that *sem-4* is expressed throughout the M lineage, and that overexpression of *sem-4* in the M lineage can lead to the fate transformation of CCs to SMs. These results indicate that SEM-4 is both necessary and sufficient to specify the SM fate, while the level of SEM-4 is critical for proper specification of M-derived CCs. The role of SEM-4 in SM specification appears to be mediated by the SoxC protein SEM-2, which is required and sufficient for SM fate specification (Tian et al., 2011): SEM-4 is required for *sem-2* expression in the SM mother and SM cells, and SEM-2 is required for *sem-4*-induced SM formation.

The SEM-4 protein contains seven zinc finger motifs of the C2H2 class. We have found that different *sem-4* alleles that affect different zinc finger motifs exhibit different effect on CC vs. SM fate specification. These observations suggest that the distinct zinc finger motifs in SEM-4 mediate distinct functions, either having different DNA binding activities or via interacting with different co-factors, or both. Current research aims at distinguishing between these different possibilities.

References:

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W4053B Identification of a novel Ral signal transduction cascade in *C. elegans* 2˚ vulval fate patterning. *Hanna Shin*[1], Rebecca E. W. Kaplan[2], Channing J. Der[2], David J. Reiner[1,2]. 1) Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX, USA; 2) Department of Pharmacology and Lineberger Cancer Center, University of North Carolina, Chapel Hill, NC, USA.

Vulval precursor cells (VPCs) are patterned through graded action of EGF from the anchor cell (AC) in conjunction with LIN-12/Notch-mediated lateral signaling. Together these signals control the highly reproducible 3˚-3˚-2˚-1˚-2˚-3˚ pattern that develops to form the vulva. A key protein in this process is LET-60/Ras, which in mammals is the most mutated oncoprotein. EGFR promotes LET-60/Ras activation to induce 1˚ fate via the canonical Raf-MEK-ERK MAP kinase cascade. We previously demonstrated that LET-60/Ras also switches effectors to activate its

effector RalGEF-Ral to induce 2˚ fate in support of Notch. In general, 1˚- and 2˚-promoting signals are strongly antagonistic. Thus, in equipotent cells Ras switches effectors between Raf and RalGEF-Ral to promote mutually antagonistic 1˚ and 2˚ cell fates, respectively. However, the Ral signaling cascade is poorly understood in all systems. We therefore screened candidate Ral binding partners from the literature. Two subunits of the Exocyst complex have been previously shown to bind Ral in other systems. We found that loss of Exo84 but not Sec5 caused the same phenotype as loss of Ral, suggesting that Ral binds to Exo84 to promote 2˚ fate. To move further downstream we analyzed a little known family of MAP4 kinases. In *Drosophila* Sec5 and Msn/MAP4K, the ortholog of *C. elegans* MIG-15, physically interact and promote JNK signaling, but function in opposition to Ral signaling. Consistent with the *Drosophila* genetics, our preliminary data in vulval development do not support the model that Sec5 and Msn function as a Ral effector. We hypothesize that the paralogous MAP4K subfamily, *C. elegans* GCK-2 and *Drosophila* Hppy, constitutes the real Ral effector. We found loss of GCK-2 phenocopies loss of Exo84, and GCK-2 functions cell autonomously downstream of Ral in vulva. Activated small GTPases often recruit effectors to membranes. Using CRISPR/Cas9, we tagged the N-terminus of endogenous GCK-2 with mNeonGreen. mNG::GCK-2 is localized in the cytoplasm of 1˚ and 2˚ cells, with perinuclear concentration. Thus, the mechanism by which Ral and Exo84 activate GCK-2 remains unclear. We will tag endogenous Ral with TagRFP to assess co-localization of mNG::GCK-2 and TagRFP::Ral in 1˚ vs. 2˚ before and after EGF induction. We also found that Ral-Exo84-GCK-2 signals through a conserved MAP3K/MLK-1-MAP2K/MEK-1-p38/PMK-1 cascade. This cascade may further activate MAPKAP kinase, MAK-2. We will generate p38::mNeonGreen to measure the activity of Ral signaling cascade through activation-dependent cytoplasmic-to-nuclear translocation of p38.

W4054C BAR-1 and CCAR-1 cooperate to properly position a subset of motor neurons along the AP axis. *Jeffrey Hung*[1,2], Matt Tanner[1,2], Antonio Colavita[2]. 1) University of Ottawa, Ottawa, Ontario, Canada; 2) OHRI, Ottawa, Ontario, Canada.

There are three classes of embryonically derived motor neurons (DD, DA and DB) that are organized into a very stereotypical pattern along the ventral nerve cord (VNC). For example, the six DD neurons (DD1-6) are evenly spaced along the AP axis and each are invariably situated between the same DA or DB neighbour. The planar cell polarity pathway (PCP) is a non-canonical Wnt pathway involved in establishing cell and tissue polarity. Recently we discovered that disruption of a PCP like pathway that includes highly conserved genes such as vang-1/Van Gogh and prkl-1/Prickle result in DD, DA and DB spacing defects. Imaging of comma to 1.5 fold stage embryos revealed that these defects are caused by delays in the cell intercalations that ultimately generates the linear profile of motor neurons along the ventral nerve cord. To identify new genes involved in motor neuron positioning or tiling we examined other conserved components of canonical and non-canonical Wnt pathways for DD position defects. We found that mutations in bar-1/beta-catenin displayed a highly penetrant phenotype in which DD1 and DD2 are clustered together at all larval stages instead of being properly spaced/tiled in the VNC. We found the same phenotype, although somewhat milder, in ccar-1/cell division cycle and apoptosis regulator-like-1 mutants. CCAR1 is a transcriptional regulator that in vertebrates has been shown to physically interact with beta-catenin to control the expression of Wnt regulated genes. In bar-1 and ccar-1 mutants DD2 is displaced anteriorly closer to DD1. PRY-1/Axin is a negative regulator of the Wnt signalling pathway as a part of a destruction complex involved in degrading BAR-1/β-catenin. Interestingly, pry-1 mutants also have a DD1-DD2 spacing defect but one in which DD1 is displaced posteriorly closer to DD2. In all three mutants, the spacing of the other DD neurons (DD3-6) are not strongly affected. These findings suggest that a non-canonical Wnt pathway may be involved in properly separating DD1 and DD2 during VNC formation or elongation of the embryo.

W4055A Centrosome-cortical contact duration affects anterior-posterior polarity in the one-cell *C. elegans* embryo. *D. M. Saturno*, D. Stephens, D. Parikh, E. Jaeger, R. Lyczak. Ursinus College, Collegeville, PA.

Anterior-posterior axis establishment occurs in the one-cell *C. elegans* embryo, triggered by the sperm-donated centrosome. While it is believed that the centrosome can cue polarity from a distance, centrosomes are pushed to contact the cortex during the polarization process. The role for this sustained cortical contact remains an open question. Centrosomes are mislocalized in one-cell *pam-1* embryos and polarity establishment fails. This phenotype is rescued however, when centrosomes are made to contact the cortex by inactivating dynein heavy chain. To examine in detail the requirement for centrosome-cortical contact, we used time-lapse confocal imaging of wild-type and *pam-1* mutant strains with GFP-tagged centrosome and PAR polarity proteins. We measured timing and duration of contact, as well as centrosome distance from the cortex and correlated these centrosome dynamics with the timing of symmetry breaking and PAR protein localization. We discovered that *pam-1* embryos had comparable centrosome-cortical distances from wild-type, but exhibited shorter centrosome-cortex durations. Very short durations in *pam-1* correlated with a delay in or lack of symmetry breaking in embryos. In addition, while centrosome distance from the cortex did not affect the extent of PAR localization, we found that duration of centrosome contact made a difference in *pam-1* mutants. Shorter duration times correlated with smaller PAR-1 posterior domains. Taken together, these results suggest that centrosome contact is required for robust polarization when *pam-1* is absent.

W4056B Suppressor screening to identify new regulators of anterior-posterior axis establishment in *Caenorhabditis elegans*. *E.Mae. Schleicher*[1], R. Alsher[1], T. Litz[1], A. Kilner[1], A. Kimble[1], K. Power[1], E. Jaeger[1], J. Lowry[2], B. Bowerman[2], R. Lyczak[1]. 1) Ursinus College, Collegeville, PA; 2) University of Oregon, Eugene, OR.

Polarity is established in the one-cell *C. elegans* embryo shortly after fertilization and determines the anterior-posterior body axis. This polarization is cued by the sperm-donated centrosome, which triggers changes in the posterior cortex. It is not well understood how the centrosome is positioned in the early embryo to trigger this polarization. Our previous work uncovered a role for the PAM-1 aminopeptidase in positioning the centrosome at the cortex to allow for proper axis polarization. PAM-1 is a highly conserved aminopeptidase that targets unknown proteins for degradation. In the absence of functional PAM-1, centrosomes are mispositioned and the anterior-posterior axis fails to establish correctly. In order to identify novel regulators of centrosome positioning and targets of the PAM-1 aminopeptidase, we took advantage of the maternal-effect embryonic-lethal phenotype of *pam-1* mutants to conduct a suppressor screen. We have identified six

suppressors, which significantly increase the *pam-1* hatch rates from 2% to between 25-96%. Two of these suppressors are dominant while the others are recessive. Using DIC microscopy we have found that each suppressor significantly rescues the polarity defects, such as the lack of pseudocleavage and the symmetric cleavage, found in *pam-1* mutants. We have also discovered that some, but not all, of our suppressors can rescue more than *pam-1* allele, pointing to different mechanisms of action. We are currently mapping the suppressor mutations to identify the molecular identity. Toward this end, we have used whole genome sequencing on two of our suppressors and are currently verifying the lesions to identify the suppressors in these strains. Future characterization of these suppressors is likely to uncover new insights into the role of this aminopeptidase in centrosome positioning and polarity establishment.

W4057C Development of Quantitative Imaging Toolkit to Monitor the Process of Symmetry Breaking. *P. ZHAO*[1,2], Z. ZHANG[2,3], H. T. ONG[2,3], T. KANCHANAWONG[2,3], F. MOTEGI[1,2,3]. 1) Temasek Life Sciences Laboratory, Singapore; 2) National University of Singapore, Singapore; 3) Mechanobiology Institute, Singapore.

Caenorhabditis elegans early zygote undergoes de novo polarization upon fertilization. Previous studies have shown that maturing centrosomes could break symmetry by destabilizing contractile network of actomyosin, leading to segregate PAR-type polarity regulators along the anterior-posterior axis. Centrosome maturation-defective mutants, however, can break symmetry during the later stage of first mitosis, and occasionally formed two posterior domains. To assess how singularity can be established and/or violated under the compromised centrosomes, we developed quantitative image-analysis tools to monitor subtle changes of cortical actomyosin and PAR proteins in response to centrosome movement with high temporal and spatial resolution. We observed in different mutant embryos that early zygote retains the competency to break symmetry throughout the first cycle, during meiotic to mitotic transition, upon mitotic entry, and even during cytokinesis. The quantitative imaging also observed roles of several mitotic kinases in global control of cortical actomyosin dynamics. We will discuss a progress of our quantitative screening to identify genes essential for cortical symmetry breaking in early zygotes.

W4058A Developmental and cancer cell invasion share regulatory pathway components. *Evelyn Lattmann*[1,2], Ting Deng[1], Charlotte Lambert[1], Michael Daube[1], Valerie Amann[2], Ines Kleiber-Schaaf[2], Mitch Levesque[2], Reinhard Dummer[2], Alex Hajnal[1]. 1) University of Zurich, Zurich, CH; 2) University of Zurich Hospital, Zurich, CH.

A critical step in metastasis formation by invasive tumor cells is the breaching of the basement membrane (BM), which is a natural barrier that separates sheets of cells. Besides its involvement in disease, BM breaching is essential for proper organ formation during normal development. Recent studies provide evidence that there are parallels between developmental and tumor cell invasion. One well-studied developmental invasion process is anchor cell invasion in *Caenorhabditis elegans*, where a specialized cell (anchor cell) in the uterus invades into the adjacent vulval epidermis. Because anchor cell invasion can easily be manipulated and observed, we use this system as a functional assay to characterize the *C. elegans* homologues of human genes, which are upregulated in invasive melanoma cells and during neural crest cell migration. From an initial list of 112 invasion-associated human genes we identified, through our functional *C. elegans* screen, 14 genes that showed an anchor cell invasion defect. Notably, three of the candidate genes encode cell cycle regulators and four genes are involved in protein sumoylation. Currently, the validated candidates are further studied using *in vitro* invasion assays with human melanoma cells and immunohistochemistry analysis of in situ and minimally invasive melanoma biopsies. Our long-term goal is to establish molecular markers of tumor invasiveness that may also be potential drug targets.

W4059B A promoter element in the *C. elegans nhr-67 tailless* gene mediates *hlh-2/daughterless* regulation of anchor cell differentiation and uterine organogenesis. *Caroline Berman*, Akshara Kartik, Sheila Clever, Bruce Wightman. Muhlenberg College, Allentown, PA.

The *tailless* family of nuclear receptors is highly conserved among animals. In humans, it functions in regulating neuronal stem cell differentiation. The *C. elegans tailless* ortholog, *nhr-67*, is expressed in a dynamic pattern in pre-uterine cells: initially in the 4 pre-VU cells during the L2, then upregulated in the anchor cell (AC) in response to the *lin-12/lag-2* Notch reciprocal signaling system. During the L3 stage, *nhr-67* expression is maintained at high levels in the AC and at low levels in VU descendants that produce the adult ventral uterus. *nhr-67* is required for expression of the *lin-12/Notch* receptor in pre-VU and VU cells and for multiple markers of AC identity, indicating that it functions in differentiation of both uterine cell types.

Deletion of a 276bp region of the *nhr-67* promoter results in a loss of *nhr-67* expression in pre-VU, AC, and VU cells. Expression of *276bp region::gfp* shows the region is necessary and sufficient for *nhr-67* expression during ventral uterine development. The region includes two E box sequences that we propose bind the HLH-2 transcription factor, which functions in AC and pre-VU development. We have performed site-directed mutagenesis to delete the E boxes and four other conserved elements from the 276bp promoter region and tested their functions directly *in vivo*. Our data demonstrate the primary role of the E box sequences in regulating *nhr-67* in the AC, pre-VU, and VU cells. *Funded by the NIGMS.*

W4060C The *fax-1* nuclear receptor of *C. elegans* functions in gonad development. *Sydney Saltzman*[1], Emily Bayer[1,2], Sheila Clever[1], Bruce Wightman[1]. 1) Muhlenberg College, Allentown, PA; 2) Columbia University, New York, NY.

The nuclear receptors constitute a class of conserved transcription factors that function in regulating animal development. The NR2E subclass nuclear receptor *fax-1* of *C. elegans* is expressed in hermaphrodite somatic gonad cells and is required for normal brood size, in addition to roles already described in neuron differentiation.

The *C. elegans fax-1* gene is the ortholog of PNR in vertebrates. We have found that *fax-1* is expressed in the migrating distal-tip cells (DTCs) of the hermaphrodite gonad from L2 through L4 stages. Loss of *fax-1* results in a low-penetrance DTC migration defect and a high penetrance brood size defect. The reduction in offspring appears to result from a defect in sperm production rather than oogenesis.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

The *vab-3* transcription factor plays a key role in regulating the migrations of the DTCs. In *vab-3* mutants, the DTCs continue to migrate into adulthood and also fail to turn off *fax-1*, suggesting that expression of *fax-1* is a property of a migrating DTC. A mutation in *fax-1* does not suppress the *vab-3* migration defect, indicating that *fax-1* is not an essential downstream mediator of *vab-3* function. We propose that *fax-1* function in DTC migration is a robustness phenomenon, in contrast to the more demanding requirement for hermaphrodite spermatogenesis. *Supported by NIGMS.*

W4061A The role of sumoylation in cell invasion. *A. Fergin*, E. R. Lattmann, C. A. Lambert, A. Hajnal. University of Zürich, Zürich, Switzerland.

Cell invasion plays crucial roles during normal development and in in many human diseases, especially in metastatic cancer. To date, the molecular mechanisms allowing cells to traverse through natural barriers like basement membranes (BM) remain poorly understood.

C. elegans is an excellent *in vivo* model to study cell invasion during normal development. During the L3 stage of hermaphrodite development, the specialized gonadal anchor cell (AC) breaches two BMs separating the gonad from the vulva precursor cells (VPCs). The AC then invades into the vulval epithelium to initiate vulval morphogenesis. Many genes regulating AC invasion as well as the structural components of the BM are highly conserved in *C. elegans*. Therefore AC invasion is a powerful model to study the first steps of tumor cell metastasis.

In order to identify novel regulators of anchor cell invasion, we performed a targeted RNAi screen and found that the components of the sumoylation pathway are important for AC invasion. By screening the genes encoding sumoylated proteins for a possible role during AC invasion, we identified *icd*-2 (inhibitor of cell death 2) and *iff*-2 (eukaryotic translation initiation factor 5A-2) as novel regulators of AC invasion. Both genes have orthologs in humans, contain classical and non-classical SUMO motifs and are essential for proper development. We are using a CRISPR/Cas9 approach to tag and further investigate the role of both of the identified proteins and sumoylation in cell invasion. Our results so far suggest that protein sumoylation is crucial for the AC invasion.

W4062B Study of antipsychotics-induced side effects in *C. elegans*. *M. Carretero*, V. Titova, R. Gomez-Amaro, M. Petrascheck. The Scripps Research Institute, La Jolla, CA.

Atypical, or second-generation, antipsychotics are the first line of defense in the treatment of schizophrenia and psychosis. One of the main side effects of treatment with antipsychotics is weight gain. Weight gain can lead to the development of a variety of conditions such as insulin resistance, diabetes, dyslipidemia, and increased cardiovascular risk. Over the past few years many studies have demonstrated that this weight gain is not only due to changes in metabolism but that treatment with antipsychotics can also induce hyperphagia through currently unknown molecular mechanisms. We developed a powerful microtiter-plate based system to measure food intake in *C. elegans* and have found that antipsychotics also induce hyperphagia in worms. The conservation of this phenotype allows us to take full advantage of the power of *C. elegans* as a model organism to study the genetics of antipsychotics-induced hyperphagia. We have used this system to analyze the signaling pathways involved in antipsychotics-induced hyperphagia, in addition to studying the effects on food intake in mutant *C. elegans* with defects in orthologues of human genes known to be affected in response to antipsychotics treatment.

W4063C Arecoline improves age-dependent motor functional decline and extends lifespan by activating GAR-2 receptor in motor neuron in *C. elegans*. *Yen-Chieh Chen*[1], Mu-Yun Tseng[2,3], Travis Mazer[4], Tsui-Ting Ching[1], Ao-Lin Hsu[2,3,4]. 1) Institute of Biopharmaceutical Sciences, National Yang-Ming University, Taipei, Taiwan; 2) Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei, Taiwan; 3) Program in Molecular Medicine, National Yang-Ming University and Academia Sinica, Taipei, Taiwan; 4) Department of Internal Medicine, Division of Geriatric and Palliative Medicine, University of Michigan, Ann Arbor, MI 48109, USA.

Aging is characterized by progressive decline in physiological functions with increasing chronological ages. Among all the age-dependent declines, loss of motor function represents one of the most prominent physiological declines in aging animals and humans. Our previous work has shown that motor neurons exhibit a progressive functional decline in the early life of *C. elegans*, while skeleton muscles remain functional until mid to late life. Here, we report that arecoline, a muscarinic acetylcholine receptor agonist can improve the age-dependent functional loss of the motor nervous system and significantly increase the lifespan of the animals. There are three different muscarinic acetylcholine receptors in worms, GAR-1, -2, -3. When we applied arecoline to these three muscarinic acetylcholine receptor mutants, we found that arecoline failed to extend only the lifespan of *gar-2* mutants, but not the other two. Therefore, this suggests that GAR-2 receptor, which is participated in the Gq signaling, might mediates the effect of arecoline on lifespan extension. Furthermore, *egl-8* (PLCβ), a downstream effector of Gq signals is also required for arecoline treatment to extend lifespan, suggesting that arecoline-induced lifespan extension may mediate through GAR-2/PLCβ pathway. Finally, we found that the over-expression of *gar-2* only in motor neuron is sufficient to rescue the arecoline-induced lifespan extension in *gar-2* mutants, suggesting that arecoline-induced lifespan extension acts specifically in motor neuron. Our findings have implicated a potential way to mitigate the age-dependent decline in motor activity. Further investigating the mechanism of action may eventually lead to the development of interventions that can increase both the heath span and the lifespan of humans.

W4064A S-adenosylmethionine synthetase-5, SAMS-5, in the regulation of longevity in *C. elegans*. *T. Ching*, C. Chen. National Yang-Ming University, Taipei, TW.

Previous studies have shown that S-adenosylmethionine synthetase-1 (SAMS-1) might be involved in the lifespan extension induced by dietary restriction (DR). In *C. elegans*, there are five S-adenosylmethionine synthetases, SAMS-1, SAMS-2, SAMS-3, SAMS-4, and SAMS-5. However, little is known with regard to the roles of other SAMs in the regulation of longevity. Through RNAi knockdown assays, we found that, like *sams-1*, inhibition of *sams-5* by RNAi or genetic deletion extends the lifespan of wildtype animals. Interestingly, unlike *sams-1* mutants,

sams-5 mutants does not exhibit the intestinal vacuoles accumulation phenotypes, which is a indication of defected lipid synthesis. The expression pattern of *sams-5* is also distinct from that of *sams-1*. In larvae stages, SAMS-5 is expressed in the intestine and a subset of neurons. However, the expression of SAMS-5 in the intestine is vanished in young adults. Genetic epistasis analysis suggests that *sams-5* is not required for the lifespan regulation of DR, in which *sams-1* plays an important role. Together, our studies suggest that *sams-5* might act through a different pathway from *sams-1* to modulate longevity.

W4065B Investigating the role of intestinal cell-to-cell communication in longevity in *C. elegans*. *C. Diehl*, K. Li, M. Peters. Oberlin College, Oberlin, OH.

In *C. elegans*, the intestine integrates nutrient uptake and systemic aging signaling. Low food or other stressors lead to the activation of key transcription factors, DAF-16 and SKN-1, initiating a set of adaptations resulting in decreased fecundity and increased lifespan. Recent research suggests that another feeding-induced intestinal process, the periodic calcium wave controlling the defecation cycle, is linked to aging and death. The terminal step of aging, death, is preceded by a fluorescent anthranilate wave (death wave) in the intestine (Coburn et al., 2012). We have investigated mechanisms underlying lifespan extension in an intestinal gap junction mutant, innexin (*inx-16),* that alters both the defecation calcium wave and the death wave.

In feeding animals, a rapid posterior to anterior calcium wave occurs synchronically with defecation. In the absence of functional *inx-16*, the intestinal calcium waves are markedly slower and sometimes fail entirely, causing serious defecation defects. Just prior to death, calcium levels rise in anterior intestinal cells leading to necrosis. The calcium and necrosis spread, producing the death wave. *inx-16* mutants do not produce these waves (Coburn et al., 2012).

inx-16 mutants exhibit increased lifespan compared to N2 which could be due to failed execution of the necrotic death wave. To determine whether adding functional intestinal INX-16 to adults could restore normal lifespan we performed an adult rescue of the mutant using a vitellogenin promoter. This adult intestinal rescue eliminated defecation defects but failed to normalize lifespan. This suggests that *inx-16's* extended lifespan is not due solely to a failure to die. We hypothesize that intestinal and/or nutrient deficiencies in early life induce a longevity program that cannot be altered later. We are investigating the nutritional state and the longevity molecular program in *inx-16* mutants. Feeding as measured by pharyngeal pumping rates appears normal. Activation of DAF-16 and/or SKN-1 is being assayed via analysis of nuclear localization in translational fusion lines. No significant increase in nuclear DAF-16 has been found in the mutants' intestines. Yolk protein production and oocyte uptake is also being scored as a downstream marker of DAF-16 and SKN-1 activation. Fluorescent imaging of vitellogenin::GFP fusions indicate defective yolk protein distribution in *inx-16* mutants and likely decreased production. This work will provide valuable insight into links between intestinal cell-cell communication, nutrient uptake and longevity.

Coburn C. et al. (2013) PloS Biology Jul;11(7):e1001613.

W4066C Bacillus subtilis and Caenorhabditis elegans are good friends. *V. Donato*, S. Cogliati, R. Grau. National University of Rosario. School of Biochemistry. CONICET. Microbiology Lab. Rosario, Santa Fe, Argentina.

It is believed that beneficial gut bacteria (probiotics) might positively contribute to the modulation of aging and the nervous system in their hosts. However, the mechanisms causing the effects due to probiotics remain poorly understood. In this way, the role of *in vivo* probiotic biofilm development during gut colonization and its effects on animal health and lifespan are poorly documented. For these reasons, we have investigated the ability of *B. subtilis,* a probiotic bacteria, to form a biofilm, to produce nitric oxide (NO) and to synthesize quorum sensing molecules in the *C. elegans* gut and we evaluated its effects on gut colonization, lifespan and in the modulation of the nervous system of *Caenorhabditis elegans*. The traslucent nematode *C. elegans* has proven to be an excellent model for the study of many central biological processes, including how the gut microbiota affects host physiology.The two wild-type *B. subtilis* strains used in this study were the domesticated and laboratory-reference strain JH642 and the isogenic undomesticated and wild *B. subtilis* strain NCIB3610. We also used mutant strains of this bacterium. N2 and mutant strains of *C. elegans* containing, as the food source, OP50 *E. coli* cells or *B. subtilis* as spores or vegetative cells or *P. aeruginosa* (PA14) were used in many essays. We developed lifespan experiments, thermotolerance and other stress essays. We cultured bacteria from worms, we studied biofilm development in the *C. elegans* gut, we used pulse-chase experiments to evaluate persistance and we measured NO and CFS under planktonic and biofilm conditions. Finally, we studied the intracellular routes that are involved in this bacteria-host interaction and the beneficial effects that this relationship produced in the nervous system of the host.Our work showed that *B. subtilis* spores are able to germinate, growth, make a biofilm, re-sporulate and persist in the intestine of *C. elegans*. Moreover, the proficiency of biofilm formation and the production of the anti-aging molecule NO and the quorum sensing pentapeptide CSF act separately to extend worm longevity. Furthermore, *B. subtilis* grown under biofilm-supporting conditions synthesizes higher levels of NO and CSF than under planktonic growth. We also could discover the routes involved in these effects and the property that this probiotic has to delay neurodegeneration produces by for example *P. aeruginosa* (PA14).In sum, *B. subtilis* can improve the health and lifespan of its host and its biofilm proficiency is a crucial factor.

W4067A Elucidating drivers of proteostasis decline by targeting age-related accumulation of insoluble protein. *Kathleen J. Dumas*, Niall Adams, Birgit Schilling, Ida Klang, Pedro Reis-Rodrigues, Karla Mark, Daniel Edgar, Dipa Bhaumik, Bradford W. Gibson, Gordon J. Lithgow. Buck Institute for Research on Aging, Novato, CA.

Defective protein homeostasis (proteostasis) is a key driver of many age-related diseases in humans and is recognized as a key element in normal aging. We developed a proteome-wide assay of proteostasis based on the accumulation of SDS-insoluble proteins during normal aging in *C. elegans,* utilizing mass spectrometry to identify specific insoluble proteins. We hypothesize that this accumulation of dysfunctional protein is a result of failure or decline in capacity of specific nodes in the proteostatic network. Comparison of multiple distinct proteome-wide

insoluble protein datasets yielded several hundred proteins which reproducibly appear in the insoluble fraction of old (aged to adult day 7 or greater) *C. elegans*. Analysis of these age-related insoluble proteins revealed significant enrichment for proteins involved in metabolism, protein folding, and translation. We have created transgenic animals expressing GFP tagged proteins from this set of reproducible age-related insoluble proteins, and are using these strains to investigate the drivers of proteostasis decline with age.

W4068B Investigation of medicinal and therapeutic effects of boronic acid compounds in an Alzheimer's Disease model of *Caenorhabditis elegans*. *D. Flaherty*, W. Lawless, L. Hall, A. McKelvy, D. Makholm, J. Larkin. Eckerd College, St. Petersburg, FL.

Alzheimer's Disease (AD) is a progressive neurodegenerative disease which affects an estimated 44 million people over the age of 65 (Alzheimer's Disease International). The aggregation of amyloid-β ($A\beta_{1-42}$) into "senile plaques" is a hallmark cellular pathology of the disease. Accumulation of $A\beta_{1-42}$ is a main target of therapeutic research due to the caustic and toxic nature of these aggregates. The *C.elegans* transgenic model GMC101 expresses full length human $A\beta_{1-42}$ in its body wall muscles, which aggregates and leads to permanent and severe paralysis, allowing for robust behavioral outcome to score for plaque burden. Similarly, strain CL2006 is an abundant expresser of Ab_{3-42} and provides a useful model for aggregate analysis.

Boronic acid compounds have been utilized in a wide range of drug therapies because of their high level of specificity and ability to interact with the proteasome. To date, these compounds have not been extensively studied in AD related pathology. We have hypothesized several potential benefits of boronic acid and boronic ester compounds to AD pathology, including enzyme inhibition and proteasome interaction. Our preliminary data indicate that boronic ester compounds have a major impact on the toxicity of $A\beta_{1-42}$ by significantly delaying paralysis in the GMC101 model. This result has been further supported via fluorescence microscopy with the use of the fibrillary stain Thioflavin T (ThT), which identified a substantial decrease in $A\beta_{1-42}$ aggregation compared to the controls. To further confirm our findings, we have also begun to conduct *in vivo* staining in live animals using the lipophilic-congo red derivative X-34, a more sensitive detector of of Aβ aggregates.

W4069C Neuronal HSF-1 cell non-autonomously regulates intestinal DAF-16 functions and longevity in *C. elegans*. *Ji -Yuen Kim*[1], Ao-Lin Hsu[1,2]. 1) Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei, Taiwan; 2) Department of Internal Medicine, Division of Geriatric and Palliative Medicine, University of Michigan, Ann Arbor, MI 48109, USA.

The insulin/IGF-1-like signaling pathway (IIS) is known to have a conserved role in aging. IIS negatively regulates the activity of the FOXO transcription factor DAF-16 and the expression of its downstream target genes. In particular, DAF-16 activity in the intestinal cells is required for the lifespan extension of the IIS pathway mutants. The heat shock transcription factor HSF-1 appears to regulate lifespan and stress resistance as well in *C. elegans*. Previous studies have shown that hsf-1 overexpression extends lifespan, while knock down of HSF-1 by RNAi shortens lifespan. HSF-1, like DAF-16, is required for the IIS pathway mutations to extend lifespan. Moreover, HSF-1 and DAF-16 might act together to activate the expression of a specific subset of genes to promote longevity. Interestingly, previous studies indicated that neural overexpression of hsf-1 is sufficient to promote longevity and it requires the activity of intestinal daf-16. However, how might neuronal HSF-1 communicate with intestinal DAF-16 remains unknown. The aim of our studies is to further dissect the molecular mechanism underlying this inter-tissue communications between neurons and intestine. First, we confirmed that intestinal DAF-16 translocates to nuclear and is activated in response to neural HSF-1 over-expression. The release of neurotransmitters and/or neuropeptides, via synaptic (SVs) and/or dense-core vesicles (DCVs) respectively, constitutes the primary mechanisms for the communication between neighboring or distant cells and organs in response to stimuli. We found that the nuclear localization of DAF-16 is significantly suppressed when several genes involved in DCV trafficking is inhibited by RNAi. Thus, a neuropeptidergic regulation of intestinal DAF-16 may be involved in the longevity phenotypes of HSF-1 OE animals.

W4070A The Spinal Muscular Atrophy Network (SMA) Regulates Insulin Signaling in Response to High-Glucose Diet in *C. elegans*. *Maegan Neilson*[1], Lauren Riley[1], Ryan Buehler[1], Emma Sikes[1], Kevin Deehan[1], Michael Mastroianni[1], John A. Hanover[2], Michael W. Krause[2], Michelle A. Mondoux[1]. 1) College of the Holy Cross, Worcester, MA; 2) National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

Spinal muscular atrophy (SMA) is a neurodegenerative disease that results in the loss of motor neurons, muscle atrophy, paralysis, and infant mortality. The causative gene of SMA is *SMN1*, which encodes a survival motor neuron protein and is implicated in the regulation of splicing. Our project seeks to establish and characterize a new, promising link between SMA and high-glucose diet in *Caenorhabditis elegans*. *C. elegans* is an excellent model to study SMA, as the *smn-1* gene is nearly 80% identical in worms and humans, and it is also a good model for to study the response to high-glucose diet due to the high level of conservation with the human insulin-signaling pathway.

Mutations in the *C. elegans* insulin receptor (*daf-2)* can lead to entry into an alternative larval stage called dauer. Previous studies have demonstrated that a high-glucose diet suppresses this effect due to the up-regulation of insulin signaling. We conducted a series of RNAi screens to identify regulators that are important for the insulin signaling response specifically on a high-glucose diet. We have identified 44 candidate genes. Interestingly, two of the most promising candidates are *smn-1* and *nekl-3*, the latter of which has been identified as a modifier of *smn-1*. This identifies a potential link between SMA, high-glucose diet, and insulin signaling.

We have completed dauer assays to verify and quantify the insulin specificity and glucose specificity of these 44 candidate genes. ~30 other genes have been identified as *smn-1* genetic interactors, and we have tested whether other members of this "SMA Network" are also involved in regulating insulin signaling. We find that the SMA Network genes are enriched for this response: ~25% are also required for the high-glucose diet suppression of dauer formation. SMA Network genes also seem to play a role in regulating other insulin-signaling phenotypes, including lifespan and stress resistance.

W4071B PROtein FEeding in CElegans (PROFECE) a new method to study gut-microbiota interaction during neuro/muscular development. *f. pio*[1], L. hueber[1], M. Mony[1], M. Cornell[1], A. Chang[1], B. Kong[1], V. Kooner[1], S. Shivji[1], A. Bikov[1], C. Rankin[2], A. Yu[2]. 1) simon fraser university, burnaby, bc, CA; 2) University of British Columbia, vancouver, bc,CA.

Similar to human, the intestine of C. elegans contain and process constantly E.coli bacteria since it fed on bacterial lawn. This observation led us to consider the bacterial lawn of C. elegans not only as a food source but as a microbiota environment. Subsequently, we can modify by genetic engineering this environment to study host-pathogen interaction in the gut of the nematode. Since a nodavirus in C. elegans called orsay was recently discovered we validated this method using this new viral system. In this study, we developed a novel method called PROtein FEeding in C. Elegans (PROFECE) that fed the worm with protein expressed in the E. coli bacterial lawn. We further investigated the effect of the viral proteins alpha and delta during development using survival assay, fluorescence microsocopy and behavioral assay with a multi-wormtracker. Survival assays shows that the viral proteins affect lifespan and provoke intestinal malfunctions and abnormalities. Finally, we observed using wormtrackers that worm fed with E. coli lawn expressing different viral proteins have behavior that is similar but different from the two well described mode of locomotion defined as roaming and dwelling in response to different type of food likelihood. Our results suggest that nematode growing in the presence of the orsay viral proteins have an altered muscular and/or neuronal function.

W4072C The role of Ca^{2+} permeability and Na^{+} conductance in cellular toxicity caused by hyperactive DEG/ENaC channels. *C. Matthewman*[1], T. Miller-Flemming[2], D. M. Miller 3rd[2], L. Bianchi[1]. 1) University of Miami Miller School of Medicine, Miami, FL; 2) Vanderbilt University, Nashville, TN.

Hyperactivated DEG/ENaCs cause neuronal death mediated by intracellular Ca^{2+} overload. Mammalian ASIC1a and *C. elegans* MEC-4(d) neurotoxic channels conduct both Na^{+} and Ca^{2+} raising the possibility that direct Ca^{2+} influx through these channels contributes to the intracellular Ca^{2+} overload. However, we showed that homologous *C. elegans* DEG/ENaC channel UNC-8(d) is not Ca^{2+} permeable yet it is neurotoxic, suggesting that Na^{+} cation influx is sufficient to induce toxicity. Interestingly, UNC-8(d) shows small currents due to extracellular Ca^{2+} block. Thus, MEC-4(d) and UNC-8(d) differ both in current amplitude and Ca^{2+} permeability. Given that these two channels show a striking difference in toxicity, we asked: *what is the contribution of Na^{+} conductance versus Ca^{2+} permeability to cell death in this channel family*. To test the contribution of Na^{+} influx to cell death we replaced Na^{+} with impermeant cation choline and compared current amplitudes and cell death in oocytes expressing either MEC-4(d) or UNC-8(d). We found that cell death is strongly suppressed in UNC-8(d) expressing oocytes under these conditions, but not in oocytes expressing MEC-4(d). These results suggest that main contributor to cell death is Na^{+} for UNC-8(d) and Ca^{2+} for MEC-4(d). To test this hypothesis, we sought to confer Ca^{2+} permeability to the UNC-8 channel by swapping its second transmembrane domain (TM2), which houses the selectivity properties of these channels, with MEC-4. Indeed we found that the UNC-8(d)/MEC-4(TM2) chimeric protein is Ca^{2+} permeable. In addition, the elimination of extracellular Na^{+} from oocytes expressing UNC-8(d)/MEC-4(TM2) did not prevent cell death. Similar results were obtained with oocytes expressing MEC-4(d). These data support the idea that MEC-4(d) Ca^{2+} permeability is conferred by TM2 and that Ca^{2+} contributes to cell death in oocytes expressing Ca^{2+} permeable DEG/ENaCs. To conclude, our data show that both Na^{+} and Ca^{2+} conductance can kill cells that express hyperactive DEG/ENaC channels. However, for Ca^{2+} permeable DEG/ENaCs, Ca^{2+} appears to play a larger role.

W4073A Knock-out of *C. elegans* sirtuin *sir-2.3* protects neurons from death. *R. Sangaletti*[1], M. D'Amico[2], D. Della Morte[3], J. Grant[1], L. Bianchi[1]. 1) Department of Physiology and Biophysics, Miller School of Medicine, University of Miami, Miami, FL; 2) Department for Life Quality Studies, Alma Mater Studiorum,University of Bologna, Rimini, Italy; 3) Department of Neurology, Miller School of Medicine, University of Miami, Miami, FL.

Neurodegeneration that follows a stroke, leads to a serious and devastating damages that affect people's quality of life. No therapeutic agents aimed at preventing neuronal death in stroke and at protecting patients at high risk are available or truly effective. Sirtuins are NAD^{+}-dependent deacetylase proteins found to influence the extent of neuronal death in stroke. However, different sirtuins appear to have opposite roles in neuronal protection. We used well characterized models of neuronal death such as *mec-4(d)*-induced death of touch sensory neurons and anoxic ischemia to test the role of sirtuins in neuronal death in *C. elegans*. We found that knock-out of this mitochondrial *sirtuin 2.3* confers neuroprotection in both models of neurodegeneration. Importantly rescue experiments demonstrated that the effect is mediated by *sir-2.3*. In addition we observed a maximum expression of *sir-2.3* mRNA in wild type animals 4-5 hours after exposure to hypoxic injury. These data confirm the involvement of the mitochondrial *sir-2.3* in neurodegeneration. Since caloric restriction protects against several neurodegenerative and cardiovascular diseases through the activation of sirtuins, we tested the involvement of caloric restriction in the neuroprotection provided by *sir-2.3* knock-out in *mec-4(d)*-induced excitotoxicity neuronal death. We used the well-known genetic model of caloric restriction *eat-2(ad1116)* mutation and we compared the extent of neuronal death in wild-type and *sir-2.3* knock-out backgrounds. Similar analysis was performed *in vitro* where cultured neurons were treated with 2-deoxyglucose (2-DG), which mimics caloric restriction. We found that in both *in vivo* and *in vitro*, caloric restriction enhanced the neuroprotective effect of knock out of *sir-2.3*, but had no effect on basal level of neurodegeneration. These data suggest a synergistic effect of caloric restriction and knock-out of mitochondrial *sir-2.3*. Experiments are under way to establish whether neuroprotection induced by the combination of caloric restriction and *sir-2.3* knock out is mediated by reduced Ca^{2+} overload in these neurons. Our study will lay the groundwork for developing future pharmacologic approaches to prevent and treat neuronal damage in neurological disorders including stroke.

W4074B The effect of sesame lignans on amyloid-beta toxicity in *Caenorhabditis elegans* model of Alzheimer's disease. *R. Keowkase*[1], N. Shoomarom[1], W. Bunargin[1], N. Weerapreeyakul[2]. 1) Srinakharinwirot Univ., Nakornayok, Thailand; 2) Khon Kaen Univ., Khon Kaen, Thailand.

Alzheimer's disease (AD), an age-related neurodegenerative disorders, is widely recognized as a serious public health problem. As lifespan increases, greater proportions of our population are affected by AD. AD is characterized by β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) observed in the brain tissue. The treatment of AD is currently limited to the symptomatic treatment with two classes of the US FDA approved drugs which are acetylcholinesterase inhibitors and N-methyl-D-aspartate receptor antagonist. These drugs have a limited efficacy and are considered to be effective only in a short period of time. At this point, there is still no approved treatment with a proven disease-modifying effect. This leads to the need for the development of effective compounds that can provide disease-modifying property. Oxidative stress is known to play an important role in AD, and there is strong evidence linking oxidative stress to Aβ. Sesame seed (*Sesame indicum*) has been known as a natural healthy food in East Asian countries. Many evidences suggested that sesame lignans including sesamin, sesamolin, and sesamol obtained from sesame seed possess antioxidant property. To test whether these sesame lignans exhibit benefit effects in AD, the nematode *Caenorhabditis elegans* (*C. elegans*) was utilized. To develop transgenic *C. elegans* model of AD, human Aβ is expressed intracellularly in the body wall muscle. The expression and subsequent aggregation of Aβ in the muscle lead to paralysis. The mechanism by which Aβ induced toxicity causing worm paralysis is believed to result from oxidative stress. The purpose of this study was to investigate the protective effect of sesame lignans (sesamin, sesamolin, and sesamol) against Aβ toxicity using *C. elegans* model of AD. Of these three sesame lignans, we found that only sesamin and sesamolin significantly delayed Aβ-induced paralysis. Sesamin and sesamolin delayed the paralysis in this model by 2 h and 1.8 h, respectively. This result suggests that sesamin and sesamolin can reduce Aβ toxicity. The mechanism by which sesamin and sesamolin delaying Aβ-induced paralysis is under investigated.

W4075C Nematode disease model of Niemann-Pick C yields pharmacological bypass suppressors. *E. O. Perlstein*. Perlstein Lab PBC, San Francisco, CA.

Perlstein Lab PBC (PLab) has built an automated model-organism-based drug discovery platform for rare genetic diseases, starting with Niemann-Pick Type C/NPC. Using CRISPR we generated null alleles of NPC1 orthologs in nematodes and flies, resulting in mutant animals that exhibit developmental delay and larval lethality, respectively. We then screened those models against a 50,000-compound library to identify small molecules that reverse disease phenotypes in whole animals. In less than a year, we discovered and validated a novel pharmacological bypass suppressor called PERL101. PERL101 is one of 14 unoptimized primary screening hits that modifies developmental phenotypes in ncr-1 null worms and cholesterol-storage phenotypes NPC patient fibroblasts. At the same time, PERL101 has excellent pharmaceutical properties such as 100% oral bioavailability, CNS penetration and tolerability in mice. Based on preliminary mechanism-of-action studies in both worms and mammalian cells, PERL101 modulates autophagy, an evolutionarily conserved pathway that has been implicated in NPC disease and other lysosomal storage disorders.

W4076A Characterizing the role of *swip-10* in the glutamatergic regulation of *C. elegans* dopamine neuron morphology. *C. Snarrenberg*[1,2], J. Balbona[1], J. Hardaway[1], A. Niedzwiecki[3], R. Blakely[2]. 1) Vanderbilt University, Nashville, TN; 2) Florida Atlantic University, Jupiter, FL; 3) Eckerd College, St. Petersburg, FL.

Precise coordination between L-glutamate (GLU) and dopamine (DA) neurotransmission drives many complex behaviors such as locomotion, reward and learning. Perturbations in the normal interactions of these neurotransmitters are believed to drive an array of brain disorders including addiction, schizophrenia and Parkinson's disease. Using the genetic model system *C. elegans*, we identified a conserved, glial-expressed gene, *swip-10,* that acts as a glutamatergic regulator of DA signaling. We have shown that mutations in *swip-10* lead to increased DA neuron excitability, elevated rates of DA vesicle fusion and the hyperdopaminerigic phenotype, Swimming induced paralysis (Swip). Glial-specific expression of wildtype *swip-10* restores normal DA neuron excitability as well as normal swimming behavior. Moreover, pre- and post-synaptic genetic disruptions of GLU signaling suppress Swip. Based on these studies, and well known findings that vertebrate glia regulate neuronal excitation via tight buffering of extracellular GLU levels, we hypothesize that *swip-10* functions in glial pathways that normally limit extracellular GLU availability and that loss of swip-10 expression drives GLU-dependent elevations in DA neuron excitation and DA release. Elevated extracellular GLU availability in the mammalian brain can alter neural morphology and ultimately lead to excitotoxic cell death. We find that *swip-10* DA neurons exhibit significant dystrophic processes, shrunken and/or missing cell soma, which are suggestive of progressive stages of excitotoxiticity. Glial-specific expression of wildtype *swip*-10 restores normal DA neuron morphology. Moreover, genetic disruption of GLU release by loss of the vesicular GLU transporter type 3 (*vglu-3),* suppresses the DA neuron morphology changes of *swip-10* mutants. Lastly, post-synaptic genetic disruption of GLU signaling through loss of select GLU receptors also suppresses *swip-10* DA neuron morphology alterations. Our studies indicate that whereas *swip-10* mutants exhibit hyperdopaminergia linked to the Swip phenotype, they also appear to be undergoing GLU-dependent morphological changes, consistent with an excitotoxic insult. Ongoing genetic, biochemical and pharmacological studies seek to further elaborate the glial pathways through which *swip-10* regulates extracellular GLU homeostasis and DA neuron morphology.

Supported by NIH Award MH095044 (R.D.B.).

W4077B Functional analysis of VPS41-mediated protection from β-Amyloid cytotoxicity. *E. F. Griffin*, K. A. Caldwell, G. A. Caldwell. University of Alabama, Tuscaloosa, AL.

According to the Alzheimer's Association 2012 report, 13% of Americans over 65 years of age, and approximately half of Americans over the age of 85, suffer from Alzheimer's Disease (AD), resulting in an estimated cost of 200 billion dollars for related health care. Additionally, AD is the most prevalent dementia, and the sixth leading cause of death in the United States. Thus, investigating mechanisms of pathophysiology and identifying potential therapeutic targets for AD is significant.

AD is characterized by the formation of plaques, composed primarily of the amyloid-β 1-42 (Aβ) peptide in the brain, resulting in

neurodegeneration. Our lab has observed that over-expression of human VPS41 in *C. elegans* provides neuroprotection from Aβ toxicity, and that depletion of VPS41 in worms expressing Aβ increases the neurotoxicity of Aβ. In yeast, VPS41 has been demonstrated to function in the tethering of vesicles, late endosomes, and AP-3-coated vesicles from the late Golgi, to the lysosome for degradation. Previously, our lab has shown that over-expression of human VPS41 is neuroprotective in a transgenic worm model of Parkinson's Disease, wherein dopaminergic neurodegeneration is induced by α-synuclein overexpression (Harrington et al., 2012, *J. Neurosci*.). VPS41-mediated neuroprotection from α-synuclein-based degeneration was abolished by mutation of putative phosphorylation sites in VPS41 and was dependent on its interactions with AP-3δ, RAB7, VPS core proteins, and VPS39. Thus, VPS41 has a role in lysosomal trafficking that impacts neuron survival.

The objective of this study is to determine how this specifically relates to cellular processing and ameliorates the impact of neurotoxic proteinaceous products. Here we report the results of a systematic RNAi screen whereby we knocked down the core components involved in lysosomal trafficking and categorized their requirement for Aβ protein toxicity. Our results indicate that the ARF-like GTPase *arl-8* mitigates endocytic Aβ neurodegeneration in a VPS41-dependent manner, rather than through *rab-7* and AP-3-coated vesicles from the late Golgi as with α-synuclein. Additionally, we suggest that the GABARAP homologue, *lgg-2*, plays a critical role in Aβ toxicity with *arl-8*. In this regard, further analysis of functional effectors of Aβ protein processing via the lysosomal pathway, along with subsequent evaluation in our worm neuronal model of AD (Treusch et al, 2011, *Science*), will assist in the elucidation of the underlying mechanism involving VPS-41-mediated neuroprotection.

W4078C Sexually antagonistic male signals manipulate germline and soma of *C. elegans* hermaphrodites. *I. Ruvinsky*, E. Aprison. Univ Chicago, Chicago, IL.

Males and females communicate via chemical signals, some of which are detrimental to the recipient. It has been reported previously that *C. elegans* hermaphrodites age more rapidly in the presence of male-secreted signals, even when no live males are present. Here we show that this is due, at least in part, to two distinct secreted signals via which *C. elegans* males manipulate reproductive physiology of hermaphrodites. The first, mediated by conserved ascaroside pheromones, counters the loss of germline stem cells that occurs during aging. The second signal, which is species-restricted, accelerates development and results in faster sexual maturation. Several conserved signaling pathways play specific key roles in these processes. The two modes of chemical communication we report promote males' evolutionary interests by ensuring that potential mates are mature and have sufficient gametes for successful reproduction. Effects harmful to hermaphrodites appear to be collateral damage rather than the goal of secreted signals produced by males.

W4079A Characterization of a CRISPR/Cas9 Mediated *C. elegans* HSF-1 Model Reveals a Complex Oxidative Response and Novel Oocyte Expression. *A. Deonarine*, M. Noble, L. Bowie, S. Westerheide. University of South Florida, Tampa, FL.

The transcription factor Heat Shock Factor-1 (HSF-1) regulates the Heat Shock Response (HSR), a cytoprotective response induced by various proteotoxic stresses. Recent data from model organisms also suggests that HSF-1 is involved in non-stress roles including the regulation of development and longevity. In order to better study HSF-1 function in a whole organism model, we have created a *C. elegans* strain containing HSF-1 tagged with GFP at its endogenous locus utilizing CRISPR/Cas9-guided transgenesis. Previous *C. elegans* models with tagged HSF-1 have relied on rescuing a mutant variant of HSF-1 in an exogenous locus, but it has been noted that mutant variants of HSF-1 can still retain some regulatory function in *C. elegans* and other organisms. This may lead to non-representational data of HSF-1 regulation collected in existing *C. elegans* models. Our endogenously tagged HSF-1 model should therefore provide more accurate data on HSF-1 function. We show here that our HSF-1::GFP is functional, as our worm strain behaves similarly to wild-type worms as well as to a previously described HSF-1::GFP model in response to heat stress, sodium azide, osmotic stress, and acute cadmium exposure. Upon further characterization of our HSF-1::GFP strain, we observe robust HSF-1 expression in the oocytes of *C. elegans*, which previously has not been reported, as well as a more complex response to oxidative damage than previously described. Further studies are planned with our model to investigate these and other potential new roles for HSF-1 in organismal biology. This model thus is unique in that it is the only endogenously-tagged HSF-1 whole-organism model currently available, and represents a novel experimental platform to study HSF-1 function within and outside of the HSR.

W4080B Identifying Intrinsic Modulators of Neuronal Resilience in the *C. elegans* Dopaminergic System. *A. Gaeta*, K. Caldwell, G. Caldwell. University of Alabama, Tuscaloosa, AL.

It has long been observed that a subset of individuals within a population respond more favorably to stress than the rest of the population. This study aims to uncover the intrinsic molecular and cellular factors that modulate the protective effects toward a specific stressor: the toxic misfolding of the human α-synuclein protein, which is implicated in the degeneration of dopamine neurons in Parkinson's Disease. Previously published work from our group has established that isogenic populations of the nematode *C. elegans* overexpressing α-synuclein from an integrated transgene exhibit reproducible and progressive age-dependent dopaminergic neurodegeneration. By utilizing this model system, we hypothesized that genetic and epigenetic factors, along with downstream targets of these factors, can be discovered that relate to the resilience exhibited by select animals in combating the stress of α-synuclein in dopamine neurons more effectively than others. We have developed a trans-generational behavioral enrichment scheme whereby individuals that express α-synuclein in dopamine neurons are selected for resilience in the context of the Basal Slowing Response (BSR), a quantifiable readout associated with dopamine neuron dysfunction. Selective propagation of animals that exhibit a BSR more like wild-type across subsequent generations has yielded heritable enrichment that has been found to last through four or more generations, after which it resets to resemble the defective BSR that individuals expressing α-synuclein in dopamine neurons characteristically exhibit. Along with examining the degeneration of dopamine neurons in strains of *C. elegans* over-expressing candidate neuro-protective genes, as well as strains of *C. elegans* under-expressing these same genes via RNAi, a better

understanding of the innate cellular mechanisms that resilient individuals employ to actively combat the stress of α-synuclein in dopamine neurons can be ascertained.

W4081C Investigating DNA damage response pathways after exposure to various heavy metals in *C. elegans*. *J. Hall*, S. Koga, S. Parag, K. Campbell, M. McMurray. Lincoln Memorial University, Harrogate, TN.

A major route of exposure to various heavy metals is through contaminated soil and water. Research has shown that these substances play roles in the induction of various diseases such as cancer, neurodegeneration and birth defects. In the cell, proteins such as metallothioneins respond to heavy metal exposure and chelate the metal to prevent cellular damage. However, little is known about the cellular response in regards to DNA damage after heavy metal exposure. To provide a better understanding of this cellular response, the induction of both cell cycle arrest and apoptosis were investigated after exposure to copper, cadmium, iron, lead, nickel and silver in the nematode *C. elegans*. Growth assays were conducted to determine EC10 and EC50 concentrations which were utilized to determine the DNA damage response pathway, apoptosis and/or cell cycle arrest, being induced upon exposure. Apoptosis and cell cycle arrest were observed in the germline after 24 hour exposure to the heavy metal. Apoptosis in the germline was induced in response to both concentrations of nickel. Additionally, apoptosis was also observed in the early embryo after nickel exposure suggesting an effect on reproduction rates. Both iron and silver exposure resulted in a slight increase in apoptosis at the EC50 concentration. Apoptosis and cell cycle assays for all metals tested will allow us to better understand the damage being caused by the metal exposure as well as mechanisms induced by the cell in response to exposure.

W4082A Functional regulation of the DAF-16 by CBP-1-dependent acetylation in response to multiple stressors. *A. L. Hsu*[1,2,3], C. Y. Liang[1], X. K. Yu[2], W. C. Chiang[3], D. Lombard[4]. 1) Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei, TAIWAN; 2) Department of Internal Medicine, Division of Geriatric and Palliative Medicine, University of Michigan, Ann Arbor, USA; 3) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, USA; 4) Department of Pathology, University of Michigan, Ann Arbor, USA.

All living organisms face a challenge to sense and respond appropriately to the environmental cues. In *C. elegans*, the FoxO transcription factor DAF-16 translocates into nucleus upon stress to regulate gene expression for organismal survival. The subcellular localization and activity of DAF-16 are tightly controlled by its post-translational modifications, including phosphorylation, acetylation, ubiquitination, methylation and glycosylation. In our previous study, we found that sirtuin family protein SIR-2.4, a homolog of mammalian SIRT6 and SIRT7, promotes DAF-16 nuclear translocation and DAF-16-dependent transcription under stress conditions. SIR-2.4 acts antagonistically with the acetyltransferase CBP-1 to negatively regulate DAF-16 acetylation. However, this modulation does not require the catalytic activity of SIR-2.4. We further identified four CBP-1-mediated acetylation sites on DAF-16 by mass spectrometry analysis. Mutations on some of these acetylation sites alter the kinetics of DAF-16 translocation upon stress, indicating a new nuclear localization signal of DAF-16. Furthermore, mutations on these acetylation sites affect both the lifespan and stress resistance of the animals, possibly due to an increased DAF-16 transactivating activity. Together, our studies identify acetylations on DAF-16 protein that significantly affect its functions in response to stress.

W4083B Environmental stresses induce transgenerationally inheritable survival advantages via germline-to-soma communications. *S. Kishimoto*, M. Uno, E. Nishida. Kyoto University, Kyoto, Japan.

Hormesis, a biologically beneficial response to low-dose exposure to stressors, has been reported to improve the functional ability of cells and organisms. Recent studies suggest that ancestral environmental conditions can influence the phenotypes of progeny. However, a key question of whether parents can transmit the beneficial memory of hormesis effects to their descendants remains largely unanswered. Here we show that hormesis effects induced in the parental generation can be passed on to the descendants in *Caenorhabditis elegans*. Animals subjected to various stressors during developmental stages exhibit increased resistance to oxidative stress and proteotoxicity. The increased resistance is transmitted to the subsequent generations grown under unstressed conditions. Remarkably, exposure of the male parents to these stressors also increases the stress resistance of the descendants and extends their lifespan. These findings suggest that the memory of hormesis effects can be transmitted to progeny through germline epigenetic alterations. Our analysis also shows that the insulin/ IGF-like signalling effector DAF-16 and the heat-shock factor HSF-1 in the parental somatic cells mediate the formation of epigenetic memory, and that the histone H3 lysine 4 trimethylase complex functions in the germline through generations to maintain the memory. Based on these results, we propose the germ-to-soma communications across generations as an essential framework for the transgenerational inheritance of acquired traits.

W4084C The *C. elegans* mitochondrial unfolded response induces dopaminergic neurodegeneration under prolonged cell-autonomous over-activation. *B. A. Martinez*, D. A. Petersen, S. P. Stanley, G. A. Caldwell, K. A. Caldwell. University of Alabama, Tuscaloosa, AL.

Mitochondrial protein folding disruption leads to recruitment of ATFS-1 in *C. elegans* to effect the mitochondrial unfolded protein response (mtUPR). Organismal ATFS-1 signaling has been linked to pathogen resistance as well as lifespan extension during prolonged electron-transport chain (ETC) disruption. However, after ETC disruption or successful pathogen avoidance, the mtUPR is presumably shut down to resume normal metabolism and protein regulation. Over-activation of this pathway, especially in the absence of stressors, is not especially well characterized from a cellular health perspective and is relevant to the mechanistic understanding of neurodegenerative disorders linked to mitochondrial dysfunction.

Here we describe consequences of over-activated ATFS-1 signaling in *C. elegans* neurons. To ascertain the role of cell-autonomous ATFS-1 we overexpressed cDNA construct variants in dopaminergic neurons and found that wild-type overexpression produces neuroanatomical changes including soma swelling. However, a constitutively active variant induces these neuroanatomical changes as well as severe age-dependent degeneration. This degeneration is not seen in variants of ATFS-1 with nuclear import or the bZIP domain mutations, suggesting that it is the

altered and ectopic gene expression induced by ATFS-1 which leads to neurodegeneration. Counterintuitively, the ATFS-1 degenerative phenotype can be attenuated by addition of a chronic stressor of the ETC such as rotenone, suggesting that cells may differentiate between chronic and acute signaling paradigms and may implicate organismal signaling mechanisms towards cellular survival. Next, we report a role for α-synuclein (αS), a Parkinson's disease-associated protein, in altering ATFS-1-mediated toxicity. We find that αS neurotoxicity is enhanced in gof ATFS-1 but reduced in a loss-of-function (lof) mutant. In addition, protein misfolding and αS::GFP bodywall aggregation is reduced in lof ATFS-1 animals suggesting differentially regulated protein handling may underlie ATFS-1 cellular toxicity. We confirmed that ATFS-1 could induce αS toxicity in a cell-autonomous fashion by crossing ATFS-1 cDNA variants into animals that express αS in dopaminergic neurons, finding degrees of age-dependent synergistic toxicity. Lastly, variants on αS possess the ability to differentially induce the mtUPR suggesting that αS may interact with the mitochondria, inducing stress, and potentially influencing cell survival through mechanisms associated with the mtUPR. These findings advance understanding of mitochondrial quality control insofar as organismal signaling and cell-autonomous health coordinate to maintain homeostasis.

W4085A Chromatin remodeling proteins influence the Heat Shock Response in *Caenorhabditis elegans*. *Mark Noble*, Andrew Deonarine, Lori-Ann Bowie, Sandy D. Westerheide. University of South Florida , Tampa, FL.

The Heat Shock Response (HSR) is an essential, highly conserved stress pathway that maintains protein homeostasis (proteostasis) by resolving misfolded proteins and detrimental protein aggregates. Recent studies in *C. elegans* have demonstrated that chromatin remodeling during the transition from larva to adulthood causes an abrupt decline in the heat shock response. To further our knowledge as to which chromatin remodeling factors may influence the HSR in an age-dependent manner, we have performed an RNAi sub-library screen of 62 chromatin remodeling factors across the larval stages to adulthood in *C. elegans*. Initiating RNAi at L1, we utilized a worm strain carrying the heat-inducible transcriptional reporter p*hsp-16.2*::GFP and the constitutive RFP construct p*dop-3*::RFP. We then treated the worms with or without heat shock at the L4/YA stage or 24 hours later during active reproduction. The regulators we identified to either positively or negatively influence the HSR in an age-dependent manner include Nucleosome Remodeling Factor (NURF) complex members, SWI/SNF family genes, and histone deacetylases. Of these hits, PYP-1, an inorganic pyrophosphatase and member of the NuRF complex, stood out as our strongest hit. PYP-1 negatively regulates the HSR only after the onset of reproduction. After this transition, *pyp-1* knockdown causes activation of both *hsp-70* and *hsp-16.2* transcriptional reporter animals during both non-stress and stress conditions. While *pyp-1* RNAi does not affect overall juvenile development and lifespan, it completely eliminates reproduction. This uncoupling of stress activation, reproduction, and lifespan by *pyp-1* knockdown appears to be a unique physiological feature, since cell physiological changes that activate stress responses typically trade-off reproduction and yield increased cell survival and longevity. We are interested in further characterizing PYP-1 function during the transition to adulthood as a way to epigenetically regulate the heat shock response upon aging and we are interested the mechanism in the uncoupling of longevity, reproduction, and development.

W4086B Determining the Role of DBL-1 TGF-β Signaling in the Response to Potential Therapeutic Compounds in the *C. elegans* Model System. *G. Ravindranathan*[1], R. A. Veettil[1], A. Gekombe[1], S. Ghosh[2], D. Dolliver[3], D. Hynds[1], M. Bergel[1], T. L. Gumienny[1]. 1) Texas Woman's University, Denton, TX; 2) Southeast Missouri State University, Cape Girardeau, MO; 3) Southeastern Louisiana University, Hammond, LA.

Development of therapeutic drugs is a challenging and lengthy process. It is vital to understand the reactions of the system being treated — both intended and side effects — and the best dosage for maximum efficacy with minimum side effects or long-term effects. Due to the complexity of human systems and ethical issues, the effect of a drug/compound cannot be directly tested without pre-clinical trials. *C. elegans* is an animal system for testing the toxicity of candidate compounds, and can provide preliminary data to justify the time, effort, and expense of pre-clinical studies in vertebrate models. We predict that *C. elegans* responds to toxic compounds using a conserved stress response pathway, the TGF-β pathway. Our goals are to 1) determine how *C. elegans* responds to potential therapeutic compounds, including nanocarriers and amidoximes (potential anti-cancer drugs), and 2) determine if the DBL-1 TGF-β pathway is required for a general stress response to toxic compounds. We first established whether potential drug delivery vehicles (three nanocarriers) and anti-cancer drugs (two amidoximes) show toxicity by determining the lethal concentration (LC50) on wild-type animals. Milder effects of these compounds on the organism were determined by assaying growth rate, body morphology, brood size, and aversion to the compounds. To determine the role of the DBL-1 TGF-β signaling pathway in the organismal response to abiotic stress, we are analyzing the effect of increased and decreased DBL-1 TGF-β pathway signaling on the animals' response to these and other established toxic compounds.

W4087C Interaction of telomerase deficiency with stress response pathways. *Maya Spichal*[1], Megan Brady[1], Chen Cheng[1,2], Shawn Ahmed[1]. 1) University of North Carolina, Chapel Hill, NC; 2) University of California, Berkeley, CA.

In the absence of telomerase telomeres shorten with very cell division leading to cell cycle arrest and senescence. Alternative lengthening of telomeres (ALT) can rescue cells from their proliferation arrest and was found to be the primary cause in 10% of all cancer types.

In *C. elegans* deficient for the telomerase reverse transcriptase subunit *trt-1*, telomeres shorten every generation affecting germline cells with little or no effect on somatic cells or lifespan (1, 2). While *trt-1* worms grown under optimal conditions become sterile after about 20 generations, *trt-1* worms grown under stressful conditions can survive indefinitely via ALT, suggesting an interaction between stress response pathways and telomere biology (3).

Additionally, telomere shortening could activate stress pathways long before complete telomere attrition as various forms of DNA damage were shown to trigger an innate immune response in *C. elegans* (4).

Under stressful conditions worms can develop into stress resistant dauer larvae to promote survival. Here we address two of the four dauer arrest stress response pathways. Preliminary results indicate that deficiency for telomerase in conjunction with deficiency for either the TGF-b

or the lifespan extending insulin/IGF pathway may each lead to distinct synthetic interactions that could be generally relevant to deficiency for telomerase in the context of aging or cancer.

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W4088A *in vivo* mechanisms of epithelial junction formation. *Jose Montoyo-Rosario*, Jeremy Nance. NYU School of Medicine, New York, NY.

Epithelial cells are specialized cells that line our organs and connect together with adhesive junctions. Loss of junctions compromises tissue structure and has been linked to human disease, such as kidney disease, cancer, and birth defects. We are investigating how cell junctions form in developing epithelia. Previous studies from our lab demonstrated that PAR-6 is required for junctions to mature in differentiating epithelial cells. PAR-6 functions together with the kinase PKC-3, but the targets of PKC-3 important for junction formation are largely unknown. Using a temperature-sensitive hypomorphic allele of *pkc-3*, we found that junctions in the spermatheca can break in mutant animals, leading to sterility. To find genes that function together with *pkc-3* to regulate junctions, we performed a suppressor screen to identify *pkc-3* mutants that are fertile at the restrictive temperature. We isolated 27 independent mutations, including six intragenic mutations in the *pkc-3* gene. We have also identified a new allele of a known *pkc-3* regulator, *lgl-1;* and identified two different alleles of *F22F4.1,* an uncharacterized gene. We are using whole genome sequencing to clone the remaining six mutants, and will characterize their function in junction maturation.

W4089B Non-autonomous roles of posterior *Hox* genes and SPON-1/F-Spondin in Q descendant migration. *Matthew P. Josephson*, Adam M. Miltner, Erik A. Lundquist. University of Kansas, Lawrence, KS.

MAB-5/Hox and LIN-39/Hox have well-defined opposing, cell autonomous roles in QL and QR migration, respectively. Here we demonstrate overlapping roles of these genes in Q descendant migration, and find a non-autonomous role for MAB-5 in this regard. The QR and QL descendants AQR and PQR migrate long distances in opposite anterior-posterior directions. The Hox transcription factor LIN-39 cell-autonomously promotes anterior migration of the QR lineage, and MAB-5/Hox cell-autonomously promotes posterior migration of the QL lineage. We have discovered parallel, roles for LIN-39, MAB-5, and another posterior Hox factor EGL-5 in migration of both QR and QL lineages. Additionally we show that MAB-5, and EGL-5 can function non-autonomously to promote Q lineage migration. Although alone, *mab-5* mutants have anteriorly-misdirected PQR (from the QL lineage), and *lin-39* have weak defects in anterior AQR migration (from the QR lineage), the *lin-39 mab-5* double mutant displayed severe anterior migration defects of both AQR and misdirected PQR. *mab-5* is not expressed in the QR lineage, and *lin-39 mab-5* defects were rescued by *mab-5* expression from posterior body wall muscles, demonstrating a non-autonomous role of MAB-5 in Q descendant migration. *egl-5* mutants displayed AQR and PQR defects that were rescued by muscle-specific *egl-5* expression, indicating a non-autonomous role of EGL-5 in AQR and PQR migration. In the triple mutant *lin-39 mab-5 egl-5,* AQR and PQR largely failed to migrate from the Q cell birthplace, indicating parallel roles of LIN-39, MAB-5, and EGL-5, likely in body wall muscles, in Q descendant migration. We hypothesize that *Hox* genes both pattern the posterior region of the animal creating an environment amenable for Q cell migration (non-autonomous role), and control how the Q cells respond to that environment (autonomous role). An RNA seq study identified *spon-1* as a potential MAB-5 target gene. *spon-1* encodes a secreted cell adhesion molecule implicated in muscle and neuronal adhesion similar to vertebrate *F-spondin.* We show that *spon-1* non-autonomously controls Q descendant migration through expression in posterior body wall muscles, and that MAB-5 activity drives expression from the *spon-1* promoter in body wall muscles, confirming the RNA seq result. Further studies are aimed at testing the idea that *spon-1* is a target of MAB-5 in body wall muscles. In sum, these studies demonstrate non-autonomous roles of Hox genes and SPON-1 in migration of the Q descendants AQR and PQR.

W4090C Analyzing phosphorylation of LIN-31, a transcription factor involved in *C. elegans* cell fate specification. *Hannah Kortbawi*, Alexandra Smallwood, Leilani Miller. Santa Clara University, Santa Clara, CA.

The development of the vulva in *Caenorhabditis elegans*, a soil nematode, is believed to be reliant on the phosphorylation of LIN-31, a winged-helix transcription factor. LIN-31 is part of the Ras/MAP Kinase (MAPK) cell-signaling pathway. When MPK-1, a protein in the Ras/MAPK pathway upstream of LIN-31, is phosphorylated, it enters the nucleus and phosphorylates LIN-31. MPK-1 can phosphorylate any of four consensus sites on the transcription factor, which are a serine or a threonine adjacent to a proline. It is not clear, however, whether or not all four consensus sites are phosphorylated by MPK-1. Understanding this is key to learning more about how LIN-31 affects vulval development. Using PIPE cloning and Gibson Assembly, a “phosphorylation library” of all of the different consensus site combinations was successfully created. This was verified by sequence analysis.

W4091A *C. elegans* immunoglobulin superfamily members, *syg-2* and *syg-1*, genetically interact with *mig-5/dishevelled* to control anteroposterior neurite growth of GABAergic motor neurons. *D. Tucker*[1], B. Ackley[1]. 1) University of Kansas, Lawrence, KS; 2) University of Kansas, Lawrence, KS.

Proper development of the nervous system requires directed axonal outgrowth. During early development in *C. elegans*, the VD GABAergic motor neuron axons are directed anteriorly. Previously, we found that *fmi-1/Flamingo*, the single *C. elegans* member of the Celsr-like cadherin

superfamily interacts synergistically with Wnt pathway components to regulate anteroposterior (A/P) axonal outgrowth of the VD neurons. In mutant animals, some VD neurons exhibit posterior neurite (PN) outgrowth instead of the stereotypical anterior neurite (AN). In a forward enhancer genetic screen for molecules that demonstrate synergy with the Wnt pathway component, *mig-5,* to direct A/P outgrowth, we recovered an allele of the cell-adhesion molecule *syg-2*. To facilitate our genetic analyses, we focused entirely on PNs that occurred in the tail of the animal, where there are normally no GABAergic axons present. In this we found that, individually, loss of function in either *mig-5* or *syg-2* resulted in a PN in approximately 2% of animals, while 25% of the double mutants had a PN. We found that mutations in the SYG-2 interacting protein, SYG-1, also demonstrated synergy with *mig-5* to direct A/P outgrowth, with a similar penetrance in the double mutants. Next we examined interactions between *syg-2* and *fmi-1.* Previously we had reported that 25% of *fmi-1;mig-5* mutants exhibited PNs, just like the *mig-5;syg-2* mutants. Double mutants of *syg-2* and *fmi-1* were not synergistic, rather they appeared to be additive (4% penetrance), although, it was also possible that these genes are functioning in a linear pathway. To determine this we examined the triple mutants of *fmi-1;mig-5;syg-2*, which exhibited PNs in 39% of animals. This suggested that *fmi-1* and *syg-2* were likely functioning in overlapping pathways, both of which are in parallel to *mig-5.* Recently however, during analysis of the *mig-5;syg-2* doubles we observed PNs emanating from DD6 (in L1 animals). Our previous work showed that the DD neurons were not affected by loss of function in *fmi-1*. Thus, it is possible the higher penetrance is due to defects present also in DD neurons. We are currently examining the L1 stage animals from the relevant mutant backgrounds to differentiate between these two models. We are also examining where *syg-1* and *syg-2* are functioning during GABAergic axon development.

W4092B A RhoGAP responds to axonal guidance signals to regulate actin nucleation during *C. elegans* morphogenesis. *Andre Wallace*, Martha Soto. Rutgers - Robert Wood Johnson Medical School, Piscataway, NJ.

The cell migrations that occur during *C. elegans* morphogenesis are meticulously regulated by rearrangement of the actin cytoskeleton. To understand how this process is regulated requires identifying the molecules that function to maintain the balance of forces during F-actin nucleation. Our lab previously showed that three axonal guidance signals, UNC-40/DCC, SAX-3/Robo, and VAB-1/Eph, function upstream of the CED-10/Rac-1 GTPase to modulate actin nucleation in a pathway centered on activation of the WAVE/SCAR complex. In an RNAi screen for regulators of the WAVE/SCAR pathway, we identified the *C. elegans* homolog of vertebrate Myosin IX, HUM-7. Loss of *hum-7* alone results in a small percentage of dead embryos, most of which have morphogenesis defects similar to what is seen in WAVE mutants. Like its vertebrate homolog, Myo9, HUM-7 has a myosin domain in its head and a RhoGAP domain in its C-terminus. Loss of *hum-7* suppressed embryonic defects in *sax-3* mutants, enhanced defects in *unc-40* and did not alter the embryonic defects observed in *vab-1* mutants. The effects of *hum-7* on these axonal guidance mutants is conserved post-embryonically since similar genetic interactions were observed in the guidance of AVM touch neuron. Loss of *hum-7* enhanced *unc-40* neuronal defects, suppressed *sax-3* but did not alter *vab-1* neuronal defects. To understand how an axonal signal acting on a RhoGAP results in morphogenesis defects, we tested which GTPase is being regulated by this candidate RhoGAP, and what loss of this protein does to F-actin levels and dynamics. While Rac is essential for epidermal enclosure migrations, genetic and biochemical tests suggest that HUM-7 is a GAP for Rho-1. Live imaging of F-actin in the migrating epidermis of the developing *C. elegans* embryo shows that loss of *hum-7* results in elevated actin levels and increased F-actin protrusions at the leading edge of the ventral cells. Similar phenotypes were observed in other mutants known to increase Rho-1 activity suggesting that HUM-7 functions through Rho-1 to modulate actin events during embryonic morphogenesis. The increased F-actin levels observed in *hum-7* mutants are similar to those observed in *vab-1* mutants. This observation, coupled with the fact that loss of *hum-7* causes no further impact on loss of *vab-1* embryonically and post embryonically suggests that HUM-7 is functioning in a pathway with VAB-1 to control RHO-1 during the ventral enclosure step of *C. elegans* morphogenesis.

W4093C Using *C. elegans* PVD Neurons to Functionally Validate Neuropsychiatric Risk Genes. *Cristina Aguirre-Chen*[1], Nuri Kim[2], Christopher M. Hammell[1]. 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) Medical Scientist Training Program (MSTP) at Stony Brook University, Stony Brook, NY.

One of the primary challenges in the field of psychiatric genetics is the lack of an *in vivo* model system in which to functionally validate candidate ***n***europsychiatric ***r***isk ***g***enes (NRGs) in a rapid and cost-effective manner. To overcome this obstacle, we performed a candidate-based RNAi screen in which *C. elegans* orthologs of human NRGs were assayed for dendritic arborization and cell specification defects using *C. elegans* PVD neurons. Of 66 NRGs, identified via exome sequencing of autism (ASD) or schizophrenia (SCZ) probands and predicted to result in a loss of protein function, the *C. elegans* orthologs of 6 NRGs were found to be required for proper neuronal development and represent a variety of functional classes, including transcriptional regulators and chromatin remodelers, molecular chaperones, and cytoskeleton-related proteins. We find that RNAi phenotypes associated with the depletion of NRG orthologs is recapitulated in genetic mutant animals, and, via genetic interaction studies, we place a subset of NRG orthologs in the SAX-7/MNR-1/DMA-1 signaling pathway. Collectively, our studies demonstrate that *C. elegans* PVD neurons are a tractable model in which to discover and dissect the fundamental molecular mechanisms underlying neuropsychiatric disease pathogenesis.

W4094A Impact of endocrine signaling on dendrite morphology during development. *C. E. Richardson*[1], K. Shen[1,2]. 1) Stanford, Stanford, CA; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

In the nematode *Caenorhabditis elegans*, the PVD sensory neuron has an elaborate dendritic arbor that enervates the majority of the worm's body wall muscles. During standard growth conditions, PVD dendrite morphology is effected through a well-characterized spatial and temporal growth program, and many of the molecular mechanisms which regulate this process have been described. We observe that adverse environmental conditions induce morphological plasticity in the development of the PVD dendritic arbor. This altered morphology is most

notable in regard to dauer diapause – the stress-resistant alternative to larval stage 3 (L3). Larvae can survive for months in the dauer stage and re-enter normal reproductive development as L4s when environmental conditions improve. There are two modes of dauer-related morphological plasticity of the PVD dendrite: first, dauer diapause induces suspension of dendrite growth; second, after recovery from dauer, PVD exhibits exuberant dendrite elaboration. Surprisingly, mutants that increase the propensity of larvae to enter dauer instead of L3, the *daf-c* mutants, exhibit exuberant dendrite growth without going through dauer, suggestive that it is the dauer signaling components rather than the dauer pause itself that regulates the exuberant growth phenotype. We are developing genetic tools to characterize the tissue-specificity of dauer signaling components in regulating PVD dendrite morphology, and we are identifying the cell-intrinsic dendrite outgrowth mechanisms that are alternately regulated to mediate this morphological plasticity.

W4095B Genetic suppression of basement membrane defects by altered function of the Myotactin/LET-805 receptor. *J. R. Gotenstein*, C. C. Koo, T. W. Ho, A. D. Chishom. University of California, San Diego, La Jolla, CA.

Basement membranes are evolutionarily ancient extracellular matrices that play key roles in tissue morphogenesis and organogenesis. In *C. elegans,* late embryonic development involves elongation of the embryo from a comma shape to a worm shape. Many basement membrane components such as type IV collagen and Perlecan are required for embryonic elongation. We previously showed that Peroxidasin/PXN-2 and F-spondin/SPON-1 are essential for epidermal elongation (Gotenstein, et al. *Development*. 2010; Woo, et al *Development*. 2008). Peroxidasins are extracellular peroxidases thought to catalyze sulfilimine cross-linking of collagen IV in the basement membrane. Complete loss of function of PXN-2 causes nonconditional lethality and arrest in late embryogenesis. F-spondins are conserved ECM proteins whose mechanisms remain unclear. We report that such *pxn-2* null mutant phenotypes can be suppressed to viability by gain of function mutations in a variety of extracellular matrix or cell-matrix receptor proteins. From large-scale screens for suppression of *pxn-2* or *spon-1* we identified over 30 suppressors, 12 of which result in missense alterations in the transmembrane protein myotactin/LET-805. The LET-805 extracellular domain contains at least 32 Fibronectin type III repeats (Hresko, et al. *JCB*. 1999); our suppressor mutations cluster in two pairs of repeats, suggesting these repeats may play a critical role in LET-805 function. *let-805* null mutants are nonconditional embryonic lethal; whereas our suppressors are viable and display no obvious phenotypes other than suppression. We find that *let-805* suppressor alleles partially suppress loss of function in other basement membrane components (type IV collagen) but not loss of function in intracellular epidermal cytoskeletal components (e.g. *vab-19*). Localization of a LET-805::GFP knockin is essentially normal in the suppressor alleles, suggesting they have a subtle effect on LET-805 expression. We will also present our analysis of additional suppressor loci from our screen. Our results reveal compensatory mechanisms in basement membrane receptor complexes that allow key structural or enzymatic components to be bypassed.

W4096C A regulatory genetic network in *C. elegans* embryos contributes to epidermal structural integrity during development. *M. K. Kelley*, D. S. Fay. University of Wyoming, Laramie, WY.

During development, biomechanical forces contour the body and provide shape to internal organs. We previously demonstrated that a regulatory network involving the conserved proteins SYM-3/FAM102A, SYM-4/WDR44, and MEC-8/RBPMS are required to maintain epidermal integrity in response to biomechanical forces in *C. elegans* embryos. Embryos and larvae that are doubly mutant for *mec-8; sym-3* or *mec-8; sym-4* have defective anterior morphology, called the Pharynx Ingressed or Pin phenotype. We showed that MEC-8 regulates the alternative splicing of multiple structural proteins including a fibrillin-like protein, FBN-1, which acts specifically in epidermal cells and is a component of the apical extracellular matrix (aECM or sheath). FBN-1 functions to withstand a variety of biomechanical forces acting on the epidermis, including an internally directed pulling force exerted by the pharynx as it undergoes stretch and elongation. Our current studies indicate that FBN-1 may attach directly to epidermal cells via integrins, which have not previously been reported to act within the epidermal aECM of embryos. Consistent with this, deletion of RGD (integrin-binding) sites in FBN-1 leads to induction of the Pin phenotype. In addition, mutations in *fbn-1*, *sym-3* and *sym-4* can enhance the "notched-head" phenotype (seen in integrin mutants) and inhibition of integrin subunits enhances Pin in *sym-3* and *sym-4* mutant backgrounds.

Our studies further indicate that SYM-3 and SYM-4 act in a parallel pathway to MEC-8 and may promote the trafficking of structurally important proteins in epidermal cells. Functional SYM-3 and SYM-4 fluorescent reporters localize to vesicle-like structures at or near the plasma membrane in epidermal cells. Furthermore, SYM-3 and SYM-4 act in a common genetic pathway with the RAB-11 GTPase, a known regulator of endocytic recycling and exocytosis, which has been shown to regulate integrin localization in other systems. Interestingly, *sym-4* is in an operon with *vha-20*/(ATP6-ap2/(P)RR, a multifunctional protein involved in vesicle acidification and planar cell polarity. Consistent with a previous study, we find that loss of *vha-20* perturbs RAB-11 localization and *vha-20(RNAi)* enhances Pin in *sym-4* mutants. In other studies, we have identified novel components of the aECM that function with FBN-1 to stabilize epidermal architecture during embryogenesis. We have also implicated roles for several cytoskeletal and cell-cell adhesion proteins in maintaining normal epidermal structure. Taken together, we have identified a network of genes that are required to maintain epidermal architecture in response to a variety of biomechanical forces during embryogenesis.

W4097A Axon guidance of the posterior lateral microtubule in *C. elegans* through VAB-1 activation by EFN-1. *E. M. Popiel*, I. D. Chin-Sang. Queen's University, KINGSTON, Ontario, CA.

Proper neural development is directed by numerous kinds of conserved axon guidance molecules, which includes a family of membrane-bound ligands and receptors called ephrins and ephrin receptor tyrosine kinases (EphRTKs). Previous research in the Chin-Sang laboratory has identified the EphRTK VAB-1 and its ephrin ligands EFN-1, EFN-2, EFN-3, and EFN-4 as key signaling molecules in the development of a pair of touch neurons in *C. elegans* called the posterior lateral microtubule (PLM) (Mohamed and Chin-Sang 2006). Loss-of-function *vab-1* mutants show a PLM overextension phenotype while constitutively active *vab-1* mutants show a strong premature termination phenotype in the PLM,

which suggests that activation of VAB-1 may play a key role in proper PLM termination during development. *efn-1* is the only ephrin ligand that shows a PLM overextension phenotype in the single loss-of-function mutant, although the triple *efn-2*; *efn-3*; *efn-4* loss-of-function mutant also shows a PLM overextension phenotype.

Since *efn-1* has the strongest PLM overextension phenotype of the ephrin ligands, we decided to investigate activation of VAB-1 by EFN-1 *in vivo*. Using ectopic expression of EFN-1 we have assessed its ability to activate the VAB-1 receptor to cause PLM termination when expressed on the same cell (*in cis*) and on neighboring cells (*in trans*). The ability of EFN-1 to activate VAB-1 to cause termination of the PLM axon both *in cis* and *in trans* in combination with our previous genetic analyses provides evidence that EFN-1 may activate VAB-1 *in vivo* to provide a stop cue for the PLM during development.

In addition to characterizing the activation of VAB-1 by EFN-1, we have also worked to identify the cellular location where the stop cue is produced to signal the PLM to halt its forward growth during development. We believe the source of the stop cue may be the seam cell V2, one of 10 specialized epithelial cells that run along the sides of the worm. This theory has been tested using genetic and molecular approaches, and our results indicate that there may be positional information exchanged between the PLM and V2, although more research is needed to clarify the nature of this interaction.

Our work adds to the current understanding of the role of EFN-1 in axon guidance in *C. elegans,* and the ability of epithelial and neuronal cells to communicate to facilitate development, and with further research this knowledge may be applied to human development.

W4098B Neurons and glia cooperate in assembly of the embryonic *C. elegans* nerve ring.. *G. Rapti*, S. Shaham. The Rockefeller University, New York, NY.

Central nervous system (CNS) assembly involves multiple cell-recognition events, including axon guidance and fasciculation. Despite extensive studies, how a connected brain emerges from these interactions remains enigmatic. Glia can act as guideposts for axon navigation, yet a detailed molecular understanding of this process is not currently at hand. To determine how pioneer neurons and glia cooperate to form a CNS, we are studying the assembly of the C. elegans CNS-like neuropil, the nerve ring (NR). The NR consists of ~170 axons and is enveloped and infiltrated by processes of four astrocyte-like CEPsh glia. CEPsh ablation does not result in neuronal death, allowing detailed investigation of glial roles in CNS assembly.

From a screen of fluorescent reporters expressed in subsets of embryonic neurons or glia we determined that the NR is populated in an orderly manner, with CEPsh extending processes early on, in conjunction with a small set of axons. Ablation studies reveal these pioneers are essential for NR entry insertion of diverse axons. We performed a forward genetic screen for mutants with NR entry defects resembling those in glia-ablated animals. In addition to known guidance factors, we isolated a new mutant that carries lesions in two genes, encoding a GTPase regulator and a pro-hormone convertase. Interestingly, each lesion alone results in only mild NR defects (5-15%); yet combining both lesions blocks 70% of NR entry of each of multiple neuron types. Importantly, full rescue of the double-mutant phenotype is achieved by combined expression of either gene in both pioneer neurons and glia. The mutant lesions result in defective axon guidance of pioneer neurons, they genetically interact with at least six known guidance pathways and they specifically affect intracellular trafficking of key guidance proteins. The synergistic nature of the double mutant, allowed us to perform screens for a novel class of axon-guidance mutants that function redundantly. We isolated over 20 such mutants, at least 14 of which appear to be in previously unknown genes. Our studies provide an inroad for studying CNS assembly in the light of neuron-glia communication and the complex redundancies of guidance pathways. Taken together, our findings suggest pivotal roles for glia in C. elegans CNS formation. This is highly reminiscent of roles of radial glia in the vertebrate brain. Furthermore, the mammalian homologs of the genes defective in our double mutant have known axon guidance roles in vertebrates, thus our studies will likely reveal conserved mechanisms promoting CNS assembly.

W4099C Novel reinforcement of Ras signaling by Rap1 in *C. elegans* vulval patterning. *N. R. Rasmussen*[1], D. Dickinson[2], D. Reiner[1]. 1) TAMHSC Institute of Biosciences and Technology, Houston, TX; 2) University of North Carolina, Chapel Hill, NC.

The small GTPase Ras is notorious as the most mutated oncoprotein in cancer. *C. elegans* and other systems were instrumental in defining the key components of Ras-Raf-MEK-ERK MAP kinase signaling cascade. However, the signaling activity of the small GTPase Ras is more convoluted than this. Decreased genetic redundancy and the anatomical simplicity of *C. elegans* provides an ideal setting to further dissect complicated Ras signaling during vulva cell fate specification. EGF induces Ras-mediated patterning of the six equipotent vulval precursor cells (VPCs) to generate the distinctive and highly reproducible pattern of 3˚-3˚-2˚-1˚-2˚-3 cell fates; presumptive 1˚ and 2˚ cells go on to form the vulva while 3˚ cells are non-induced and quiescent. Initial cell fate specification of the pattern is thought to be promptly followed by a reinforcement phase to enact final commitment, all prior to the first VPC division. We hypothesize that additional signaling mechanisms are executed to reinforce initial Ras-directed cell patterning and overcome contradictory signals within presumptive 1˚ VPCs. The small GTPase Rap1 (Ras proximal) is 100% identical to Ras in the core effector-binding region. Yet previous studies were inconclusive about Rap1 and Ras sharing effectors, particularly the S/T kinase Raf. Since *C. elegans* encodes a single Rap1 isoform that is not required for viability or fertility, we are using this system to define the role of Rap1 in the EGF-Ras signaling system. We N-terminally tagged endogenous Rap1 using CRISPR/Cas9. Tagged Rap1 localized to the plasma membrane of the six VPCs, consistent with published data of Ras promoter being active in VPCs, but providing more cell biological detail. Vulval-specific expression of mutationally-activated Rap1 was sufficient to hyper-induce VPCs. Consistent with Rap1 acting cooperatively with Ras, Rap1(RNAi) or deletion decreased 1˚ hyperinduction in a sensitized hyper-induced background. We also found that vulval-specific RNAi targeting the RapGEF, PXF-1, reduced hyper-induction, consistent with PXF-1 functioning to activate Rap1 in the vulva. We analyzed a transgenic transcriptional promoter::GFP fusion of the RapGEF, PXF-1. Prior to EGF signal, GFP was expressed at low levels in all six of the VPCs. After EGF signal, GFP expression was increased in presumptive 1˚ but decreased in presumptive 2˚ VPCs. Therefore, we hypothesize that Ras-Raf-MEK-ERK in presumptive 1˚ VPCs increases PXF-1 expression, and hence Rap1 activation, specifically in these cells

while Rap1 signaling is excluded from presumptive 2˚ VPCs. Thus the Ras-Raf signal is reinforced by a positive feedback loop. Our findings establish a novel aspect of Ras signaling, pointing the way to unconventional treatments for Ras-driven cancers.

W4100A Mechanosensing during *C. elegans* embryogenesis: Hunting for a putative mechanosensor. *S. Suman*, M. Labouesse. Institut de Biologie Paris-Seine, Paris, FR.

Mechanosensing refers to a process whereby mechanical inputs are sensed and transduced as a chemical or electrical signal to initiate or accelerate a downstream pathway. Many important biological events are mediated by this process including tissue differentiation, morphogenesis and also in pathophysiological conditions such as tumor progression, but their mechanisms at a molecular level remain poorly understood.

Mechanotransduction signaling *in vivo* can be triggered by body-wall muscle contractions (elongating *C. elegans* embryos), cell shape changes (*Drosophila* embryos), hydrodynamic forces (ventricle of mouse brain), or blood flow (heart development in zebrafish). During *C. elegans* development, embryos elongate 4-fold without additional cell division or migration. This process is highly robust and invariable. Previous studies demonstrated that hemidesmosomes (cell attachments to the extracellular matrix) play a critical role in mechanosensing by stabilizing the scaffolding protein GIT-1 in response to muscle input, but little is known about how it works at the molecular level (Nature, 2011 (471) 99-103). Therefore we aimed to characterize a putative binding partner of the earliest known effector (GIT-1) of mechanosensing. For this, we used both *in vitro* and *in vivo* (CRISPR-CAS9) tools to identify its binding partners (if any). Interestingly, we found that a core component of the hemidesmosome *i.e.*, VAB-10 strongly interacts with GIT-1 molecularly and genetically. Far-Western blotting suggests that purified VAB-10 and GIT-1 interact molecularly *in vitro*. Genetic interaction studies revealed that a mutation affecting the SH3 domain present within a spectrin repeat of VAB-10 strongly interacts with mutations in *git-1, pix-1* and also with *pak-1* (players of the mechanotransduction pathway). In double mutants background, embryos arrested prior to the two-fold stage, a phenotype also noticed in hemidesmosome-deficient embryos. This result suggests that the SH3 domain of VAB-10 provides an interaction platform for diverse players in response to physical cues in mechanosensing pathway. We would also like to extend our studies in the context of characterizing mechanosensing properties and molecular cause of such phenotypes using a combination of biophysical tools (AFM) *in vitro,* and genetic tools (transgenic line) *in vivo* respectively. Thus, understanding how mechanosensors and their signaling cascades operate should elucidate a cellular phenomenon in a better way.

W4101B A Genetic Screen for Temperature-Sensitive, Morphogenesis Defective Mutants in *C. elegans*. *Molly Jud*[1], Josh Lowry[1], Erin Clifford[1], Yuqi Yang[1], Hong Shao[2], Nhah Tran[2], Zhirong Bao[2], Bruce Bowerman[1]. 1) University of Oregon, Eugene, OR; 2) Memorial Sloan Kettering Cancer Center, New York, NY.

Embryonic morphogenesis in *C. elegans* is the process by which an ovoid mass of cells reshapes itself to take on the final elongated larval shape. Embryonic cells are quite dynamic during this process, undergoing both positional and morphological changes as they differentiate and form mature tissues. Many genes required for this dynamic behavior are also required earlier in development, complicating the identification of specific morphogenetic requirements using either non-conditional alleles or RNAi. Conditional alleles can bypass early genetic requirements and thus provide an excellent tool for dissecting later stage genetic pathways. We have isolated a collection of ~1,000 temperature-sensitive embryonic lethal (TS-Emb) mutants as a valuable resource for the identification of genes that influence embryonic morphogenesis. We now seek to identify all of the morphogenesis defective mutants within this collection, and to positionally clone as many of them as possible. In the longer term, we propose to build a systems-level model of embryonic morphogenesis.

To date, we have identified 68 mutants with penetrant embryonic morphogenesis defects. Most of these mutants arrest without extensive elongation, while others arrest after significant elongation (≥2-fold stage). We are initially classifying these terminal phenotypes using DIC microscopy. We also are scoring heterozygous embryonic lethality at the restrictive temperature and segregation frequency after outcross to ensure we are working with single gene, loss of function alleles. So far, nearly all alleles observed have behaved as expected for recessive, loss of function mutations.

We are using a high throughput whole genome sequencing-based approach to positionally clone mutants identified in this screen. This approach has been previously described[1,2] and applied to TS-Emb mutants affecting various stages of development[3,4], and both provides genome wide SNP mapping data and uncovers all homozygous SNPs within the mapping region. Genetic complementation tests against known loss of function alleles are then used to determine gene identity. So far, we have cloned 10 alleles representing 7 genes: *glp-1* (3 alleles), *let-19* (2 alleles), *klp-18, chaf-1, zwl-1, rib-1, hlh-1.* We anticipate that some of these genes will have direct roles in morphogenesis, while others may represent partial loss of function mutations in genes with earlier more general requirements in metabolism or cell division. Ultimately, we aim to clone 200+ mutants using this approach. These alleles will provide a resource that augments other efforts to build a detailed model of embryonic morphogenesis in *C. elegans*.

W4102C Male Specific Neurogenesis Depends on the Sexual State of the Seam in *C. elegans*. *Noah J. Reger*, Douglas S. Portman. University of Rochester, Rochester, NY.

In order to generate two functionally distinct sexes, sex-determination mechanisms must work in conjunction with generic developmental pathways to bring about unique changes in cellular differentiation and morphogenesis. How sex-determining factors generate dimorphic features from an otherwise identical cellular lineage is poorly understood. An example of this is ray neurogenesis in *C. elegans*. In males, the posterior seam cells give rise to neural precursors that generate rays, sensory organs required for mating. The DM gene *mab-3* and the Hox gene *mab-5* function in the male seam to activate the proneural gene *lin*-32, which triggers the ray sublineage. However, the activity of these genes does not appear to be restricted to males, making it unclear why ray neurogenesis is sex-specific. In order to better understand how the sexual identity of the seam affects ray neurogenesis, we used seam-specific promoters to drive expression of activators or inhibitors of the

master sex-determination regulator *tra-1*. This cell-autonomously "flips" the state of the sex-determination pathway, genetically reversing seam sexual identity. Interestingly, we found that masculinization of the hermaphrodite seam resulted in the development of ray neurons as well as a loss of alae formation in the posterior of the animal, indicating that "maleness" in the seam is sufficient to bring about ray neurogenesis. Masculinized hermaphrodites also displayed a mild tail retraction phenotype and appeared to form a male tail seam or SET cell. Surprisingly, these animals also have an exploding vulva phenotype, suggesting a possible role for seam sexual identity in vulva morphogenesis. Conversely, feminization of the male seam resulted in a significant loss of rays and the generation of ectopic alae as well as a reduced fan. Together, these data demonstrate that the sex-determination pathway functions in the seam to promote male specific neurogenesis. Using this sex-reversal strategy, we hope to identify how *tra-1* regulates gene expression in the seam to promote male-specific activation of *lin-32*.

W4103A Scaffolding Cells and Associated Molecular Factors in *C. elegans* Nerve Ring Development. *Kris Barnes*, Pavak Shah, Li Fan, Anthony Santella, Nhan Nguyen-Tran, Zhirong Bao. Memorial Sloan Kettering, New York, NY.

The embryonic development of the *C. elegans* nerve ring has remained an open and largely unexplored problem since John White mapped the nervous system in 1986. Genetic mutants with abnormal post embryonic nerve ring morphologies exist, but the specific cellular events in the developmental process and the function of associated molecular factors have not been well characterized. This is in part due to the difficulties involved with combining high resolution imaging with already time consuming forward genetic screens. We hypothesize a scenario where specific cells act as a scaffold to draw in surrounding axons and promote outgrowth along a concise and consistent path. This process must involve at least 3 main components: the development of the scaffold itself, local paracrine signaling which causes spatially separated axons to be drawn in to the scaffold, and a stage where cell specific juxtacrine factors promote the outgrowth of axons along specific paths. Initial anatomical observations point to several possible areas of interest which lie adjacent to the proto nerve ring including the GLR's, the pharyngeal primordium cells, and surrounding rows of neuroblasts which also lie parallel yet lateral to the incoming axon fibres. We aim to establish a functional relationship at the level of causation to determine which of these areas are involved in the scaffold. Furthermore, multiple incoming axon bundles are being studied for their spatial and temporal relation, and juxtacrine factors of interest are being identified with localized expression. We are finding specific genetic and anatomical perturbations leading to impairment of these processes, including results from mutant analysis and automated cell specific ablation experiments.

W4104B Dissecting *paired-box* and *odd-skipped* transcriptional networks. *Amy C. Groth*, Elizabeth J. Del Buono, Elizabeth A. Schoell, Gabriella M. Scoca. Eastern Connecticut State University, Willimantic, CT.

Paired-box (*pax*) and *odd-skipped* (*odd*) are two conserved families of developmental transcription factors. We are studying these two transcriptional families in *C. elegans*, in order to uncover genetic redundancies and functional interactions. In mammals, ODD proteins affect the development of a variety of tissues, including kidney, heart and soft palate, and are implicated in pancreatic and gastric cancer. Mammalian PAX proteins also affect the development of a wide variety of tissues, including palate and kidney. These two transcriptional pathways have been shown to interact in higher animals. Loss of function of *Odd* genes leads to altered levels of Pax2/8 in frog and mammalian kidneys. Mutation of *Pax9* and *Odd-skipped related 2* (*Osr2*) lead to cleft palate in mice, and PAX9 positively affects the expression of OSR2 in that tissue. *C. elegans* has 5 *pax* genes, *pax-1, pax-2, pax-3, egl-38 and vab*-3. Genetic interactions between *pax-2* and *egl-38* have been previously studied, but not between the remaining family members. Preliminary data shows that RNAi against *pax-3* increases lethality in a *vab*-3(*e1062)* mutant strain. It has been previously reported that knockdown of either *C. elegans odd* genes (*odd-1* or *odd-2*) leads to high levels of early larval lethality, due to defects in intestinal development. In our hands, an *odd-1*(*tm848*) mutant that deletes most of the gene or enhanced RNAi against *odd-1* do not result in high-level larval lethality. Fluorescent reporters of each *odd* gene (generous gift of the Rothman lab) show expression in adult posterior intestinal nuclei, with the brightest expression of ODD-1::GFP in Int9R. ODD-2 additionally shows strong expression in what appears to be the three rectal gland cells. RNAi against *odd-1* reduced the expression of ODD-2::GFP in the posterior intestinal cells, but not the rectal gland cells. Studies have been initiated to identify interactions between *odd* and *pax* in *C. elegans*. RNAi against the *pax* gene, *vab-3*, in an ODD-1::GFP strain resulted in variable brightness and number of GFP-expressing intestinal nuclei. This variability is consistent with a previous finding of a variable transgene expression phenotype found by RNAi against *vab-3*. RNAi against *pax-1* led to a slight deformity in the posterior intestine. Three *pax* genes have putative ODD binding sites in their promoters; experiments are planned to study the effect of *odd* mutants/RNAi on the expression of these genes.

W4105C The Transcription Factors LIN-31 and LIN-1 Play a Role in C. elegans Vulval Morphorgenesis. *L. M. Miller*. Santa Clara Univ, Santa Clara, CA.

The *C. elegans* hermaphrodite vulva is an ectodermal organ that connects the internal gonad with the external environment. Vulval morphogenesis involves cell-fate specification, cell adhesion, cell fusion, cell migration, and cell invasion. While much progress towards understanding signaling pathways has been made, an understanding of later morphogenetic events and how they are connected to cell fate specification is limited. The different fates of the six VPCs are determined in response to several extracellular signals and divide three times in a particular pattern to produce 22 descendants that begin as a linear array, then a tube, and finally a stack of seven toroids, with the anchor cell at the apex of this tube connecting to the uterus. The anchor cell then fuses to the utse uterine multinucleated cell, causing the opening of the tube. While the biochemical events that transduce the signal in the Ras/MAPK pathway are well characterized, it is ***not well understood*** how two transcription factors downstream of MAPK (LIN-31 and LIN-1) activate their downstream targets and play a role in vulval development. Specifically, the latter events (cell arrangements, invagination, toroid formation, cell migration, and cell-cell fusion events) are still ***not well understood***.

LIN-31 contains a winged-helix (WH) DNA-binding domain (DBD), a small acidic domain (AD), a putative MAPK D-domain docking site

(MAPKDS), four MAPK consensus phosphorylation sites (MAPK sites), and a homology region (RII). Site-directed mutagenesis revealed that the DBD, AD, and RII are important for LIN-31's function in vulval development. In addition, heterologous transcription assays in yeast indicated that that both the AD and RII function can act as transactivation domains. In contrast, we observed no measurable effect on LIN-31 function when we disrupted all four MAPK sites or the MAPKDS.

Since LIN-1 is also phosphorylated by MAPK during vulval development, we tested the effect of the loss of LIN-31 phosphorylation in a genetic background containing a point mutation in the LIN-1 MAPK docking site. In this ***sensitized*** genetic background, this mutation by itself does not display a strong Egl phenotype, however, when ***both*** LIN-31 and LIN-1 are defective for phosphorylation, we observe a ***strong Egl phenotype***. The identification of a sensitized background in which to study LIN-31 phosphorylation has also allowed us to identify a putative MAPK docking site on LIN-31, and preliminary confocal analysis of the *lin-31; lin-1* double phosphorylation-defective mutant suggests that the strong Egl phenotype is due to defective toroid formation.

W4106A Protein phosphatase 2A is crucial for muscle organization in *C. elegans*. *Hiroshi Qadota*[1], Yohei Matsunaga[1], William Vander Pols[1], Emily Swartzbaugh[1], Kristy Wilson[1], David Pallas[2,3], Guy Benian[1]. 1) Department of Pathology, Emory Univ, Atlanta, GA; 2) Department of Biochemistry, Emory Univ, Atlanta, GA; 3) Winship Cancer Institute, Emory Univ, Atlanta, GA.

Body wall muscle is fundamental for nematode locomotion. Among the more than several hundred proteins required for sarcomere assembly, maintenance and function, there are muscle specific giant proteins (>700 kDa). *C. elegans* has 3 giants; twitchin, UNC-89, and TTN-1. UNC-89 consists of 53 Ig domains, 2 Fn domains, 2 kinase domains, and KSP repeat sequence. UNC-89 is localized at M-lines, the structure in the middle of the sarcomere where thick filaments are cross-linked. Mutations in *unc-89* result in sarcomere disorganization (including absence of M-lines) and reduced locomotion. To understand how UNC-89 is positioned and how it carries out its functions, we are conducting a systematic identification of interacting molecules by using the yeast two-hybrid (Y2H) system, and have identified a number of interesting interactors (Gieseler et al. WormBook).

By using 1/3IK-Ig53-Fn2 region of UNC-89 as bait to screen a Y2H library, we identified PPTR-1 and PPTR-2. PPTR-1 and PPTR-2 are protein phosphatase 2A (PP2A) regulatory subunit B subunits (B56). A deletion mutant of *pptr-1, tm3103,* shows sarcomere disorganization. Anti-PPTR-1 antibodies localize to I-bands. Since antibodies generated to a highly conserved region of human B56 that is likely to react with both PPTR-1 and PPTR-2 stain I-bands and M-lines, we hypothesize that PPTR-2 may localize to M-lines. We also characterized muscle functions of other PP2A regulatory subunits. Antibodies to SUR-6 (B subunit)(provided by Kevin O'connell) localize to I-bands. A ts mutant of *sur-6*, *or550*, shows sarcomere disorganization at the restrictive temperature. Mutants of *rsa-1* (B'' subunit) display a characteristic myosin heavy chain A (MHC A) staining pattern. Normally, MHC A is located at the center of A-bands, localized as a straight single line. In *rsa-1* mutants, MHC A localizes as two parallel lines with the M-line in the center. Since some *unc-54* (MHC B) mutants (*s74*) show a similar split MHC A staining pattern, an RSA-1-associated PP2A complex might regulate phosphorylation of myosin heavy chains. The PP2A A subunit (PAA-1) and C subunit (LET-92) are also expressed in muscle. We addressed their muscle function by RNAi feeding from the L1 stage. Worms fed bacteria expressing *paa-1* or *let-92* dsRNA show muscle detachment from the basement membrane, resulting in folded "jack-knife" appearance. Taken together, protein dephosphorylation performed by PP2A might be crucial for many aspects of sarcomere assembly/maintenance.

W4107B CRISPR/CAS-9 mediated engineering of the *lin-3 egf* locus enables the analysis of tissue-specific functions. *S. Spiri*, L. Mereu, M. Morf, A. Hajnal. Universitiy of Zürich, Zürich, CH.

lin-3 encodes the only member of the epidermal growth factor (EGF) family in *C. elegans*. LIN-3 has a number of essential functions during larval development, hermaphrodite fertility and behavior. One of the best studied functions is the role of LIN-3 during primary (1°) vulval fate specification. After vulval induction, *lin-3* is also expressed in the inner-most 1° VulF cells to determine the uv1 subfate of cells in the ventral uterus. However, due to the many essential functions of *lin-3* during early larval development, it is difficult to detect potential later functions of LIN-3 after vulva induction. *lin-3* is a large locus with three different transcriptional start sites and many splice variants, making it difficult to create transcriptional reporters that reproduce the wild-type expression pattern. Using CRISPR/CAS9, we inserted an mNeongreen reporter into the *lin-3* locus to create an endogenous and fully functional LIN-3 reporter. This reporter has enabled us to analyze the expression pattern and subcellular localization of LIN-3.

Tissue-specific RNAi data has indicated that LIN-3 secreted by the VulF cells is required for dorsal lumen formation during vulval morphogenesis. To verify these RNAi data we generated a tissue-specific knockout strain by inserting into the *lin-3* locus two FRT recombination sites flanking part of the EGF domain. By expressing the *flp* recombinase under control of the heat-shock promoter we can now induce the loss of LIN-3 function at different developmental stages. In addition, we are expressing the *flp* recombinase under the control of different promoters to investigate the tissue-specific functions of LIN-3. This system will allow us to explore novel functions of LIN-3 in a highly specific manner.

W4108C Unearthing the cues to tissue identity within the gonadal sheath. *L. G. Vallier*, V. Tailor, M. Saini. Hofstra, Hempstead, NY.

A fertilized one-celled embryo contains all the genetic information to form the tissues and cells of the entire organism. Each division of the embryo results in a more restricted potential and a more specialized possibility for each of the two daughter cells to form a set of tissues and cells. In *C. elegans*, there are notably several master regulators of key cell fate decisions that have been well studied such as the Notch gene, *lin-12*, which for example, regulates the AC-VU decision to produce a wild-type vulva. More commonly, multiple genes act together in a combinatorial fashion to regulate the expression of a tissue or cell-type. In *C. elegans*, the gonad comprises two reflexed arms, each of which is mostly encircled by the gonadal sheath, a tissue composed of five pairs of cells. Gonadal sheath pair (Sh) 1 is necessary for maintaining the mitotically dividing stem cell population of the germline while Sh4 and Sh5 are necessary for ovulation of the mature oocytes into the

spermatheca. Loss of the sheath results in extremely reduced fertility: at most one – two progeny per hermaphrodite. Because of its simple structure we have used the gonadal sheath as a model to examine the genes that are required for establishment and maintenance of tissue identity. We approached our goal in several ways. First, using an RNAi screen to look for genes that were necessary for the presence or maintenance of the gonadal sheath, we uncovered approximately 40 candidate genes. Only one of these had been previously localized to the sheath. Next, approximately one-half of these candidate gene products are predicted to interact with gene products that have predicted or been shown to localize to the gonadal sheath. Finally, we used a datamining approach to look at unique expression patterns that have been reported in the literature. Approximately 246 genes have been reported to localize to or affect the gonadal sheath. After checking the literature and databases, only the localization pattern of 88 of the 246 genes could be confirmed in the literature and databases. Twenty-eight of those genes were not ubiquitously expressed in all five sheath pairs but had unique sheath pair expression patterns. Taken together, these data reveal potential new relationships of combinatorial gene expression patterns that begin to uncover signals that mark the identity of the gonadal sheath.

W4109A RNAi-based screens identify tube morephogenesis genes in the *C. elegans* spermatheca. *Md. Asaduzzaman Khan*[1], Fangyuan Wu[1], Rong Ying[1], Verena Gobel[2], Hongjie Zhang[1]. 1) Faculty of Health Sciences, University of Macau, Taipa, Macau, China; 2) Mucosal Immunology and Biology Research Center, MGH, Harvard Medical School, Boston, MA, USA.

Tubes are the building blocks of many internal organs in multicellular organisms, and defects in tube morphogenesis cause devastating diseases. To understand the molecular mechanisms of tube formation, our previous screen in *C. elegans* has identified hundreds of molecules required for tubulogenesis in intestine and/or excretory canals. Here in this study, we are aiming to identify genes involved in the formation and the size and shape regulation of a different tubular organ - the spermatheca - through a genome-wide RNAi screen.

The hermaphrodite spermatheca is composed of 24 epithelial cells organized into an accordion-like stretchable tube that contains sperm. Its ability to expand during the passage of oocytes demonstrates unique resilience and flexibility of a tubular epithelium. The spermatheca develops post-embryonically starting from late L3/early L4 stages, providing the following advantages for a tube morphogenesis screen: (1) it can be specifically targeted with conditional RNAi for those embryonic and larval lethal genes, conveniently bypassing their functions in growth regulation, signaling, transcriptional regulation, and cell fate determination; (2) it will help to recover non-lethal genes, whose knockdown may hardly affect the development of intestine and excretory canals; (3) its specific features may allow us to identify molecules involved specifically in tube resilience and flexibility, the characteristic existing in a number of human internal organs too.

The screen is designed to target non-lethal genes using standard RNAi condition and essential genes using conditional RNAi initiated after completion of embryogenesis on the *C. elegans* strains expressing distinct fluorescent markers that outline the structure of the spermatheca, such as LET-413, AJM-1 and ERM-1. Worms subjected to RNAi knockdown are first evaluated for morphological defects in gonad system under a dissecting fluorescence microscope followed by detailed characterization on spermathecal phenotypes by a confocal microscope. We are expecting to identify specific genes affecting spermatheca structure, size and shape etc. Here some preliminary results of our tube morphogenesis screen on the hermaphrodite spermatheca will be presented.

W4110B Developing an assay for high throughput detection of dauer larvae in *C. elegans*. *Maxwell Colonna*, Francesca V. Ponce, Goncalo Gouveia, Arthur S. Edison. Complex Carbohydrate Research Center, University Of Georgia, Athens, GA.

To enable NMR metabolomic studies in the model organism *Caenorhabditis elegans*, large-scale cultures are a prerequisite to produce small molecule extracts of sufficient concentration for analysis. Extensive growth results in a variable distribution in the distinct life stages of *C. elegans* (L1, L2, dauer, L3, L4, young adult and adult) that are present at any given time. The COPAS Biosorter, a specialized large particle flow cytometer, collects some phenotypic data of such cultures. While the sorter allows us to count and identify the normal developmental stages of sampled worms based on time of flight (TOF) and extinction (EXT) measurements, we cannot intuitively quantify how many dead or dauer worms result from these dense cultures. We treated samples with a fluorophore-conjugated lipid analog that has been previously shown to insert into the epicuticular layer of a normally developing nematode, but is excluded by dauer larvae. Combined with dauer-specific morphology data collected by the Biosorter, we are able to estimate the number of dauer worms present in a dauer "spiked" sample by calculating the number of non-fluorescent events that appear within the size and density range of dauer worms. This poster depicts our efforts to develop a robust, high-throughput assay for the presence of dauer larvae that utilizes the detection capabilities of the COPAS Biosorter to exploit biophysical and morphological differences between normally developing worms and dauer larvae. Along with fluorescent vitality dyes that can simultaneously identify the number of dead animals present in a sample, we aim to collect an inclusive set of phenotypic data from a single experiment that can be related to the resulting metabolite profile of each culture.

W4111C A developmental map of accessible chromatin in *C. elegans*. *Yan DONG*, Jürgen Jänes, Alex Appert, Djem Kissiov, Julie Ahringer. The Gurdon Institute, University of Cambridge, Cambridge, UK.

Genome-wide mapping of regulatory regions is important for understanding developmental gene regulation. Regulatory elements such as promoters and enhancers are often nucleosome-depleted, rendering them accessible to digestion by a low concentration of DNase I. Regions of open chromatin are also susceptible to transposition of sequencing adaptors by the transposase Tn5 (ATAC-seq). Nucleosome occupancy or positioning can be mapped by digestion using MNase. In *C. elegans*, chromatin accessibility data is currently limited to a single stage generated using DNase-chip whereas MNase maps are only available for two developmental stages.

We have used ATAC-seq, DNAse-seq, and MNase-seq to map genome-wide accessibility at six developmental stages: embryos, four larval stages and young adults. Using ATAC-seq, we define more than 30,000 accessible sites across development, which show agreement with developmental TF ChIP binding patterns from the modENCODE project. Most accessible sites are present at multiple stages, with stage specific

sites primarily occurring in embryos and young adults.

Although many accessible sites are detectable at multiple stages, the majority of sites change in accessibility across development. We observe two common patterns of developmental changes — regions that decrease over development and regions that increase over development. Regions with increasing accessibility are characterised by lower G+C content compared to regions with decreasing accessibility. We further characterise regions of open chromatin based on relative accessibility to DNase I, nucleosome occupancy, transcription factor or chromatin remodeller binding, and histone modifications.

W4112A CEC-4 reads histone H3K9 methylation to promote heterochromatin organization. *Jennifer C. Harr*[1], Adriana Gonzalez-Sandoval[1,2], Daphne S. Cabianca[1], Susan M. Gasser[1,2]. 1) Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland; 2) Faculty of Natural Sciences, University of Basel, Basel, Switzerland.

Heterochromatin is localized at the nuclear periphery in most eukaryotic organisms. A distinct compartmentalization of euchromatin and heterochromatin occurs upon cellular differentiation. The functional relevance of sequestration of chromatin at the nuclear periphery is unknown. While a variety of studies have begun to identify factors involved in anchoring chromatin at the nuclear periphery, the functional characterization of this mechanism, particularly in differentiated tissues, has yet to be described. In *C. elegans* embryos, we have identified the histone H3K9 methylation reader CEC-4 as a factor that maintains chromatin positioning at the nuclear periphery. CEC-4 binds selectively histone H3 bearing mono-, di- or tri-methylation on lysine 9 and is localized independently of chromatin to the nuclear periphery. In *cec-4* mutant strains the anchoring of chromatin is perturbed both for an integrated heterochromatic array and for endogenous chromosomes, although gene expression is not affected. Beyond larval stages, additional anchoring pathways compensate for the loss of CEC-4. We carried out a forced muscle cell transdifferentiation assay by heat-shock induction of the MyoD homologue, HLH-1, to address the function of reduced anchoring in early embryos. *cec-4* mutant embryos had the ability to continue developing into a larva-like stage, unlike the wild-type embryos. Therefore, we propose that the anchorage of chromatin at the nuclear periphery in early *C. elegans* embryos helps to stabilize cell fate decisions.

CEC-4 is expressed throughout development with variable expression levels across differentiated tissues and muscle cells in larvae and adult worms. Muscles exhibit particularly high levels of CEC-4. Live cell imaging of *cec-4* mutants harboring a heterochromatic reporter suggests that CEC-4 contributes to chromatin anchoring in differentiated muscle and gut cells. H3K9 methylation has been implicated in nuclear organization across species, although CEC-4 is not conserved. We are currently investigating the additional mechanisms that tether chromatin to the nuclear periphery in order to find other factors that are involved. RNAi of all known nuclear envelope proteins in worms, individually and in pairwise combinations, did not disrupt CEC-4 localization. Mutations that act synergistically with CEC-4 to delocalize chromatin include other histone modifying enzymes and readers. We are currently exploiting BioID (proximity-dependent biotin identification) to screen for protein- protein interactions of CEC-4 to characterize its role in perinuclear anchoring throughout development.

W4113B In search of *C. elegans* histone H3 lysine 27 methylation (H3K27me) 'readers'. *Arneet L. Saltzman*[1,2], Mark W. Soo[1], Jeannie T. Lee[1,2,3]. 1) Department of Molecular Biology, Massachusetts General Hospital; 2) Department of Genetics, Harvard Medical School; 3) HHMI.

The Polycomb group proteins form chromatin-modifying complexes that function in the inheritance of gene expression patterns. Originally identified in *Drosophila* for their role in body patterning through Hox gene repression, Polycomb group proteins act in part through repressive modifications to the N-terminal tails of histones. In particular, methylation of lysine 27 on histone H3 (H3K27me) can be recognized or 'read' by the chromo (chromatin organization modifier) domain, found in the *Drosophila* Polycomb and related mammalian Chromobox proteins. Previous work has shown that H3K27me is important for germ cell fate and worm development, yet a 'reader' of this mark in *C. elegans* has remained elusive. Several *C. elegans* genes have been identified by the sequence similarity of their encoded chromodomains to those of Polycomb and Chromobox proteins (Agostoni *et al*. 1996), however the binding preferences of these chromodomain-containing factors are not known. To determine whether these candidates have specificity for methylated histone tail residues, we are using a biochemical approach in which recombinant proteins are assayed for binding to a panel of histone tail peptides. To understand the potential roles of these proteins in development and chromatin modification pathways, we are creating knockout and tagged knock-in strains that will allow us to identify the genomic binding sites and protein interaction partners of these proteins by ChIP-Seq and mass spectrometry, respectively. Our results suggest that the *C. elegans* genome encodes several chromodomain 'readers' of histone H3K27 methylation. Uncovering the degree of functional overlap versus cell type or target locus-specificity among these related factors may provide an informative model for the regulatory complexity also observed in mammalian chromatin modification readers.

W4114C TGF-β signaling promotes competence for sleep in *C. elegans*. *D. Goetting*, C. Van Buskirk. California State University Northridge, Northridge, CA.

Members of the transforming growth factor beta (TGF-β) family of secreted factors play critical roles in development across species. In the nematode *C. elegans*, the DAF-7/TGF-β ligand is part of a signaling cascade that determines developmental, metabolic, and behavioral decisions based on environmental conditions. During larval development, favorable environmental conditions activate a conventional TGF-β signaling cascade that promotes reproductive growth by stimulating the production of a hormone, dafachronic acid (DA). In the absence of this signal, the animal delays reproduction and growth by entering into an alternate life stage known as dauer. In addition to its roles during development, TGF-β signaling has known roles during the adult stage, including pathogen avoidance and longevity. Here, we describe a previously unknown physiological role for TGF-β signaling in sleep behavior. *C. elegans* is known to sleep following exposure to noxious environmental conditions that cause cellular stress, such as heat. This stress-induced sleep (SIS) response is mediated by activation of epidermal growth factor (EGF) signaling in a sleep-inducing neuron called ALA, and induction of EGF-overexpression (EGF-OE) promotes a

robust sleep response in the absence of noxious stress. We have identified that *daf-7*/TGF-β mutants are impaired for SIS, and intriguingly, that various aspects of the sleep program are differentially affected. By exposing *daf-7* mutants to a variety of stressors and examining their sleep behavior, we have determined that *daf-7* mutants are severely impaired for locomotor quiescence but only mildly impaired for feeding quiescence. We wished to ascertain where in the SIS pathway DAF-7 is acting. To test this, we overexpressed the EGF ligand and examined *daf-7* mutants for sleep behavior. Interestingly, we found that *daf-7* mutants are still slightly impaired for sleep in response to EGF-OE. This suggests that the primary effects of DAF-7 on SIS may be downstream of EGF. To determine whether the role of DAF-7 in sleep regulation is dependent on DA hormone signaling, we examined *daf-36* and *daf-12* mutants, which are defective in the production of DA and its receptor, respectively. Both mutants displayed very mild defects in SIS, indicating that the effects of DAF-7 are attributable partially to dafachronic acid production during development and partly to an unidentified mechanism. I am currently analyzing changes in *daf-7* expression in response to stress. We speculate that DAF-7 may be conferring competency on the ALA neuron to mediate discrete aspects of EGF-induced sleep. Therefore, I plan to analyze reporter genes corresponding to ALA-expressed components of stress-induced sleep.

W4115A Optogenetics of gene regulation in *C. elegans*. *A. K. Lam*, K. Dvorak, V. Prahlad, B. T. Phillips. University of Iowa, Iowa City, IA.

Precise spatial and temporal activation of target genes at specific tissues or cells are critical in the study of developmental processes in vivo. Currently, various chemical and genetic methods of gene activation used in nematodes lack precise spatiotemporal specificity. We adopted a light dependent gene activation system for a more precise and controlled gene activation. Using light-sensitive protein dimerization of *Arabidopsis* cryptochrome 2 and the bHLH protein CIB1, the CRY2-CIB system activates gene expression through the interaction of two fusion proteins, CRY2-LexA DNA binding domain and CIB-VP16. In the presence of blue light, the two fusion proteins bind and activates the transcription of target genes under the LexA promoter. To track activation of target genes, we created a LexA activated target gene followed by SL2 splice site linked to mCherry florescent reporter. Preliminary results showed positive target gene activation in transgenic following a global exposure of blue light. Further studies will be done to observe target gene activation in a tissue specific manner in various nematode developmental stages.

W4116B Modeling Craniofacial Diseases in *C. elegans*. *Aditi Chandra*[1], Tyler Hansen[1], Sharon Kim[1], Victoria Scanlon[2], Andrew Wilkie[3], Ann Corsi[2], Andy Golden[1]. 1) NIDDK/NIH, Bethesda, MD; 2) The Catholic University of America, Washington, DC; 3) University of Oxford, Oxford, UK.

Millions across the world are currently living with one of 7,000 rare diseases, the majority of which are thought to be monogenic in origin. Due to their rarity, many of these diseases are not well studied. As a result, patients with a monogenic disease often face limited treatment options and a slim chance towards remission. However, with recent advances in genome editing, it is now possible to model many inherited diseases in a variety of organisms. Given this, we are utilizing *Caenorhabditis elegans* as an avenue to model monogenic diseases and develop treatments for patients. The nature of *C. elegans* makes it an efficient, inexpensive, and simple system to accomplish this task. In this study, we have applied this novel approach to explore Barber-Say, Ablepharon Macrostomia, and novel Saethre-Chotzen-like syndromes, which arise due to mutations in the two human TWIST genes. These syndromes are autosomal dominant in nature. We use *C. elegans* to model conserved mutations in *hlh-8,* the TWIST ortholog.

Human TWIST1 and TWIST2 are bHLH transcription factors involved in many important pathways, including craniofacial development. As a result, humans with TWIST dysfunction have severe bone development defects, most obvious in the skull manifested as craniofacial deformation. Using our system, we modeled six mutations found in patients with the above three syndromes. The mutations, all at codon 29 of *hlh-8*, disrupt the basic domain, which binds DNA. Interestingly, we observed different, distinct phenotypes for each allele, even though these mutants only differed by the amino acid at codon 29. The three phenotypes we observed were one or a combination of the following: egg-laying defective (Egl), constipation (Con), and a tail deformation. The most severe allele exhibited all three phenotypes whereas the mildest only displayed an Egl phenotype. Medium severity alleles exhibited an Egl phenotype with a variably penetrant Con and tail deformation phenotype. This unexpected spectrum of alleles poses some interesting biochemical questions for future investigation. A previously studied null *hlh-8* mutation behaved as a recessive allele with all three of these phenotypes. Our genetic analysis is consistent with these new alleles having a dominant negative nature, which suggests the human syndromes are due to defective TWIST proteins interfering with wild-type TWIST function. Future plans include suppressor screens of these alleles and modeling other diseases using this methodology.

W4117C Genome engineering with the CRISPR/Cas9 system in *C. elegans*. *Daniel J. Dickinson*, Bob Goldstein. Department of Biology and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.

The CRISPR/Cas9 system is fast becoming an indispensible tool for research in model organisms, including the nematode *C. elegans*. In just the past few years, a large number of different strategies for CRISPR in *C. elegans* have been described. The purpose of this presentation is to aid users in choosing the most effective strategies for their applications. We will summarize our own contributions to genome editing techniques for the worm, along with the work of others, and provide recommendations for which approaches to try first for different types of genome modifications. In addition, we'll present some novel applications and improvements to our selection-based protocol (Dickinson et al. 2015), including improved cloning methods, a streamlined approach for structure-function experiments, and a conditional balancer strategy that facilitates functional studies of essential genes.

W4118A Recombineering in C. elegans: genome editing using in vivo assembly of linear DNAs. *A. PAIX*, H. Schmidt, G. Seydoux. HHMI - Johns Hopkins University School of Medicine, Baltimore, MD.

Recombineering, the use of endogenous homologous recombination systems to recombine DNA in vivo, is a commonly used technique for genome editing in microbes. Recombineering has not yet been developed for animals, where non-homology-based mechanisms are thought

to dominate DNA repair. Here, we demonstrate that in C. elegans, linear DNAs with micro-homologies (35 bases) participate in a robust local gene conversion mechanism that can be exploited for recombineering. We find that double-stranded PCR amplicons and bridging single-stranded oligonucleotides (ssODNs) recombine readily to form novel DNA assemblies in vivo that can repair double-strand breaks induced by CRISPR/Cas9 on chromosomes. We use this method to create precise GFP knock-in alleles using a universal linear GFP template and two gene-specific "bridge" ssODNs linking GFP to the Cas9 cleavage site. In vivo assembly can also be used to assemble novel knock-in fusions and gene replacements, and is efficient enough for use without selection (scarless edits). Our findings are consistent with the budding yeast models for synthesis-dependent strand annealing and template switching during repair-induced DNA replication. We conclude that, like microbes, metazoans possess robust homology-dependent repair mechanisms that can be harnessed for recombineering and genome editing.

W4119B Digital resources for high-throughput analysis of 3D spatial and temporal cell division dynamics in early embryos. *K. Kyoda*, H. Okada, S. Onami. RIKEN Quantitative Biology Center, Kobe, JP.

During animal development, cells are genetically controlled to generate the three-dimensional morphological structures. A collection of quantitative data of spatiotemporal morphological dynamics when a wide variety of individual genes are perturbed would provide a rich resource for understanding animal development. Here we created a collection of quantitative data of cell division dynamics in early *C. elegans* embryos when each of all 351 essential embryonic genes was silenced individually by RNA interference (RNAi). To collect five sets of quantitative data from RNAi-treated embryos for each gene, we applied our computer image processing system to ~2,000 sets of four-dimensional differential interference contrast microscopy images. The collection finally consists of 33 sets of quantitative data for wild-type embryo and 1,142 sets of quantitative data for RNAi-treated embryos for 243 genes. Each data contains the 3D coordinate values of the outlines of nuclear regions and the changes of the outlines over time. We first performed computational phenotype analysis by using this collection. Statistical analysis identified over 6,000 RNAi-induced phenotypes for 437 phenotypic characters, which can be numerically extracted from the collection. The phenotypes include three-dimensional (3D) spatial and temporal phenotypes that are difficult to be identified manually. We next predicted sets of genes involved in the same cellular processes from the collection. By applying hierarchical clustering method to the profiles of the extracted phenotypic characters, we found 7 independent clusters corresponding to the individual cellular processes such as polarity/asymmetric division, DNA replication and chromosome maintenance/segregation. In addition, we developed novel computational methods for inferring a coarse-grained model of embryogenesis by finding correlations between phenotypic characters. The inferred model represents the order in which the phenotypic characters relate and appear in the course of development. Our collection provides novel opportunities for performing high-throughput analysis of 3D spatial and temporal cell division dynamics during animal development. In the near future, all data and results will be available at the Worm Developmental Dynamics Database (http://so.qbic.riken.jp/wddd).

W4120C SSBD: an open database of quantitative data and microscopy images of biological dynamics. *Y. Tohsato*, K. Ho, K. Kyoda, S. Onami. RIKEN Quantitative Biology Center, Kobe, JP.

Recent technological advances in digital microscopy and image analysis have produced a large amount of quantitative data of spatiotemporal dynamics of biological objects such as molecules, cells, and organisms. These quantitative data can be used to reveal the nature of dynamical behaviors of biological phenomena. However, these data are difficult to reuse for further analysis because they are often scattered over the Internet in different formats. There is a crucial need in bringing these data together in a coherent and systematic manner. We developed Systems Science of Biological Dynamics database (SSBD) as an open repository for quantitative data and microscopy images (http://ssbd.qbic.riken.jp). SSBD provides quantitative data in a unified format (BDML: Kyoda *et al.*, 2015) and through REST API (Representational State Transfer Application Programming Interface). SSBD also provides image data in the original format by using OMERO platform. SSBD currently provides 313 sets of quantitative data and 194 sets of microscopy images for single molecules, nuclei, and whole organisms in a wide variety of model organisms from *E. coli* to mouse. For example, SSBD includes 186 sets of nuclear division dynamics data of wild-type and RNAi-treated *C. elegans* embryos during the first three rounds of cell division and the corresponding 4D differential interference contrast microscopy images. One set of nuclear division dynamics data of wild-type *C. elegans* embryo from 4- to 350-cell stage, and 11 sets of behavioral dynamics data of goa-1 *C. elegans* adults are also included. A web-based viewer allows users to visualize quantitative data in time and 3D space on-demand without any additional plugin. In addition, SSBD provides software tools for data visualization and analysis such as Phenochar and BDML4DViewer. Phenochar is a software tool for extracting phenotypic characteristics from the data in BDML format. BDML4DViewer is an ImageJ plugin to read and visualize quantitative data in BDML format. The open-source version of SSBD for managing quantitative data is also available at http://github.com/openssbd/. It enables other research groups to setup their own databases independently for storing and sharing their data. SSBD will support data-driven biology by accelerating the reuse of quantitative data and microscopy images, and development of software tools for data analysis.

W4122B Calcium imaging of a dopamine-regulated chemosensory circuit in *Caenorhabditis elegans*. *C. Kunkel*, J. Kim, M. Baidya, P. Turturro, M. Chao. California State University San Bernardino, San Bernardino, CA.

The neurotransmitter dopamine regulates chemosensory avoidance behavior in the model organism *Caenorhabditis elegans*. Avoidance behaviors are mediated by the polymodal ASH nociceptive sensory neurons, and behavioral avoidance of stimuli detected by ASH is less robust when dopamine signaling is impaired. Our broad research goals are to understand the underlying molecular and cellular mechanisms of how dopamine modulates the activity of neural circuits. Using a custom microfluidics device and the transgenically-expressed Ca^{2+} sensor GFP variant G-CaMP, we have begun to characterize the ASH neural response of wild type and dopamine-deficient *cat-2* mutant *C. elegans* when

treated with exogenous dopamine and stimulated with the osmolyte glycerol. Our preliminary data suggest that exogenous dopamine perfused into the recording buffer in the microfluidics device affects ASH neuron excitability in *cat-2* mutants. Our ongoing efforts now focus on optimizing our assay conditions (including type of stimuli to use and recording buffer components) and identifying molecular elements in ASH neurons that mediate this response, including the relevant dopamine receptor(s).

W4123C Differential Gene Expression within a Single Sex-Specific Class of *Caenorhabditis elegans* Neurons. *D. K. Reilly*[1], E. M. Schwarz[2], J. Srinivasan[1]. 1) Worcester Polytechnic Institute, Worcester, MA; 2) Cornell University, Ithaca, NY.

Organisms must be able to accurately assess a constantly changing environment as they navigate their surrounding – avoiding danger, remaining in beneficial areas, and determining whether or not a potential mate is in the vicinity. *C. elegans* males sense nearby hermaphrodites through pheromones of the ascaroside family of small-molecule social cues. The sex-specific ascarosides, namely ascarosides #3 and #8, are sensed via the male-specific CEM neurons. Laser cell ablation studies have confirmed that the CEM neurons are necessary and sufficient for sensation of ascaroside #8, whereas ascaroside #3 sensation requires ASK neurons as well. While the four CEMs are considered to be of a single class of neurons, recent work has shown that individual CEMs respond uniquely to these cues (Narayan et al, 2016). The neurophysiological properties of these individual neurons are stereotyped, yet summation of the four CEMs suggests a heterogeneous response to ascarosides #3 and #8. To understand the molecular mechanisms of these differences in response to the stimuli, we isolated transcriptomes from each of the four individual CEM neurons. We then performed RNA-seq analysis to determine genes with elevated expression levels in these sex-specific sensory neurons. We created GFP-promoter fusions to confirm expression within a single CEM neuron for each gene. We identified five potential receptor candidates necessary for sensing the two ascarosides using the CEM transcriptomic data and gene ontology analysis. We intend to extend these findings to manipulate individual CEM neurons with optogenetic tools to dissect how they communicate with each other to drive male behavior.

W4124A High throughput chemical genomics in *C. elegans* to screen for novel bioactives and their targets. *H. Zahreddine Fahs*, F. Refai, R. White, PG. Cipriani, S. Kremb, F. Piano, KC. Gunsalus. New York University Abu Dhabi, Abu Dhabi, UAE.

Biologically active chemicals are the basis of most known therapeutics and are powerful tools to study cell biological processes. However the discovery of new bioactive compounds and characterization of their molecular targets remain a challenge. We are using small molecule and natural products libraries to identify novel compounds that affect animal development and study their modes of action. We have established a high-throughput automated platform for chemical and functional genomic screening that accommodates both cell-based and whole-organism (e.g. microbial, worm and zebrafish) assays. We are focusing on potential disease therapeutics (e.g. cancer, diabetes) and broad-spectrum anthelmintics (to target parasitic nematodes that affect human, crops, and livestock) using the free-living nematodes *C. elegans* and the distantly related *P. pacificus* as model animals, since they offer a rapid and powerful platform for lead compound discovery and mode of action studies. Given the short life cycle of the worm, our high-throughput screening (HTS) platform enables one person to screen 20,000 chemicals per week and perform one genome-wide RNAi screen every 2 weeks. Results are scored using automated image analysis with DevStaR, a software package we developed that employs computer vision and machine learning to quantify embryonic lethality in mixed-stage worm populations (*White et al., 2013*). We validated our screening approach in a pilot screen of an FDA-approved drug library, which confirmed the effects of known anthelmintics on *C. elegans* and *P. pacificus*. We then screened a library of ~32,000 small molecules that were selected using a computational approach to predict bioavailability in nematodes (*Burns et al. 2010*). Preliminary analysis based on worm survival and developmental phenotypes suggests a 4% hit rate. Positive hits will be assayed for toxicity in zebrafish and mammalian cells. To uncover molecular targets of bioactives and mechanisms of resistance, we will use forward and reverse genetic screens to identify suppressors (or enhancers) of chemical sensitivity. We are also performing chemical screens using sensitized *C. elegans* genetic backgrounds to identify molecules that specifically interact with targeted pathways. In parallel, we screened a library of 300 uncharacterized *Bacillus thuringiensis* (Bt) strains (isolated by Mireille Kallassy – USJ, Lebanon). Bt is a spore-forming bacterium that synthesizes crystal inclusions, some of which are toxic against insects (used as bioinsecticides), nematodes and cancer cells. These crystal proteins are safe to humans, biodegradable, and constitute a promising alternative to chemical anthelmintics. We found 95 Bt strains that hinder the development of *C. elegans* or *P. pacificus* and are analyzing these to characterize new virulence factors and mechanisms of action.

W4125B Ribosomal DNA copy number as an unexplored potential source of heritable phenotypic variation. *E. Morton*, C. Mok, S. Lee, R. Waterston, C. Queitsch. University of Washington, Seattle, WA.

Complex phenotypes such as lifespan, disease susceptibility, and mutation penetrance vary substantially even among isogenic individuals. The molecular determinants behind this variation have long been sought, along with the ability to predict how one individual will differ from another. In *Caenorhabditis elegans*, variation in lifespan and mutation penetrance among nominally isogenic individuals has recently been linked to differences in expression of molecular chaperones. These expression differences are heritable, implying a stable epigenetic or unstable genetic source. We propose copy number variation in ribosomal RNA genes as an underlying, unexplored genetic cause of phenotypic variation. Ribosomal RNA genes (rDNA) exist as tandem gene arrays in all eukaryotes, and their repetitive nature predisposes them to instability and thus copy number variation among individuals. Differences in rDNA copy number indeed exist among laboratory N2 worms only ten generations removed from a common ancestor. rDNA copy number is known to associate with global gene expression in flies and humans. rDNA also associates with genome integrity and replicative lifespan in yeast. Furthermore, the nucleolus itself, which is comprised of rDNA, is a major hub of stress response and protein homeostasis. Despite its obvious biological significance, rDNA and its copy number variation are understudied, due largely to the inherent technical challenges involved in genotyping repetitive DNA. We have addressed this problem by developing a high-throughput technology to genotype rDNA copy number in *C. elegans*, employing molecular inversion probes to count repeat

units. This cost-efficient and universally applicable method provides rDNA copy number estimates that agree with whole genome sequencing-based estimates. Facilitated by this technology, we have begun exploring the potential phenotypic consequences of rDNA copy number variation. Our preliminary data show an association between rDNA copy number and two different biomarkers of longevity. Our results suggest that rDNA copy number may be a contributor to phenotypic variation among nominally isogenic individuals. We provide a technology to make this class of genetic variation accessible for in-depth exploration of the phenotypic consequences of rDNA copy number variation in *C. elegans* as well as for future studies of aging and disease susceptibility in other organisms, including humans.

W4126C Textpresso: mining full text for efficiently obtaining information from the biological literature. *Paul Sternberg*[1], Hans-Michael Müller[1], Yuling Li[1], Kimberly Van Auken[1], Christian Grove[1], Karen Yook[1], Ranjana Kishore[1], Seth Carbon[2], Chris Mungall[2], Suzanna Lewis[2]. 1) Division of Biology and Biological Engineering, California Inst of Technology, Pasadena, CA; 2) Genomics Division, Lawrence Berkeley National Lab, Berkeley,CA USA.

We all face a continual increase in the number of papers whose content we need to know. Curators at biological databases such as WormBase also need to efficiently locate and extract information from this expanding corpus of papers. To help reduce the tedium and cost of scouring the literature for specific experimental results, we developed the Textpresso text mining system available at www.textpresso.org. We have completely rewritten the system to scale to millions of papers (e.g., all of PubMedCentral) and to help biocurators at the model organism databases and the Gene Ontology Consortium (GOC) make annotations to genes, cells, molecules and so forth. Textpresso indexes individual sentences with keywords and categories of terms that allow highly specific searches. Category searches in *C. elegans* Textpresso allow you to find all sentences in any *C. elegans* paper that mentions, for example, a worm gene and a human disease, or a drug, a cell type and a phenotype. Textpresso also indexes the Drosophila, mouse, zebrafish and yeast literature. We are now integrating the new system (TextpressoCentral) into the GOC's Common Annotation Framework. This framework was designed to work for all biological literature and allows annotation using the Noctua curation tool developed to support GO's more expressive LEGO curation paradigm, which relates gene products to specific functions in context. We will explain the use of Textpresso for individual researchers and for biocurators, and howTextpresso will make your life better by making it much easier to find particular information quickly. For example, by automating the process of skimming thousands of papers for some detailed information, Textpresso will allow you to spend more time reading papers.

W4127A Metablomics meets genomics in *Pristionchus pacificus*: A highly specific esterase is involved in the synthesis of dauer inducing small molecules. *J. M. Meyer*[1], N. Bose[2], J. Y. Yim[2], A. Artyukhin[2], F. C. Schroeder[2], R. J. Sommer[1]. 1) Max-Planck Institute for Developmental Biology, Tuebingen, DE; 2) Boyce Thompson Institute, Cornell University, New York, USA.

All animals have evolved abilities to react towards changes in their environments. Nematodes for example, can arrest their development under adverse environmental conditions and enter a long-lived, stress-resistant "dauer" stage. This "dauer stage" plays a major role as survival but also as dispersal strategy of nematodes. Small molecules are crucial for the regulation of dauer entry and additionally, have been shown to function as cues in chemical communication through which they can regulate complex behaviors. Recent studies in *Pristionchus pacificus* and comparison to Caenorhabditis *elegans* indicated that small molecule structure and function evolve rapidly. One hallmark is that small molecule structures in *P. pacificus* are far more complex than their *C. elegans* analogs. Also, the collection of worldwide isolates of *P. pacificus* provided strong evidence for extreme natural variation of these pheromones across genotypes. In this context, a new type of intraspecific competition among sympatric strains was identified with a new role of small molecules in nematode evolutionary ecology. Thus, small molecules are important for the regulation, ecology and evolution of dauer development. Despite this, very little is known about the enzymes and pathways involved in the synthesis of small molecules. Here, we study intra-species variation in small molecules production in *P. pacificus* on La Réunion Island. In the last 5 years, we extensively sampled a nematode population by collecting more than 300 local strains, which were subsequently characterized through a combination of genomic (RAD-sequencing) and secrotomic (HPLC/MS/MS) approaches. We found that these highly related strains still showed massive differences in their small molecule secretomes. Most importantly, ubas#1 and ubas#2, two compounds shown to play a crucial role in dauer regulation, were absent from a certain number of strains. Combining both datasets we were able to utilize genome-wide association studies (GWAS) to identify a candidate region. Using nanopore and whole genome re-sequencing we confirmed the identity of the causative gene as a putative esterase that is involved in the synthesis of ubas#1 and ubas#2. We used the CRISPR/CAS9 system to inactivate the esterase-encoding gene and found the resulting mutant to be ubas#1 and ubas#2 deficient. Thus, through a multifaceted experimental approach we have identified a key enzyme involved in the synthesis of complex, dauer-inducing small molecules in *P. pacificus*.

W4128B *Caenorhabditis* sp. 34 is a sister species to *C. elegans* with marked differences in morphology and ecology. *A. Sugimoto*[1], R. Tanaka[2], K. Tsuyama[1], S. Namai[1], R. Kumagai[1], T. Shimura[1], N. Kanzaki[3], T. Kikuchi[2]. 1) Tohoku University, Sendai, JP; 2) University of Miyazaki, Miyazaki, JP; 3) Forestry and Forest Products Research Institute, Tsukuba, JP.

For comparative and evolutionary studies, satellite species that are closely related to established model organisms are valuable. In the genus *Caenorhabditis*, several species (e.g., *C. briggsae*, *C. brenneri* and *C. remanei*) have been used as satellite models for *C. elegans*, but no phylogenetic "sister" species to *C. elegans* has been isolated. Recently we discovered a new species *Caenorhabditis* sp. 34 from syconia (fresh fruits) of the fig *Ficus septica* in Ishigaki Island, Japan. *C.* sp. 34 is gonochoric and shares typological key characters with other Elegans supergroup species, but strikingly, adults are nearly twice as long as *C. elegans*. The optimal culture temperature (27°C) for *C.* sp. 34 is significantly higher than that of *C. elegans* (20°C). Dauer larvae were detected in the fig syconia, but generally not observed in laboratory culture conditions. Despite these marked ecological and morphological differences, phylogenetic analysis based on the genome sequences revealed that *C.* sp. 34 is a long-sought sister species to *C. elegans*. We assembled a draft genome sequence of *C.* sp. 34 using data from a mixture of sequencing technologies (Illumina and PacBio). A similar number of protein-coding genes to the *C. elegans* genome was predicted,

but 7TM GPCR domains are diminished in *C.* sp. 34, whereas transposon-related domains are highly enriched. To make *C.* sp. 34 a genetically tractable satellite organism, we have been developing genetic and molecular techniques/tools by transferring the ones established in *C. elegans*. Heritable transgenic lines were obtained by microinjection of commonly used *C. elegans* marker plasmids into female adult gonads, although the frequency was lower than that in *C. elegans*. Both soaking and feeding RNAi in *C.* sp.34 worked as efficiently as in *C. elegans*. Taken together, its high-quality genome information, genetic tractability, and characteristic morphology and ecology make *C.* sp. 34 an attractive satellite model organism for a wide range of studies in comparative and evolutionary biology.

W4129C Large-scale genetic interaction maps for *C. elegans* embryonic development. *P. G. Cipriani*[1,2], E. Munarriz[3], K. Erickson[1], J. Lucas[1,2], N. Smit[1], F. Ahmed[2], J. Reboul[4], K. Gunsalus[1,2], F. Piano[1,2]. 1) New York University, New York, NY; 2) New York University Abu Dhabi, Abu Dhabi, UAE; 3) University of Buenos Aires, Buenos Aires, Argentina; 4) INSERM, France.

Genetic interactions are functional associations between genes that are revealed upon simultaneous perturbation of two genetic loci. Genetic interactions provide information about how pathways buffer each other to maintain an organism's homeostasis and can be used to identify previously unknown components and regulatory relationships within molecular networks. While only around 15% of genes appear to be essential on their own, expression studies tell us that over half of the transcriptome is typically expressed in any individual cell. To learn more about how essential and non-essential genes work together in embryonic development, we devised and implemented a high-throughput (HTP) system for RNAi-based genetic interaction studies. We first completed a pilot study testing a large set of predicted genetic interactions, and we have recently completed genome-wide enhancer and suppressor screens using 24 strains harboring temperature-sensitive alleles of genes that are essential for different aspects of early embryogenesis. The primary screen consisted of over 50 genome-wide RNAi screens in the conditional strains and over 30 additional screens in the N2 control, at multiple temperatures; we rescreened thousands of potential enhancers and suppressors in secondary assays with at least eight replicates. Suppressor interactions that were reproducible in multiple assays have been confirmed using both manual scoring and automated image analysis with our DevStaR algorithm (White et al., 2013). The final suppressor network links over 500 genes through more than 900 interactions; the enhancer network is not yet finalized, but based on results from our pilot study, we expect it to be much larger. We have archived all experimental results to enable quantitative scoring of embryonic lethality, and we have built a database for the millions of images collected with a web interface that allows querying and visualization of all the data collected in the screens. Analysis and follow-up studies of suppressor and enhancer interactions are ongoing.

W4130A Truncation of the RUNX transcription factor RNT-1 disrupts dopaminergic signaling in *Caenorhabditis elegans*. *Sarah Baas Robinson*[1], J. Andrew Hardaway[1], Sarah Sturgeon[1], Tessa Popay[1], Daniel Bermingham[1], Phyllis Freeman[1,2], Randy D. Blakely[1]. 1) Vanderbilt University, Nashville, TN; 2) Fisk University, Nashville, TN.

Dopamine (DA) modulates brain circuitry that supports cognition, reward, motor control, and arousal. Perturbed DA signaling is believed to contribute to addiction, ADHD, schizophrenia, and Parkinson's disease. The presynaptic DA transporter (DAT) is a major determinant of DA signaling and increasing evidence indicates that altered regulation of DAT may contribute to risk for these disorders. We have pursued the nematode *Caenorhabditis elegans* as a model system to elucidate novel mechanisms regulating DAT and/or DA signaling. DA is important for swimming behavior in the worm, as ablation of the *C. elegans* gene encoding DAT (*dat-1*) results in swimming-induced paralysis (Swip). To determine novel presynaptic regulators of DA signaling, we undertook a chemical mutagenesis screen and identified a mutant, *vt34*, that exhibits robust DA-dependent Swip. *vt34* exhibits biochemical and behavioral phenotypes consist with reduced DAT-1 function, with reversal of Swip observed in *vt34* mutants crossed to either *cat-2*, which encodes the DA biosynthetic enzyme tyrosine hydroxylase, or *dop-3*, which encodes a D2 subtype DA receptor. Additionally, *vt34; dat-1* double mutants exhibit an enhanced Swip phenotype. Unlike *dat-1* animals, however, *vt34* has altered male tail morphology and reduced body size, which we found arises from a premature stop codon in the Runx transcription factor ortholog RNT-1. *rnt-1(ok351)* and *rnt-1(tm388)* mutants, as well as mutations in RNT-1's binding partner, *bro-1*, also exhibit Swip. Both bioinformatics and reporter expression studies support the expression of *rnt-1* by DA neurons. Lastly, *vt34* and *rnt-1* mutants exhibit reduced mRNA levels of *dat-1* and *cat-2*, further supporting a role for *rnt-1* in regulating pathways that dictate the capacity for DA signaling. This work was supported by NIH awards T32 MH065215 (SBR), F31 MH093102 (JAH) and MH095044 (RDB).

W4131B Genome-wide mapping in *C. elegans* using a bulk segregant approach. *E. Ben-David*[1], A. Burga[1], L. Kruglyak[1,2,3]. 1) Department of Human Genetics, University of California, Los Angeles, Los Angeles, California 90095, USA; 2) Howard Hughes Medical Institute, University of California, Los Angeles, Los Angeles, California 90095, USA; 3) Department of Biological Chemistry, University of California, Los Angeles, California 90095, USA.

Despite decades of research, the genetic architecture underlying most complex traits – traits with contribution of multiple genetic and environmental factors – remains poorly understood. Many factors, including complex population structure, difficulty in acquiring large sample sizes, genetic heterogeneity and others, have complicated the study of complex traits in humans, and model organisms have provided an excellent tool for studying such traits. In *C. elegans*, quantitative trait loci (QTL) mapping has been used to identify genetic variation between strains that underlie many aspects of the worm's physiology and behavior. So far, QTL mapping in *C. elegans* has been performed using panels of recombinant inbred lines (RILs). Such panels, while providing an excellent tool for mapping differences across strains, are usually limited to comparisons across two backgrounds, and are arduous to generate, limiting the scalability of the approach to multiple strain backgrounds or transgenic animals.

To deal with these limitations, we developed a bulk segregant approach for mapping genetic traits in *C. elegans*. Our approach is based on mutants in which reproduction is obligate sexual, that are allowed to mate for multiple generations. The resultant segregant pool can be subjected to selection, and groups of segregants showing differential phenotypes can be used for mapping. Using simulations, we compared

the power of this approach to other mapping approaches under different assmuptions. We then applied this method to a cross between the lab Bristol (N2) strain and a wild Hawaii isolate (CB4856), and identified QTLs associated with fitness. Finally, in a cross between two transgenic strains under different backgrounds, we identified a distant-acting QTL underlying differences in the expression of a fluorescent marker.

W4132C Rational design of protein coding sequences that evade piRNA-mediated germline silencing. *Daniel J. Dickinson*, Bob Goldstein. Department of Biology and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.

In the *C. elegans* germline, multiple mechanisms act to silence foreign DNA. Because these pathways can silence experimentally introduced transgenes, they pose a technical challenge to biologists studying the germline and early embryo. Developing strategies to overcome or bypass silencing of transgenes would facilitate research in multiple fields that use *C. elegans* embryos as a model system. One pathway that can silence single-copy foreign sequences involves recognition of the foreign DNA by genomically encoded 21U-RNAs, which initiate silencing through the Piwi Argonaute PRG-1 (Shirayama et al. 2012). The diversity of 21U-RNAs is high enough to target essentially any DNA sequence. Endogenous germline genes are protected from silencing by a second pathway that involves sequence-dependent licensing by 22G-RNAs bound to the Argonaute CSR-1 (Seth et al. 2013). Newly generated transgenes can adopt either an expressed or a silent state as a result of competition between these two pathways. In some cases, recognition of the GFP portion of a transgene by the PRG-1 pathway can lead to complete silencing. Because silencing is sequence-dependent, we thought it should be possible to design protein coding sequences that evade recognition by PRG-1 and thereby escape silencing. Designing sequences that lack 21U-RNA recognition sites is difficult because the number of 21U-RNAs is very large, and their base pairing specificity (i.e., mismatch tolerance) has not been well characterized. However, we reasoned that bona fide germline-expressed genes should be depleted of high-affinity 21U-RNA binding sites, and might also be enriched for 22G-RNA binding sites that could facilitate expression. Therefore, we developed an algorithm that constructs a coding sequence for any protein of interest by assembling short "words" found in germline-expressed genes. We refer to coding sequences generated in this way as "germline optimized." We constructed synthetic transgenes to test whether germline-optimized sequences are resistant to silencing. We obtained robust germline expression of bacterial proteins that were otherwise efficiently silenced. Moreover, by using a germline-optimized GFP coding sequence, we were able to obtain expression of *gfp::cdk-1*, a model transgene that is particularly prone to silencing. We have produced a web-based tool that allows users to evaluate the degree of germline optimality of existing transgenes and to design germline-optimized coding sequences for any protein of interest.

W4133A High-resolution microfluidic imaging platform for high-throughput drug discovery using *C. elegans* disease model. *S. Mondal*, E. Hegarty, C. Martin, S. K. Gokce, N. Ghorashian, A. Ben-Yakar. University of Texas at Austin, Austin, TX.

High-throughput screening of *C. elegans* for drug discovery and gene function would require technological advancements for high-resolution imaging operating at high speeds. To enable both high-speed and high-resolution imaging of multiple *C. elegans* populations, we developed an automated and large-scale microfluidic imaging platform. The platform includes a large-scale microfluidic chip with 96 wells designed in standard microtiter plate format and densely packed trapping channels for each well. The channels are uniquely designed to immobilize approximately 4,000 animals simultaneously in 3 minutes. The automated imaging platform takes 15 z-stack images of all trapped animals, capturing their whole volume in less than 16 minutes with a resolution of a micron. An automated graphical user interface (GUI) loads all the images, identifies single animal and perform image processing steps for phenotyping. Using this platform, we screened more than 100,000 animals of a polyglutamine aggregation (PolyQ) model using a total number of 25 chips. We tested the efficacy of ~1,000 FDA approved drugs in improving the aggregation phenotype in the PolyQ model to identify possible proteostasis modulators with a Z'-factor of 0.80. The study resulted in four potential hits using the protein aggregation model. The platform provides a leap forward for high-throughput screening of other *C. elegans* disease models with cellular or sub-cellular phenotypes.

W4134B Pilot study to map the *Caenorhabditis elegans* metabolome to its genome. *T. Carter*[1], A. Edison[1], F. Ponce[1], G. Gouveia[1], F. Tayyarri[1], E. Andersen[2]. 1) University Of Georgia, Athens, GA; 2) Northwestern University, Evanston, IL.

A complete systems biology analysis requires the integration of different levels of omic information. *C. elegans* is an ideal model organism to develop systems biology methods because of the ease of culturing, the ability for genetic manipulation, and the wealth of existing information generated over decades of study. There are several approaches that one could take to relate the metabolome to the genome, including the use of existing mutants in metabolic pathways, targeting pathways of interest using CRISPR/Cas9 or RNAi, or the use of recombinant inbred lines (RILs). In this methods development study, we used 12 RILs generated by the Andersen lab from parental N2 and Hawaiian (CB4856) strains. These have been sequenced and analyzed by several different methods in the Andersen lab. For our study design, we grew 6 independent biological replicates from each of the 12 RIL strains collected at two time points for a total of 144 samples. These were started from synchronous populations and extracted at two separate time points (6 replicates each) in an effort to distinguish metabolite differences at different developmental stages of the organism. The final populations are "semi-synchronous" and represent distributions of developmental stages, which are then characterized by a COPAS Biosorter. The extracted samples are analyzed using a combination of NMR and LC-MS to obtain metabolomic and glycomic information, which will be deconvoluted into developmental stages. The spectral data are processed and statistically analyzed using a MATLAB metabolomics toolbox used by the Edison Lab. We will report the initial analysis, identification, and quantification of metabolites as a function of genotype and development. This pilot study is the first step in the generation and refinement of mapping the differences in metabolic pathways of *C. elegans* to differences in the genetic make-up of the organism, which will require greater numbers of genetic lines.

W4135C Development of systems biology in *Caenorhabditis elegans*. *A. Edison*, F. Ponce, G. Gouveia, M. Colonna, F. Tayyari, A. Le Guennec, R. Taujale, O. Bifarin, A. Martin, J. Helmken. Deptartments of Genetics and Biochemistry, Institute of Bioinformatics and Complex Carbohydrate Research Center, The University of Georgia, Athens, GA.

Systems biology is the integration of multiple levels of omics data with environmental factors and phenotypic responses. *C. elegans* is an ideal organism for these studies. There are several obstacles to a complete systems biology analysis, especially at the level of metabolomic analysis. Metabolites span a range of chemical classes including amino acids, sugars, lipids, organic acids and are expressed in a very wide range of concentrations ranging from <fM to >mM. There are no existing methods to cover this broad metabolite diversity. Mass spectrometry is extremely sensitive but is not able to reliably identify unknown metabolites that are not well characterized in databases. NMR is capable of unknown metabolite identification but suffers from low sensitivity. We are developing several approaches to help address these problems: "Deep annotation" is a chemical approach to combine the strengths of NMR and MS to relate molecular formulas to compound identifications using large pooled cultures of worms and preparative chemical fractionation techniques. "Developmental distribution" is an approach to extract developmental stage-specific analytical data from several replicates of mixed population cultures that are characterized by a worm sorter. We are applying these approaches to targeted mutations in glycosyltransferases as well as other systems such as recombinant inbred and mutation accumulation lines. I will provide an overview of how these approaches fit together to relate metabolites to genes and phenotypes.

W4136A Metabolomics of developmental stages of *Caenorhabditis elegans* using mixed populations. *Francesca V. Ponce*[1], Fariba Tayyari[1], Goncalo Gouveia[1], Andrew Martin[1], Cord Helmken[1], Chaevien Clendinen[2], Facundo M. Fernández[2], Osman Sheikh[1], Lance Wells[1], Arthur S. Edison[1]. 1) Complex Carbohydrate Research Center, University of Georgia, Athens; 2) School of Chemistry and Biochemistry & Petit Institute for Bioengineering & Bioscience, Georgia Institute of Technology.

Metabolomics analyses attempt to study the complete set of metabolites produced by an organism during its life cycle under all possible conditions. The nematode *C. elegans* is an ideal organism for comparative metabolomics studies. However, detailed analysis of the metabolic traits associated with different life stages of the worm is lacking in part due to low throughput methods to generate and study synchronous worm populations and also due to low sensitivity of some analytical techniques (e.g. NMR), which rely on large amounts of sample for adequate detection. We are developing approaches to combine the use of the large particle flow cytometer COPAS Biosorter, NMR and mass spectrometry to attempt to identify some metabolic traits associated with the different developmental stages of *C. elegans* by sampling and comparing several mixed-stage cultures. The Biosorter can measure the extinction (EXT) and time-of-flight (TOF) of individual nematodes in a population and total population size, which can then be used as descriptors of developmental stages within a mixed population of animals. Samples of synchronous worm populations of 200 thousand individuals were collected at five different time points during a 96-hour period to capture the different developmental stages (L1, L3, L4, Adult, Adult and their progeny) for NMR and MS analyses. A subset of each population was analyzed with the COPAS Biosorter. The frequency distributions of EXT and TOF of each subset were used as a proxy for the developmental stages present in the mixed stage population collected at the last time point. We were able to distinguish distinct features present in different collection time points laying out a platform for the identification of metabolic traits associated with different developmental stages present in a mixed stage culture. This approach allows for standardization and introduction of phenotypical and developmental variables to metabolomics studies. This will aid in the effective characterization of metabolic traits involved in each developmental stage of the *C. elegans* life cycle.

W4137B The EAT-2 and GAR-3 acetylcholine receptors have distinct effects on pharyngeal muscle peristalsis. *A. Kozlova*, M. Lotfi, P. Okkema. University of Illinois at Chicago, Chicago, IL.

Pharyngeal muscles exhibit two distinct types of contractions called pumps and peristalses. A peristalsis is a wave-like contraction followed by rapid relaxation that travels posteriorly through the isthmus, so that only a small portion of the isthmus lumen is open at any time. This contraction is remarkable, because it occurs within individual pm5 muscle cells that extend the entire length of the pharyngeal isthmus. Peristalses occur only after several pumps, and they are dependent on signals from the M4 motor neuron. We are examining how M4 and the isthmus muscles interact to produce these peristalses. M4 is a cholinergic neuron, and we have found that *cha-1* mutants lacking acetylcholine (ACh) fail to pump or peristalse. Chemical stimulation of either muscarinic or nicotinic ACh receptors (mAChRs and nAChRs) in *cha-1* mutants is sufficient to activate peristalsis. We have examined several mutants affecting AChR genes expressed in the pharyngeal muscles and have found that mutants defective in the nAChR *eat-2* and the mAChR *gar-3* exhibit distinct peristaltic defects. *eat-2* mutants pump less frequently than wild-type animals, but most of the pumps are followed by peristalses. The *eat-2* peristalses are prolonged suggesting that EAT-2 stimulates isthmus muscle relaxation. In comparison, *gar-3* mutants fail to respond to the mAChR agonist arecoline indicating GAR-3 mediates peristalsis in response to exogenous arecoline. *gar-3* mutation also suppresses the prolonged peristalses observed in *eat-2* mutants, which suggests that these prolonged contractions are stimulated by GAR-3. To understand better how these AChRs affect peristalsis, we examined Ca^{2+} dynamics in the isthmus muscles using GCaMP3. Wild-type animals display a broad increase in Ca^{2+} in the center of the isthmus during pumps followed by wave-like Ca^{2+} transients in the posterior isthmus during peristalses. We found that changes in Ca^{2+} concentration during peristalsis in *gar-3* mutants are similar to those in wild type, and this is consistent with our observation that *gar-3* mutants do not have any defects in peristalsis under normal conditions. In contrast, *eat-2* mutants have larger changes in Ca^{2+} concentration in the posterior isthmus during peristalsis, and these Ca^{2+} transients are prolonged in *eat-2* mutants when compared to those in wild-type animals. Based on our observations, we conclude that GAR-3 is the receptor which mediates peristalsis in response to exogenous arecoline, but not essential for these contractions under normal conditions. Surprisingly, we found that EAT-2 may be involved in stimulation of isthmus muscle relaxation, rather than isthmus muscle contraction.

W4138C Identification of genetic variation in *Caenorhabditis elegans* bleomycin sensitivity. *S. C. Brady*, S. Rosenberg, S. Zdraljevic, E. C. Andersen. Northwestern University, Evanston, IL.

Individuals within a population vary across a broad range of traits, including many that are influenced by multiple genetic factors as well as the environment. Complex traits that are medically relevant include susceptibilities to many common diseases, such as cancer, along with sensitivity to drugs, such as chemotherapeutics. However, the identification and validation of genetic loci underlying complex traits in humans is difficult. *Caenorhabditis elegans* is a tractable model that can be used to identify the genetic factors that cause variation in complex traits by pairing high-throughput phenotypic assays with a broad range of genetic tools.

Like humans, strains of *C. elegans,* vary in sensitivity to the chemotherapeutic drug bleomycin. The laboratory strain, N2, is sensitive to the drug whereas a genetically diverged strain, CB4856, is more resistant. We examined bleomycin sensitivity in a panel of 350 whole-genome sequenced recombinant inbred advanced intercross lines (RIAILs) generated from a cross between the N2 and CB4856 parents. Recombinant strains exhibited variation in bleomycin sensitivity as determined by changes in growth rate, offspring production, and feeding rate. Approximately 1,600 markers across the genome were used to correlate genotype with phenotype. Linkage analysis identified a quantitative trait locus (QTL) on chromosome V with a 162-kb confidence interval that is strongly correlated with bleomycin sensitivity. Within this interval, we identified five candidate genes — three genes with nonsynonymous variants and two genes with varied gene expression between N2 and CB4856.

I have developed a multi-pronged approach to identify any and all causative variants that contribute to bleomycin sensitivity. This approach involves experimental and computational methods, including building nearly isogenic lines (NILs) to empirically narrow the interval and determine the effect of the chromosome V QTL on bleomycin sensitivity as well as creating CRISPR-generated knock-out alleles of each candidate gene in both genetic backgrounds. Ultimately, I aim to identify the gene(s) within the 162-kb QTL on chromosome V in which variation explains differential sensitivity to bleomycin, thus improving our understanding of variation in human response to this chemotherapeutic.

W4139A Genetic and molecular tools for *Caenorhabditis* sp. 34, a sister species of *C. elegans* with a larger body size. *K. Tsuyama*[1], S. Namai[1], R. Kumagai[1], T. Shimura[1], N. Kanzaki[2], T. Kikuchi[3], A. Sugimoto[1]. 1) Tohoku University, Sendai, Miyagi, JP; 2) Forestry and Forest Products Research Institute, Tsukuba, Japan; 3) University of Miyazaki, Miyazaki, Japan.

Caenorhabditis sp. 34 is a sister species of *C. elegans* recently isolated from syconia of the fig *Ficus septica* on Ishigaki Island, Japan. Although their genome sequences are the closest each other, there are striking developmental and morphological differences between *C.* sp. 34 and *C. elegans*. For example, *C.* sp. 34 adults are two times longer than *C. elegans*; *C.* sp. 34 has female and male, unlike *C. elegans* that has hermaphrodite and male. The genome similarity and phenotype differences make this organism highly attractive as a satellite model for *C. elegans* in comparative and evolutionary biological studies.

To make *C.* sp. 34 more tractable, we are developing genetic and molecular tools for *C.* sp. 34, by emulating the rich experimental techniques established in *C. elegans.* **1) Immunofluorescence.** A panel of antibodies against *C. elegans* proteins was used for immunofluorescence of *C.* sp. 34 embryos, many of which successfully recognized expected structures in *C.* sp. 34. Notably, polyclonal antibodies against *C. elegans* PGL-1/3 recognized P granule-like granules in *C.* sp. 34 embryos, but their size was smaller and more dispersed than their *C. elegans* counterparts, implying the distinct dynamics of germ granules between these two species. **2) Transgenesis.** We examined transgenesis in *C.* sp. 34 by microinjecting transgenic marker plasmids commonly used in *C. elegans*. Although the frequency was lower than that in *C. elegans*, stable transgenic lines were obtained for all transgenes tested, and their expected phenotypes (Rol for *rol-6(su1006)*) or expression patterns (Psur-5::GFP (universal), Punc-122::GFP/RFP (coelomocytes), Pmyo-3::mCherry (body wall muscle), Podr-1::GFP (AWC neuron)) were detected. Thus, promoters and 3'-UTR sequences of *C. elegans* appear generally functional in *C.* sp. 34. **3) RNAi.** We tested RNAi in *C.* sp. 34 using the soaking and feeding methods. The soaking RNAi of the *tbg-1* (g-tubulin) ortholog of *C.* sp. 34 resulted in early embryonic lethality at a high penetrance, with phenotypes similar to those of *tbg-1(RNAi)* in *C. elegans*. Feeding RNAi for the GFP coding sequence in the GFP-transgenic *C.* sp. 34 animals efficiently reduced the GFP signal. Thus, *C.* sp. 34 is highly sensitive to RNAi via ingested dsRNAs.

Taken together, many of the genetic techniques, molecular tools and resources of *C. elegans* can be directly transferred to *C.* sp. 34. The relative feasibility of importing *C. elegans* techniques and resources will allow rapid establishment of *C.* sp. 34 as a satellite model species for evolutionary studies. Now we plan to use these techniques and tools for dissecting the genetic bases for morphological and developmental differences between *C.* sp. 34 and *C. elegans*.

W4140B Neurologic and Genetic Analysis of *Ginkgo biloba* Extract Effects in *Caenorhabditis elegans*. *H. Cathcart*, A. Battle, M. Thomas, C. Thurber. Abraham Baldwin Agricultural College, Tifton, GA.

Ginkgo biloba extract (GBE), which is rich in antioxidants is often used as an alternative treatment to postpone the development of Alzheimer's disease (AD) symptoms, but little is known regarding its protective mechanisms. The nematode, *Caenorhabditis elegans*, is a valuable tool for studying AD because it expresses an APP ortholog, apl-1, which has been implicated in the insulin signaling pathway and in larval development. Our study was two-fold. 1) We examined the touch withdrawal response of *C. elegans* strains overexpressing apl-1 that were GBE treated and untreated. An eyelash was used to gently tap on the posterior and anterior ends of the nematodes to determine habituation and memory. 2) RNA sequencing was used to determine the expression level of genes affected by GBE in both wild type (N2) and mutant strains. Total RNA was extracted using the Purelink© RNA Mini Kit, while libraries and sequencing were performed at the New York Genome Center. By combining these phenotypic and genotypic methods, we expect to identify genes and mutations responsible for molting and memory defects in this important model species.

W4141C Evaluation of single-cell RNA sequencing measurements for use in developmental lineage reconstruction. *H. Dueck*, E. Torre, S. Shaffer, A. Raj, J. Murray. University of Pennsylvania, Philadelphia, PA.

The process of development in multicellular organisms may be specified by a lineage tree, whose structure represents the developmental trajectory of each cell, and by the dynamic gene expression changes that occur in individual cells progressing along these trajectories. Transcriptome measurements from single developing cells may provide sufficient information to infer an unknown lineage and estimate lineage-specific expression dynamics. Single-cell RNA sequencing provides a method to make such measurements; however, the adequacy of scRNA-seq measurements for developmental lineage reconstruction is unknown. In particular, technical noise may generate spurious expression similarity between cells and limited sensitivity may prevent detection of critical regulators that are expressed at low levels.

Here, we evaluate the adequacy of Drop-seq [1] for developmental lineage reconstruction. This recently developed scRNA-seq method provides high-throughput measurements at low cost and so could be used to measure the transcriptomes of individual cells densely sampled over developmental time, even for large lineage trees. We generated expression measurements for thousands of melanoma cells using both Drop-seq and single-molecule florescence *in situ* hybridization (smFISH) for hundreds of genes. We compare the population distribution of expression levels estimated using these methods and, treating smFISH as a gold standard, provide a gene-level assessment of Drop-seq measurements in terms of sensitivity, precision and accuracy. We consider the relative strengths of *in situ* imaging and sequencing methods, as well as the possibility of integration of these different data types. As proof of concept, we are generating Drop-seq measurements for individual C. elegans cells from dissociated mixed-stage embryos. We will compare these data to dynamic, single cell expression measurements of the developing worm generated by automated lineage-tracing microscopy. Through this comparison, we will further assess potential sampling bias in the Drop-seq method and the ability to detect key developmental genes.

1. Macosko, Evan Z., Anindita Basu, Rahul Satija, James Nemesh, Karthik Shekhar, Melissa Goldman, Itay Tirosh, et al. *Cell* 161, no. 5 (May 21, 2015): 1202–14.

W4142A Chromsosomal context influences X chromosome targeting by the C. elegans Dosage Compensation Complex. *S. E. Albritton*, L. W. Winterkorn, A. L. Kranz, S. Ercan. New York University, New York, NY.

Multi-subunit condensin complexes are essential for chromosome condensation during both mitosis and meiosis and have also been implicated as having important roles in transcriptional regulation during interphase. Metazoans contain two condensin complexes, I and II, which specifically localize to different chromosome regions where they perform different functions. Caenorhabditis elegans contains a third condensin complex, Condensin DC, whose localization is uniquely restricted to the hermaphrodite X chromosome where it acts as part of the Dosage Compensation Complex (DCC) to repress X-transcription. The regulatory mechanisms by which Condensin DC is targeted specifically to the X chromosome are not yet fully understood.

As part of the DCC, Condensin DC interacts with at least four other non-condensin proteins, including two zinc-finger-containing proteins, SDC-2 and SDC-3, which act to recruit Condensin DC to approximately 100 recruitment sites across the X-chromosome. Evidence suggests that this initial targeting is sequence-dependent. Sites of initial recruitment, termed recruitment elements on X (rex), are enriched for a 10bp recruitment motif. Our analyses indicate that this motif is four times enriched on the X-chromosome as compared to autosomes and is often clustered at rex-sites. However, the motif is not unique to the X-chromosome; both the X chromosome and the autosomes contain many perfect matches that are not bound by the DCC. Further, we show that insertion of a rex-site in single-copy onto an autosome fails to detectably recruit DCC. Increasing the number of inserted rex-site copies overcomes the inability recruit on autosomes. We conclude that motif sequence, while important for DCC recruitment, is not sufficient to recruit the complex on its own. We hypothesize that chromosomal context of the X chromosome facilitates the specificity of DCC recruitment.

W4143B Identification of *lin-35* (Rb) suppressors. *Cynthia Becker*, Lo Te-Wen. Ithaca College, Ithaca, NY.

Proper development of an organism requires the precise regulation of many genetic factors. Mis-regulation of gene expression often results in human diseases such as cancer. This can be caused by mutations in key classes of genes required for cellular development. Tumor suppressor genes are one class of genes known to play a role in cancer prevention. In mammals, retinoblastoma (Rb) is a tumor suppressor gene that, when inactivated, promotes cancer. We are using *C. elegans* as a model to study Rb function. We performed an RNAi screen to identify suppressors of *lin-35*, the *C. elegans* Rb homolog.

Our study builds directly on an RNAi suppressor screen previously published by Polley and Fay (2012). Polley and Fay exploited the genetic interaction between *lin-35* and *slr-2*. *lin-35* and *slr-2* act redundantly in the intestine and as a result, simultaneous mutations in both genes result in a synthetic lethal phenotype. In order to circumvent the synthetic lethality and to maintain the strain, Polley and Fay expressed a wild-type copy of *lin-35* in the double mutant as an extrachromosomal array with a GFP marker. Worms that contain the array, and therefore a wild-type copy of *lin-35*, can live past the larval stage. This array is lost in about 30% of offspring resulting in non-green offspring that would die as young L1 larvae, unless the lethality is suppressed by RNAi knockdown of another gene.

This screen is exceptionally well-suited for teaching genetics concepts and techniques. As a teaching tool, the preliminary screen was performed in a genetics lab course at Ithaca College. In Spring 2015, the genetics class screened the supplemental X chromosome Ahringer RNAi library. They identified 171 out of 371 genes as potential suppressors. In the Lo lab, through additional experiments, we have identified 50 of the strongest suppressors of the *lin-35* lethal phenotype. For each experiment, we included known suppressors of *lin-35* as positive controls to ensure all reagents and techniques functioned properly. RNAi bacteria that did not target a gene was included in each experiment as a negative control.

Ongoing research including additional viability and larval arrest experiments will further identify the most relevant suppressors. Ultimately, these studies will help identify components of the Rb tumor suppressor pathway and better inform future human disease treatments.

W4144C Mutagenesis of GATA motifs controlling the endoderm regulator *elt-2* reveals distinct dominant and secondary *cis*-regulatory elements. *Lawrence Du*, Sharon Tracy, Scott Rifkin. University of California San Diego, La Jolla, CA.

Cis-regulatory elements (CREs) are crucial links in developmental gene regulatory networks, but in many cases, it can be difficult to discern whether similar CREs are functionally equivalent. We found that despite similar conservation and binding capability to upstream activators, different GATA *cis*-regulatory motifs within the promoter of the *C. elegans* endoderm regulator *elt-2* play distinctive roles in activating and modulating gene expression throughout development. We fused wild-type and mutant versions of the *elt-2* promoter to a *gfp* reporter and inserted these constructs as single copies into the *C. elegans* genome. We then counted early embryonic *gfp* transcripts using single-molecule RNA FISH (smFISH) and quantified gut GFP fluorescence. We determined that a single primary dominant GATA motif located -527 bp upstream of the *elt-2* start codon was necessary for both embryonic activation and later maintenance of transcription, while nearby secondary GATA motifs played largely subtle roles in modulating postembryonic levels of *elt-2*. Mutation of the primary activating site increased low-level spatiotemporally ectopic stochastic transcription, indicating that this site acts repressively in non-endoderm cells. Our results reveal that CREs with similar GATA factor binding affinities in close proximity can play very divergent context-dependent roles in regulating the expression of a developmentally critical gene *in vivo*.

W4145A Direct and positive regulation of *bed-3* by BLMP-1 in *C. elegans*. *H. T. Fong*, J. Yang, W. H. Tan, T. Inoue. National University of Singapore, Singapore, SG.

C. elegans BLMP-1, the ortholog of the mammalian zinc finger transcriptional repressor PRDM1/BLIMP1, plays an important role in the heterochronic pathway regulating developmental timing. Previously, we have reported that BLMP-1 positively regulates *bed-3*, a gene required for molting and vulval development. By performing *in vivo* reporter assays and quantitative PCR, we have shown that *bed-3* transcription is downregulated upon BLMP-1 knocked down. Using electrophorectic mobility shift assay (EMSA), we identified two BLMP-1 binding motifs in the 200bp SF2-9 enhancer region located within *bed-3* intron 3. In addition, through RNAi screening and double knock-downed assays, we identified candidate chromatin factors and co-activators which may act with BLMP-1 to regulate *bed-3* transcription. We anticipate that analysis of these components will reveal the mechanism by which BLMP-1 functions as a positive regulator of *bed-3*, and give us better understanding of how the heterochronic pathways regulates molting and vulval development.

W4146B Cellular proteomes drive tissue-specific regulation of the heat shock response. *E. Guisbert*, C. Grant, J. Ma, K. Kim Guisbert. Florida Institute of Technology, Melbourne, FL.

The heat shock response (HSR) senses protein misfolding in the cell and induces a set of conserved heat shock proteins, many of which help to restore protein homeostasis (proteostasis). The HSR is a cell-autonomous response, but under some conditions, it can be coordinately regulated across tissues and throughout the whole organism. Currently, the distinctions between cell-autonomous and coordinate HSR regulation are not well established.

Previously, we identified a tissue-specific HSR regulatory network in *Caenorhabditis elegans*. Here, we analyze several of these regulators and uncover mechanisms that drive their tissue-specific HSR regulation. We find that muscle-specific regulation of the HSR by the TRiC/CCT chaperonin complex is not driven by muscle-specific expression of the TRiC/CCT complex itself, but rather by actin, one of its the primary cellular substrates. Actin knockdown reduces the sensitivity of muscle to TRiC/CCT and broad overexpression of an actin isoform expands the tissue-specificity of TRiC/CCT regulation of the HSR by sensitizing intestinal cells. Additionally, we characterized the actin overexpression line and found that it has several interesting phenotypes, including shortened lifespan, supporting other recent research suggesting that maintenance of the actin cytoskeleton is an important factor for maintaining longevity. Futhermore, we show that intestine-specific regulation of the HSR by components of the secretory pathway is driven by the vitellogenins, some of the major secreted proteins in the intestine.

Together, these data reveal that tissue-specific regulation of the HSR reflects a balance between the protein homeostasis (proteostasis) machinery and the specific requirements arising from the cellular proteome. This tissue-specific regulatory mechanism is similar to the well-established regulation of the HSR by the HSP70 and HSP90 chaperones that is also dependent on their cellular substrates but is not tissue-specific. Our findings that distinct cellular proteomes influence HSR regulation have important implications because the HSR has been implicated in a variety of tissue-specific diseases including cancers and neurodegenerative diseases.

W4147C A sexually dimorphic transcriptional switch integrates information about microbial environment and nutritional state to regulate exploratory behavior of *C. elegans*. *Z. A. Hilbert*, D. H. Kim. MIT, Cambridge, MA.

In their natural environments, *C. elegans* integrate a wide array of information to make behavioral decisions. We have previously reported that exposure to the pathogenic bacteria *Pseudomonas aeruginosa* can alter the neuronal expression pattern of the DAF-7/TGFß ligand, inducing expression in the ASJ neuron pair of hermaphrodite animals (Meisel, J.D. *et al. Cell* (2014)). Here, we show that males exhibit the induction of *daf-7* expression in the ASJ neuron pair even in the absence of *P. aeruginosa*. This sexually dimorphic transcriptional switch is turned on in an age-specific manner in adult males and is required for the male-specific mate searching behavior. Moreover, we have found that expression of *daf-7* in the male ASJ neurons is dynamically regulated by the availability of bacterial food. We observe that starved male animals repress the activation of *daf-7* expression in ASJ that promotes mate-searching behavior. In contrast, when these starved animals are challenged with a pathogenic food source, they rapidly up-regulate *daf-7* expression in the ASJ neuron pair indicating that a worm will prioritize pathogen avoidance over seeking food when starved, which in turn is prioritized over mate searching. Our data suggest that sex, development, nutritional state and microbial environment are all integrated in the hierarchical regulation of a transcriptional switch in the ASJ neuron pair to regulate exploratory behavior.

W4148A Dynamic trans-splicing in *C. elegans*. *Marija Jovanovic*, Jiarui Li, Justin White, Jack Chen. Simon Fraser University, Burnaby, Canada.

Trans-splicing occurs when two distinct RNA molecules, which originate from different genomic regions, are spliced together. This phenomenon occurs in a wide range of organisms, including nematodes and mammals. In *C. elegans*, a particular form of trans-splicing takes place. During the post-transcriptional processing of pre-mRNA, a highly conserved splice leader sequence is spliced to the 5' end of transcripts. Earlier efforts that quantified trans-splicing in *C. elegans* suggest that 70% of genes are trans-spliced. The goal of this research is to examine whether trans-splicing in *C. elegans* is static or dynamic in developmental stages in different tissue types and to ascertain the functional impact of trans-splicing in *C. elegans*. In this project, we examined trans-splicing events for all genes and study the dynamics of trans-splicing in different conditions, using Iso-seq data we have generated and taking advantage of existing RNA-seq data generated by the modENCODE projects.

Our research used a combination of computational analysis and bioinformatics software to characterize trans-splicing events. We used Iso-seq data to study the nature of trans-splicing events in the context of full-length transcripts, the results of which were used to determine parameters for predicting and annotating trans-splicing acceptor sites. We predicted candidate trans-splicing acceptor sites based on the genome sequences and location of trans-splicing sites. Using RNA-seq data and the genome-sequence-based prediction of trans-splicing acceptor sites, we annotated trans-splicing events under various conditions, including developmental stages and tissue types. These results enabled us to compare the trans-splicing status across stages and conditions so that we can elucidate the dynamics of trans-splicing activities. Our analysis revealed that trans-splicing in *C. elegans* is a dynamic and regulated process, which may play a role in maintaining the level of translated genes. In this presentation, we will elaborate a genome-wide analysis of trans-splicing in *C. elegans*, with a focus on the dynamics of trans-splicing, tissue- and developmental-stage specific trans-splicing, and constitutive trans-splicing, aiming to elucidate the functional impact of trans-splicing in *C. elegans*.

W4149B Hox proteins generate neuronal diversity by regulating the transcriptional output of a single terminal selector gene. *P. Kratsios*[1,2], S. Y. Kerk[2], O. Hobert[2]. 1) University of Chicago, Chicago, IL; 2) HHMI, Columbia University, New York, NY.

Understanding how the expression of neuron class-specific genes is established during development is of key importance to the problem of neuronal diversity. Exploiting the molecular diversity of distinct motor neuron (MN) classes in *C.elegans*, we show that the evolutionarily conserved COE (Collier, Olf, Ebf) - type terminal selector UNC-3 is required for MN diversity by directly regulating the expression of MN class-specific terminal identity genes, such as ion channels and neurotransmitter receptors. We further find that Hox proteins cooperate with UNC-3 and remarkably employ two distinct, region-based strategies to generate MN diversity. In the *C.elegans* ventral nerve cord (VNC), the more anteriorly expressed *Hox* genes, *lin-39*/Scr/Dfd and *mab-5* (Antennapedia-type), as well as the Hox cofactor *ceh-20*/Exd/Pbx are required for expression of MN class-specific terminal identity genes. Our mutational analysis shows that *lin-39* and *mab-5* – similarly to UNC-3 – directly activate MN class-specific genes, revealing a coactivation strategy that generates MN diversity along the VNC. Conversely, the posterior *Hox* gene *egl-5/Abd-B* acts as a repressor of *lin-39* and *mab-5 expression*, thereby diversifying posteriorly located MN subclasses from their anterior homologs. Genetic removal of *egl-5* results in homeotic transformation of posterior MN subclasses as evidenced by molecular and anatomical criteria. Intriguingly, we find that *unc-3* and *Hox* orthologs are coexpressed in mouse MNs along the spinal cord, suggesting that this intersectional, region-based gene regulatory principle we uncovered in *C.elegans* may be conserved across phylogeny to generate neuronal diversity.

W4150C X-box promoter motif searches: from *C. elegans* to humans to novel candidate ciliopathies. *G. Lauter*[1], K. Tammimies[1,2], A. Bieder[1], D. Sugiaman-Trapman[1], R. Torchet[1], M.-E. Hokkanen[3], J. Burghoorn[1], E. Castrén[3], J. Kere[1,4], I. Tapia-Páez[1], P. Swoboda[1]. 1) Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; 2) Centre of Neurodevelopmental Disorders (KIND), Pediatric Neuropsychiatry Unit, Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden; 3) Neuroscience Center, University of Helsinki, Helsinki, Finland; 4) Molecular Neurology Research Program, University of Helsinki, Helsinki, Finland and Folkhälsan Institute of Genetics, Helsinki, Finland.

Cilia are highly conserved organelles protruding from dendrites of certain sensory neurons in *C. elegans* and from the cell surfaces of various cell types in other species. Since cilia are implicated in essential cellular processes, defects of ciliary structure and function have been identified as the underlying causes of severe genetic disorders collectively called ciliopathies. We make use of the X-box promoter motif recognized by the RFX transcription factor DAF-19, the major regulator of ciliogenesis in *C. elegans*, to identify genes involved in human ciliopathies. Using a C. *elegans* derived X-box search, we have identified several hundred human genes with conserved X-box motifs. The identified X-box genes are candidate ciliary genes as is the case in *C. elegans*, *Drosophila melanogaster* and other species. Currently, we focus on genes associated with dyslexia, the most common reading disorder. In the promoter regions of human *DYX1C1*, *DCDC2* and *KIAA0319*, three of the most replicated dyslexia candidate genes, we identified conserved X-box motifs. We demonstrate their functionality as well as ability to recruit RFX TFs using reporter gene and electrophoretic mobility shift assays. Furthermore, we uncover a complex regulatory interplay between human RFX1, RFX2, RFX3 and their effect on the endogenous expression of *DYX1C1* and *DCDC2* in human retinal pigment epithelial (RPE-1) cells. Induction of ciliogenesis increases the expression of RFX TFs and dyslexia candidate genes. Endogenous DYX1C1 localizes to the base of the cilium while DCDC2 localizes along the entire ciliary axoneme. Finally, over-expression of human DCDC2 leads to ectopic branching of ciliated neurons in *C. elegans*. Our results demonstrate that the evolutionarily conserved X-box promoter motif can be used to identify ciliary candidate genes across species.

W4151A *Caenorhabditis elegans* BMP Transcriptional Program Implicates Collagen Remodeling in Body Size Regulation. *U. Madaan*[1], M. Meade[1], E. Yzeiraj[1], C. Rushlow[2], C. Savage-Dunn[1]. 1) Queens College, Flushing, NY; 2) New York University, NY.

Cell-cell communication is required for proper development and survival of an organism. One form of cell-cell communication uses signal transduction pathways such as the TGF-β pathway. TGF-β pathways are critical for developmental processes such as cell growth, cell division and organ development. TGF-β ligands signal through a type I and type II receptor and intracellular signal transducers called Smads. Binding of the ligand to its receptors allows for phosphorylation of Smads which then complex together and are shuttled to the nucleus and regulate target genes of the TGF-β pathway. The TGF-β ligands are conserved across animal phyla and a homolog of TGF-β in the nematode *C. elegans* is DBL-1. We previously established DBL-1 signaling in the epidermis of the worm as necessary and sufficient for normal body size. However, it is not clear which genes are directly responsible for changes in body size and what is the mechanism leading to a small worm. **We present evidence implicating cuticle collagen genes as the effector genes responsible for the small body size phenotype resulting from defects in DBL-1 signaling.** First, we show through EMSA and analysis of ChIP-seq data that specific collagen genes are direct or indirect targets of Smad regulation. Next, we establish through RNAi inhibition and overexpression that DBL-1-regulated cuticle collagen genes are positive and negative regulators of body size. Future work aims to determine whether changes in the exoskeleton structure of the worm affects DBL-1 signaling.

W4152B Activation and Repression of Target Gene Expression in Neurons by the *C. elegans* RFX Transcription Factor, DAF-19. *K. Mueller*[1], Z. Ek-Vasquez[1], S. Scholtz[1], H. Olson[1], P. Phirke[2], D. Sugiaman-Trapman[2], P. Swoboda[2], E. A. De Stasio[1]. 1) Lawrence University, Appleton, WI; 2) Karolinska Institute, Stockholm-Huddinge, Sweden.

DAF-19, the only RFX transcription factor found in *C. elegans*, is required for the formation of neuronal sensory cilia. Four isoforms of the DAF-19 protein have been reported, and the *m86* nonsense (null) mutation affecting all four isoforms has been shown to prevent cilia formation. Transcriptome analyses employing microarrays of L1 and adult stage worms were completed using RNA from *daf-19(m86)* worms and an isogenic wild type strain to identify additional putative DAF-19 target genes. Using transcriptional fusions with GFP, we compared the expression patterns of several potential gene targets using fluorescence confocal microscopy. Expression patterns were characterized in various genetic backgrounds in order to determine isoform-specific expression patterns. Additionally, we completed rescue experiments using cDNAs encoding specific DAF-19 isoforms in a *daf-19* null genetic background. Our data indicate that at least five new genes are activated and four new genes are repressed by DAF-19 in both ciliated and non-ciliated neurons. Few of these genes are known to contain a classical X-box motif through which DAF-19 gene regulation is thought to occur. We are currently developing isoform-specific CRISPR/Cas9 mutants to further explore the precise mechanisms by which different DAF-19 isoforms regulate their target genes.

W4153C Regulation of anterior lineage genes in *C. elegans* embryogenesis. *J. D. Rumley*, A. Zacharias, J. I. Murray. University of Pennsylvania, Philadelphia, PA.

Embryonic patterning requires many signaling pathways to control cell fates. A fundamental step in development is patterning the body axes. Antero-posterior axis patterning depends on the Wnt pathway, which acts through the transcription factor TCF and its coactivator β-catenin. Classic Wnt targets are transcriptionally activated when Wnt signaling is active through the TCF:β-catenin complex binding to *cis* regulatory regions and are repressed when Wnt signaling is inactive through TCF binding without β-catenin. In contrast, emerging evidence suggests the existence of direct "opposite" targets, activated by TCF without β-catenin and repressed by TCF:β-catenin.

In the *C. elegans* embryo, almost all cell divisions are patterned by a Wnt pathway variant, called the Wnt/β-catenin asymmetry pathway, in which nuclear level of POP-1/TCF is higher in the unsignaled anterior sister cell and nuclear level of SYS-1/β-catenin is higher in the signaled posterior sister cell. Many genes expressed during embryogenesis are differentially expressed between anterior and posterior sister lineages. Several genes expressed in posterior lineages are classic Wnt targets, expressed when Wnt signaling is active. On the contrary, the regulation of anterior lineage genes is not well understood. I am testing whether these genes are direct opposite Wnt targets and how they are regulated. RNAi depletion of either POP-1 or SYS-1 shows that these factors are necessary for proper regulation of anterior genes but does not distinguish direct from indirect regulation. I will identify anterior gene enhancers, and determine if these enhancers are bound by POP-1 and if any POP-1-bound sites are necessary for anteriorly biased expression.

This work will clarify mechanisms regulating expression of anterior sister lineage genes in the *C. elegans* embryo, and may have implications for Wnt pathway regulation of genes expressed in unsignaled cells in other species.

W4154A Transcriptomic Analysis of *C. elegans* transgenic animals overexpressing human alpha-synuclein (A53T): Comparison to genes regulated in human Parkinson's Disease brain tissues. *C. Wang*[1], J. Peltonen[2], L. Heikkinen[3], G. Wong[1]. 1) University of Macau, Macau, MO; 2) University of Eastern Finland, Kuopio, Finland; 3) University of Jyväskylä, Finland.

Parkinson's Disease is a devastating neurodegenerative disorder characterized by loss of dopaminergic neurons in the *substania nigra pars compacta*. We modeled this disease by constructing a transgenic *C. elegans* strain overexpressing human alpha-synuclein gene with mutation A53T in dopaminergic and other neurons using a pan neuronal promoter. We isolated total RNA and performed RNA-seq transcriptomic analysis on young adult animals and compared the gene expression changes to controls. We observed 1793 genes up- and 92 down-regulated using a Bowtie-Cufflinks-Cuffdiff workflow. Gene set enrichment analysis using David 6.7 indicated that up-regulated annotation clusters included phosphatase activity (46 genes, $p<10^{-20}$), PapD-like (31 genes, $p<10^{-23}$), DUF856 (7 genes, $p<10^{-7}$) and down-regulated included (serine/threonine protein kinase (4 genes, $p<0.02$). Comparing the list of regulated genes from *C. elegans* to the top 11 genes related to human Parkinson's disease confirmed an overlap of 4 genes: DGKQ/GAK, diacylgycerol kinase (*dgk-1*), MAPT, microtubule-associated protein tau (*ptl-1*), SYT11/RAB25, synaptotagmin-11 (*snt-1*), and STK39, SPS1-related proline-alanine-rich protein (*gck-3*). Taken together, our results suggests

that *C. elegans* could be a useful model to identify gene expression changes in human Parkinson's disease and ultimately to understand the pathophysiology of this disease.

W4155B Overlapping microRNA networks during nematode development. *A. E. Kerscher*, J. Jo, K. Breving, J. Gordon, N. Feuer, E. Humes. Eastern Virginia Medical School, Norfolk, VA.

MicroRNAs (miRNAs) are a highly conserved class of non-coding RNAs that regulate essential cellular events such as proliferation and differentiation. The *Caenorhabditis elegans* (*C. elegans*) animal model provides a unique opportunity to determine how distinct groups of miRNAs, such as those belonging to the *lin-4* and *let-7* families, function in overlapping signaling networks during post-embryonic development. *lin-4* and *let-7* are well characterized and direct cell fate determination in *C. elegans* during the larval transitions and act as key regulators of temporal gene expression. Additional *lin-4* and *let-7* family members display overlapping expression patterns in the developing hypodermis and reproductive system in nematodes, and we hypothesize that combinations of miRNAs across these families (and sharing little sequence homology to one another) control common developmental events. Our deletion studies indicated that the *lin-4* homologue, miR-237, and the *let-7* family members, miR-48 and miR-84, function together likely with chromatin remodeling genes to direct cell cycle progression in the germline as well as formation of the egg-laying structures. Our work also suggested that members within the *lin-4* miRNA family, namely *lin-4* and *mir-237,* which share a common "miRNA seed", functionally compensate when tested in a *lin-4* loss-of-function *in vivo* rescue assay. These studies may provide insight into why mammalian homologues for *lin-4* and *let-7* are often targeted in a wide range of human cancers.

W4156C Identification of microRNAs that regulate ovulation in *C. elegans*. *Katherine Maniates*, Benjamin Olson, Jordan Brown, Tyler Crawford, Katalin Kenney, Allison Abbott. Marquette University, Milwaukee, WI.

MicroRNAs regulate many developmental and physiological processes in *C. elegans* including ovulation, a rhythmic process that occurs every 20 minutes in adult worms. Even though microRNAs are known to be necessary for ovulation, the individual microRNAs that regulate ovulation are still largely unknown. One challenge of this work is that microRNAs can function redundantly and many individual microRNAs mutants do not display observable phenotypes. We have taken several approaches to identify individual microRNAs that function in the process of ovulation. First, we have performed a synthetic sterility screen of microRNA mutants using knockdown of a "hub gene," *hmg 1.2* RNAi. On this sensitized genetic background, we identified five microRNAs as candidates to regulate ovulation in *C. elegans.* These candidates are being further analyzed to characterize their role in ovulation. Second, we have analyzed microRNAs that were identified from small RNA sequencing of RNA isolated from mature oocytes. One abundant family of microRNAs in the oocyte is the miR-44 family. It is composed of four miRNAs: *mir-44, mir-45, mir-61,* and *mir-247,* that share a common seed sequence and thus are predicted to regulate shared target mRNAs. Triple *mir-44* family mutants have significantly reduced ovulation rate and brood size, suggesting a role in regulating ovulation. *mir-44* and *mir-45* are within about 9kB of each other so double mutants were not able to be generated. Because miR-44 and miR-45 have identical mature miRNA sequences, we sought to obtain a double mutant using CRISPR. CRISPR was used to introduce a mutation in the *mir-45* gene in worms carrying a deleting in *mir-42/44.* This *mir-45 mir-42/44* double mutant is currently being analyzed to characterize ovulation defects. The goal is to identify pathways and targets regulated by specific microRNAs in the process of ovulation.

W4157A Investigating the role of KIN-20 in microRNA biogenesis, LIN-42 regulation and developmental timing. *Christiane Olivero*, Katherine Cascino, Laura Aseltine, Joseph Spina, Priscilla Van Wynsberghe. Colgate University, Hamilton, NY.

The *C. elegans* heterochronic pathway is composed of multiple proteins and microRNAs that regulate developmental timing. microRNAs are ~22 nucleotide RNAs that post-transcriptionally silence gene expression, and are thus essential in diverse biological processes. Previous work in our lab and others has shown that the heterochronic pathway member and circadian rhythm Period protein homolog, LIN-42, regulates microRNA biogenesis. Like other period proteins, levels of LIN-42 oscillate throughout development. In other organisms this cycling is controlled in part by phosphorylation and dephosphorylation events. KIN-20 is the *C. elegans* homolog of the *D. melanogaster* Period protein kinase *doubletime*. Worms containing a large deletion in *kin-20* develop slower and have a significantly smaller brood size than wild type *C. elegans*. Here we analyze the effect of KIN-20 on the expression of the canonical heterochronic miRNAs let-7 and lin-4. We find that let-7 and lin-4 levels are significantly decreased and that mRNA levels of the let-7 target *lin-41* are increased in *kin-20* mutant worms relative to wild type. Additionally, we find that primary let-7 and primary lin-4 levels are unchanged in *kin-20* mutant worms suggesting that KIN-20 impacts miRNA biogenesis downstream of miRNA transcription. Current work seeks to determine if these effects are dependent on LIN-42. To further explore the relationship of KIN-20 and LIN-42, we analyzed the effect of KIN-20 on endogenous LIN-42 expression. Based on the homologous system in *D. melanogaster*, we hypothesized that KIN-20 phosphorylates LIN-42 to trigger LIN-42 degradation. Surprisingly, we find that KIN-20 effects on LIN-42 differ depending on the isoform analyzed. Altogether these results help uncover the mechanisms by which these conserved circadian rhythmic genes interact to ultimately regulate rhythmic processes, developmental timing and microRNA biogenesis in *C. elegans*.

W4158B A conserved yet uncharacterized RNA binding protein modulates microRNA activity during *C. elegans* development. *A. Y. Zinovyeva*, M. Kranick, K. Nilges, M. Gulley. Kansas State University, Manhattan, KS.

MicroRNAs are small non-coding RNAs that post-transcriptionally regulate expression of many genes. MicroRNA activity is essential for animal development as alterations in microRNA expression and function have been associated with many developmental pathologies and diseases, making microRNAs of great interest as diagnostic and therapeutic targets. However, little is known about how microRNA function itself is modulated by specific developmental and physiological signals. We wish to understand how cellular factors may integrate these signals to modulate microRNA-mediated gene repression. Using proteomics approaches, we identified a conserved yet uncharacterized RNA binding protein, HRPK-1, as an Argonaute ALG-1 interacting factor. Loss of *hrpk-1* function results in a number of developmental phenotypes, including

defects in embryonic development, fertility, and organ morphogenesis, all phenotypes reminiscent of defects seen in specific microRNA mutants. Knockdown of *hrpk-1* by RNAi enhances the embryonic lethality associated with *mir-35-41* mutants, suggesting that *hrpk-1* function is required at least for miR-42 activity. RNAi knockdown of *hrpk-1* also enhances the cell fate defect observed in a reduction-of-function allele of a neuronal microRNA, *lsy-6*, implying that *hprk-1* similarly normally promotes lsy-6 microRNA activity. Interestingly, *hrpk-1* mutants showed no reduction in mature microRNA production, suggesting that HRPK-1 function may not be required for microRNA biogenesis. Because HRPK-1 has predicted RNA binding domains and appears to function downstream of microRNA biogenesis, we hypothesize that HRPK-1 may modulate microRNA activity by interacting with miRISC on target mRNAs.

W4159C Using *C. elegans* cuticle collagen genes to dissect temporal regulation of gene expression during development. *P. Abete Luzi*, D. M. Eisenmann. University of Maryland Baltimore County, Catonsville, MD.

The precise temporal control of gene expression is an essential aspect of metazoan development. After embryogenesis, the ecdysozoan nematode *C. elegans* goes through four larval stages (L1 - L4), molting its outer cuticle covering between each, before becoming an adult. The cuticle in each stage is unique, and the major components of the cuticle are nematode-specific cuticle collagen proteins, which are expressed from a large gene family in this species. Examination of modENCODE project RNAseq temporal developmental data showed that during *C. elegans* development a majority of cuticle collagen (*col*) genes (116/187) display a strong peak of expression in only one developmental stage. This set of temporally co-regulated similar genes provides a powerful system to study temporal regulation of gene expression. Our work is focused on a subset of *col* genes that peak during the L4 larval stage: *bli-1, col-38, col-49, col-63*, *col-138* and *col-175*. Transcriptional YFP reporters for these genes showed peaks of expression in the hypodermis during the L4 stage. Deletion analyses narrowed the necessary promoter regions to fragments of 262, 222 and 282 bp in *col-38*, *col-63* and *col-49*, respectively. In *C. elegans*, developmental timing is controlled by the heterochronic pathway of proteins and miRNAs. The most downstream effector of this pathway is LIN-29, a zinc finger transcription factor required for the larval-to-adult 'switch' in hypodermal cells. As *lin-29* was previously shown to regulate expression of an adult cuticle *col* gene, we investigated the role of *lin-29* in expression of our L4-specific *col* genes. We examined the effects of both *lin-29* loss- and gain-of-function on L4 *col* YFP transcriptional reporters as well as on endogenous *col* gene transcription levels, and found that LIN-29 is required for robust expression of *col* genes in the L4 stage, and sufficient for misexpression of L4-*col* genes at other developmental times. We also found that LIN-29 protein binds in gel shift assays to the smallest *col* gene promoter fragments necessary for *in vivo* expression. These fragments each contain several putative LIN-29 DNA binding motifs, and the requirement for these sites for *in vitro* binding and *in vivo* expression is being tested. Taken together, these results strongly suggest direct regulation of stage-specific *col* genes by LIN-29 during the L4 stage. In order to expand our work on the temporal regulation of gene expression during the L4, we are currently performing a genome-wide search for LIN-29 target genes using temporal misexpression of LIN-29 and RNAseq. Results will be reported.

W4160A Role of nuclear Argonaute proteins in the inheritance of acquired stress resistance in *C. elegans*. *E. Okabe*, S. Kishimoto, M. Uno, E. Nishida. Kyoto University, Kyoto, JP.

As organisms are constantly exposed to many environmental stresses, dealing with stresses is an intrinsic protective mechanism to adapt oneself to the environment. Through exposure to milder stresses, animals increase stress resistance and viability, and thereby live longer. In *Caenorhabditis elegans*, it has been reported that exposure of adult worms to mild stresses can increase stress resistance. Recently, it has been becoming clear that the acquired stress resistance in the parental generation can be inherited to the next generation. However, the molecular mechanisms underlying the inheritance of acquired stress resistance remain largely unknown. Because endogenous small RNAs have been shown to play an important role in transgenerational epigenetic inheritance, we focused our attention on nuclear Argonaute proteins whose functions are essential for transcriptional regulation by endogenous small RNAs. We exposed larvae to hyperosmosis, and then measured the resistance to proteotoxity. Our measurements showed that exposure to hyperosmosis increased the stress resistance, and that the increased stress resistance in the parental generation was inherited to the offspring. We next knocked down each of the two nuclear Argonaute proteins, *hrde-1* and *nrde-3*, in the parental generation. Either *hrde-1* RNAi or *nrde-3* RNAi resulted in suppression of the increased stress resistance in the offspring. Our results suggest that nuclear Argonaute proteins play a role in the transgenerational inheritance of acquired stress resistance. We are now examining underlying mechanisms in more detail.

W4161B The effects of different food types on the reproductive physiology of *C. elegans*. *S. Mishra*, K. Marbach, E. Allen, J. Alcedo. Wayne State University, Detroit, MI.

Different aspects of an animal's life, like development, growth and reproduction, are directly influenced by the quality of its food. In the worm *Caenorhabditis elegans*, different types of food have been shown to affect different physiological processes, such as longevity, feeding and reproduction (Maier *et al*., 2010). For example, *C. elegans* grown on two different strains of *E. coli* bacteria (CS180 and OP50) show marked differences in their reproductive patterns. Worms grown on CS180 have a smaller brood size compared to worms grown on OP50. However, the animals on CS180 have faster rates egg-laying of as compared to animals on OP50. Currently, we are determining the mechanism(s) through which food quality could alter an animal's rate of egg-laying. Since egg-laying behavior is mediated by the amine neurotransmitter serotonin, we are also investigating how food quality affects serotonin signaling in these animals. Thus, we will use both molecular genetics and metabolomic approaches to define the metabolites/molecules present in the bacterial food sources, which are responsible in altering the reproductive physiology of *C. elegans.*

Maier, W., Adilov, B., Regenass, M., and Alcedo, J. (2010). A neuromedin U receptor acts with the sensory system to modulate food type-dependent effects on *C. elegans* lifespan. PLoS Biol *8*, e1000376.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

W4162C Fluorescent beads are a versatile tool for staging *C. elegans* in different life histories. *I. nika*, T. Gibson, R. Konkus, X. Karp. Central Michigan University, Mount Pleasant, MI.

C. elegans life history is dependent on environmental cues transduced through several signaling pathways. In favorable environmental conditions *C. elegans* develops continuously through four larval stages before molting into the adult stage. In contrast, adverse environmental conditions promote entry into the stress resistant dauer larva stage immediately following the second larval molt. Dauer larvae possess distinct properties including cellular and developmental arrest, extended lifespan, and discontinued feeding. If environmental conditions improve, dauer larvae recover to post-dauer L3 larvae, which are developmentally identical to continuously developing L3 larvae. Precise staging of larvae developing through either continuous or dauer life histories is essential for the study of development and/or dauer-related processes. Here, we describe a staging method taking advantage of the inability of dauer larvae and molting larvae to feed. Fluorescent beads are added to the bacterial food source, and dauer or molting larvae are identified by a lack of beads within their digestive tract. Bead-lacking worms are sorted using a dissecting microscope or a wormsorter. We show that lack of beads correlates with two molting markers: lack of pharyngeal pumping, and expression of *mlt-10::GFP-pest.* We then describe how this assay can be used to isolate dauer larvae formed by any of three common methods: starved plates, exogenous pheromone, and dauer-constitutive mutations. Lack of beads correlates well with other known markers of dauer formation, including greatly reduced pumping rate, presence of dauer alae, and SDS resistance. We find that using beads rather than SDS-resistance to identify dauer larvae enables the recovery of SDS-sensitive mutants, including cuticle mutants and partial dauer larvae formed by mutations within the dauer formation pathway. This method therefore provides a simple, transgene-free way to distinguish particular stages during development through continuous or dauer life histories.

W4163A Sex-specific maturation of the *C. elegans* nervous system. *H. Steinert*, D. Portman. University of Rochester, Rochester, NY.

As animals transition into adulthood, males and females undergo sex-specific developmental changes that affect both morphology and behavior to maximize fitness. Juveniles primarily adopt new behaviors upon maturation through modification of existing neural circuits, which must occur at the appropriate time during development. In the *C. elegans* nervous system, most neural circuits are fully formed by the L3 larval stage; however, there are a few examples of neurons with delayed maturation. One is that the embryonically derived HSNs generate synapses onto the vulval muscle at the L3 and L4 larval stages. In addition, several existing neural circuits appear to undergo significant modulation through gene expression changes, particularly in males, during the juvenile-to-adult transition. For example, juvenile males and hermaphrodites have similar levels of the chemoreceptor *odr-10* in the sensory neuron AWA; however, upon maturation males specifically downregulate expression of this chemoreceptor. Similarly, males upregulate expression of *srj-54* in the interneuron AIM during this transition. These gene expression changes likely mediate the activation of adult behaviors; however, it remains unclear how their onset is regulated. In nematodes, it is known that the heterochronic pathway controls developmental timing by regulating stage specific events. Although this pathway has been carefully characterized in seam cells, its functions in the nervous system are not well understood. We are using *odr-10, srj-54,* and other genes as molecular markers for nervous system maturity, allowing us to investigate whether the heterochronic pathway regulates nervous system maturation. Surprisingly, our results suggest that several important heterochronic genes, such as *daf-12 and lin-41*, may not be important for this process. However, we find that *lep-5*, a newly identified heterochronic gene, is essential for juvenile-to-adult changes in the expression of *odr-10* and *srj-54*. Thus, a previously uncharacterized branch of the heterochronic pathway may be important for developmental timing in the nervous system.

W4164B FAX-1 and UNC-42 transcription factors regulate developmental arrest in *C. elegans*. *Bruce Wightman*[1], Emily Bayer[1,2], Sheila Clever[1]. 1) Muhlenberg College, Allentown, PA; 2) Columbia University, New York, NY.

The *fax-1* nuclear hormone receptor and *unc-42* homeobox gene control interneuron identities in *C. elegans*. *fax-1* is the ortholog of *unfulfilled* in *Drosophila* and PNR/NR3E3 in vertebrates, where it functions in the development and function of mushroom bodies and photoreceptors, respectively. The *fax-1* and *unc-42* transcription factors function in specifying the identities of an overlapping subset of nematode interneurons, including the command interneurons AVA and AVE, which function in coordinated movements. Both genes are required for the expression of neuron-specific genes, including glutamate receptors subunits, and axon pathfinding.

Mutations in both *fax-1* and *unc-42* cause an incompletely-penetrant slow-growth phenotype that arises from temporary arrest after hatching at the L1 stage. L1 arrest has been shown to be controlled by the insulin-like signaling pathway that also controls dauer formation and longevity. The *daf-2* insulin receptor is a primary mediator of insulin signaling in *C. elegans.* Strong *daf-2* mutations cause L1 arrest, while weak *daf-2* mutations cause dauer-arrest. Both *fax-1* and *unc-42* mutations cause a fully-penetrant L1 arrest in combination with a weak *daf-2* mutation. The L1 arrest can be reversed by a mutation in the *daf-16* forkhead transcription factor, which functions downstream of *daf-2*. These observations indicate that the *fax-1* and *unc-42* transcription factors may function in insulin-signaling or another pathway that controls developmental progression. Given that both genes are required for the development of a limited set of interneurons, these experiments suggest a previously unappreciated role for interneuron function in regulating developmental temporal progression and arrest. *Supported by NIGMS.*

W4165C Dystrophin interactors in worms and flies. *K. Edwards*, S. Goel, A. Rodriguez, L. Barickman, M. Villarreal, B. Rodemoyer, A. Vidal-Gadea. Illinois State Univ., Normal, IL.

Duchenne muscular dystrophy (DMD) is a fatal genetic disorder caused by mutations in the dystrophin gene and affecting up to one in 3500 males. Understanding the role of dystrophin (Dys) in the etiology of DMD will benefit from a comprehensive approach combining the individual advantages of multiple model systems. Therefore we are developing a pipeline to compare Dys interactor function in *C. elegans* and *Drosophila*. Lack of dystrophin in *C. elegans* leads to impaired burrowing, muscle decline, and death. In the DMD suppressor strain JPS518

(Beron et al. 2015; Genes, Brain & Behavior 14:357), one such interacting mutation displays large "blooms" of ectopic f-actin accumulation at the termini of the body wall muscles in response to muscle challenge. This suggest a novel mechanism for the restoration of muscle function in dystrophic worms. In Drosophila, Dys has been shown to be essential for two developmental events, the correct specification of the wing crossveins and the elongation of the egg during late oogenesis. Both phenotypes extend to new Dys insertion alleles from the Drosophila Gene Disruption Project. A GFP protein trap in the endogenous Dys locus shows that Dys has relatively low abundance and is very specifically localized at the basal side of epithelia such as the egg chamber follicle cells (as in Schneider et al. 2006; Dev. 133:3805). Dys-GFP accumulates laterally only in certain large cells, e.g., the egg chamber nurse cells and the salivary gland. Dys-GFP signal shows diverse, non-uniform patterns on the membrane; in the oocyte follicle cells it is circumferentially banded like actin. The Dys phenotypes and distributions allow for sensitive genetic and/or localization assays of candidate interactors such as the adaptor protein Capon/NOS1AP (*C. elegans* DYC-1). The Drosophila ortholog of Capon (CG42673) was identified and found to have conserved PTB and coiled coil domains, indicating participation in a protein complex, but it lacks the C-terminal Nitric Oxide Synthase binding region known in vertebrates. Capon LOF allelic combinations are viable and lack the short-egg phenotype seen in Dys mutants, but they do have defects in wing venation. The results indicate that a comparative approach will provide greater insights into the signaling and structural roles of the Dys complex and its functional partners.

W4166A Comparative genomics reveals novel genes associated with sensory cilia. *B. P. Piasecki*[1], T. Sasani[1], B. O'Flaherety[1], J. Henriksson[2], E. A. De Stasio[1], P. Swoboda[2]. 1) Lawrence University, Appleton, WI; 2) Karolinska Institute, Stockholm, Sweden.

A comparative genomics-based screen was conducted to identify evolutionarily conserved ciliary genes that facilitate sensory-specific roles. Using reciprocal BLAST analyses, the genomes of organisms that do not make cilia (the plant *Arabidopsis thaliana* and the yeast *Saccharomyces cerevisiae*) and that retain motile but not sensory cilia (the moss *Physcomitrella patens*) were subtracted from the genomes of organisms that have retained sensory cilia (the worm *Caenorhabditis elegans* and the alga *Chlamydomonas reinhardtii*). These analyses revealed a list of approximately 300 genes that are found exclusively in organisms with sensory cilia but not motile cilia. Importantly, over 10% of the genes on this list have previously been implicated in sensory cilia-specific roles, thus providing numerous internal positive controls, which demonstrates that this list is enriched with sensory-specific ciliary genes. A subset of uncharacterized candidate genes are currently being studied in *C. elegans*, which retains cilia exclusively on a set of neurons termed ciliated sensory neurons (CSNs). We are currently generating a number of promoter- and gene-to-green fluorescent protein (GFP) fusion constructs in order to determine the expression and localization patterns of the proteins encoded by these genes. Two of these candidate genes, which are found in worms (*C. elegans*) and algae (*C. reinhardtii*) but not in moss (*P. patens*) have been termed *wam-1* and *wam-2*, respectively. Expression of *wam-1* appears to be localized exclusively in the support cells of ciliated dopaminergic neurons, while expression of *wam-2* has yet to be fully characterized. Our analyses have successfully revealed novel genes involved in ciliary sensory-specific processes in animals.

W4167B Coordinating microtubule organization with cell cycle state. *Maria Sallee*, Jessica Feldman. Stanford Univ., Palo Alto, CA.

Microtubule organizing centers (MTOCs) generate specific arrangements of microtubules that are essential for many cellular functions, including division, polarization, and transport. Different subcellular sites serve as the MTOC to accommodate these processes; dividing animal cells establish an MTOC at the centrosome, while many differentiated cells designate a non-centrosomal site as the MTOC. A conflict arises when differentiated cells divide — the centrosome must be re-established as the MTOC for mitosis to occur. Regulating this switch in the subcellular site of the MTOC (the "MTOC switch") is important for controlling proliferation in epithelial cells, and a dysregulated switch may contribute to disease states such as cancer. We are using the developing *C. elegans* intestine as a model to understand the mechanistic nature of this MTOC switch. In polarized embryonic intestinal cells, the site of the MTOC switches very stereotypically from the membrane to the centrosome as they divide, and then back to the membrane as they exit mitosis. This binary MTOC switch offers a simple and elegant system to study how the MTOC location is regulated by and coordinated with the cell cycle. We hypothesize that cell cycle genes directly control MTOC location, and that MTOC location is a critical determinant in regulating a cell's potential to divide. Using fluorescently-tagged markers, we can observe the MTOC switch as the cell cycle progresses in real time, and have determined that the membrane switch occurs in G1, while the centrosome switch occurs in G2/M phase. Our hypothesis predicts that cell cycle regulators must interface with MTOC components at some level to coordinate the MTOC location with cell cycle state. We are now testing several candidate regulators of the MTOC switch, including a surprising candidate, the microtubule-severing enzyme *mei-1*/Katanin. In *mei-1(0)* embryos, intestinal cells initially undergo the membrane switch, but soon after, they aberrantly activate the centrosome switch and divide. Intestinal cell number is normal prior to the E16 intestinal stage, suggesting that the additional intestinal cells are not due to early cell fate specification defects. The centrosome switch and division defects are rescued by GFP-MEI-1, and also observed in mutants for the other subunit of the Katanin complex *mei-2*, suggesting that the defects are caused specifically by loss of the Katanin complex. MEI-1 localizes to the membrane MTOC, suggesting a potential role at the membrane in maintaining the MTOC state. In addition to *mei-1*, we are currently investigating several candidates and carrying out an unbiased genetic screen to identify additional MTOC regulators. This work will help reveal the molecular mechanism underlying the coordination of MTOC location with cell cycle state.

W4168C Mechanisms of SYS-1/β-catenin centrosomal localization in early embryonic blastomeres. *J. W. Thompson*, Bryan Phillips. University of Iowa, Iowa City, IA.

Asymmetric cell division, the unequal distribution of cell fate determinants between daughter cells, is a critical system underlying the development and maintenance of the varied tissue types of a multicellular organism. Despite widespread utilization of such divisions, the mechanisms responsible for induction of asymmetry are less understood due to the complex, pleiotropic, and often functionally redundant signaling systems involved. Throughout *C. elegans* development, the Wnt/β-catenin asymmetry pathway induces the asymmetric distribution of Wnt signaling components to polarize a mother cell and therefore differentially regulate Wnt target genes in its daughter cells. For instance,

a proper Wnt signaling response results in accumulation of cytoplasmic SYS-1/b-catenin in the proximal 'signaled' cell, which, along with co-activator POP-1/TCF, activates genes to induce the corresponding cell fate. Despite its asymmetric regulation immediately post-division, cytoplasmic SYS-1 localizes symmetrically to mitotic centrosomes. Recent data from our lab indicate that centrosomal localization of SYS-1 serves as a clearance mechanism to increase the robustness of Wnt-mediated polarity. However, the method of SYS-1 trafficking to and accumulation at the centrosome is unknown. In the early embryo, this centrosomal localization of the SYS-1 is dependent on the centrosomal scaffolding protein RSA-2. When SYS-1 is prevented from loading to the centrosome by loss of RSA-2, it accumulates in the approximate region of kinetochore microtubules. This localization suggests that SYS-1 may be directly transported to centrosomes along microtubules via the action of molecular motors. Specifically, we focused on dynein and dynactin components as the primary minus-end directed motor machinery. We conducted an RNAi and chemical screen specifically aimed at depleting these microtubules and microtubule transport proteins and quantified relative and proportionate SYS-1 centrosomal accumulation. This screen identified several dynein subunits that have a role in proper centrosomal localization of embryonic SYS-1/b-catenin and its regulators. Further, the preliminary data from microtubule-destabilizing and ATP-depleting chemical treatment indicate that both ATP and microtubules are important for SYS-1 centrosomal localization. Together, these results suggest an active role for the multimeric dynein/dynactin cargo binding complex in SYS-1/b-catenin regulation and signaling status.

W4169A The effects of luteolin on the V-ATPase and the acidification of the FB-MOs in *C.elegans* sperm. *Melissa Henderson*, Kenneth Dexter, Marybeth Babos. Lincoln Memorial University-DeBusk College of Osteopathic Medicine, Harrogate, TN.

The V-ATPase is a 14 subunit complex responsible for the acidification of intracellular vesicles and organelles. The complex is also found on the surface of osteoclasts involved in bone resorption during osteoporosis and overexpressed in some tumors. The connection of the V-ATPase with these diseases has launched a need to find inhibitors of this complex as potential therapeutics. During spermatogenesis in *C.elegans* the fibrous body-membranous organelles (FB-MOs) act as secretory vesicles responsible for the delivery of proteins to the surface of the sperm prior to fertilization. The FB-MOs undergo acidification in spermatids facilitated by the V-ATPase. This necessary change in pH can be indicated by the use of LysoSensor Blue (Molecular Probes) staining and the spermatids ability to mature into mature spermatozoa. Here we have established a method to screen potential inhibitors of the V-ATPase complex. Luteolin is a flavonoid compound found in vegetables, fruits, and medicinal herbs. We have examined the inhibitory effects of luteolin on the V-ATPase complex in the sperm in *C.elegans*. Growth assays were used to examine the toxicity of the compound. In addition to the staining for acidification of the FB-MOs, morphology of the germ cells undergoing spermatogenesis as well as DAPI staining allowed for the comparison to *spe* mutants. This novel approach to screening inhibitors of the V-ATPase will facilitate the screening for new therapeutic compounds.

W4170B The calponin family member CHDP-1 promotes membrane expansion and interacts with Rac/CED-10 to regulate protrusion formation. *Y. l. Guan*. Chinese Academy of science, Beijing, CN.

Eukaryotic cells extend a variety of surface protrusions to direct cell motility, which is mediated by coordinative actions between plasma membrane and underlying actin cytoskeleton. Here, we found that with its amphiphilic motif, the single calponin homology (CH) domain-containing protein CHDP-1 anchors on plasma membrane and induces membrane deformation in *C. elegans*. Through its CH domain, CHDP-1 associates with the small GTPase Rac1/CED-10, which is a key regulator for actin cytoskeleton arrangement. CHDP-1 preferably binds to the GTP-bound active form of CED-10 protein and the membrane localized GTP-CED-10 is decreased when CHDP-1 is removed. Thus, by coupling membrane expansion to the Rac1-mediated actin dynamics, CHDP-1 promotes membrane protrusion *in vivo*.

W4171C *Caenorhabditis elegans* extracellular matrix proteins regulate polycystin localization/activity and cilia integrity. *Deanna Michele De Vore*, Maureen Barr. Rutgers University, Piscataway, NJ.

PKD2 encodes a transient receptor potential polycystin (TRPP) channel receptor protein found in primary cilia of mammalian cells and sensory cilia of *C. elegans* neurons. In humans, PKD2 mutations result in Autosomal Dominant Polycystic Kidney Disease (ADPKD). Given the ancient and evolutionarily conserved role for polycystin-2 in cilia, we are using *C. elegans* as a model to identify new genes required for ciliary receptor localization. We recently found that extracellular matrix components are important for *C. elegans* PKD2 localization and function. ECM formation, secretion, and integrity have been shown to be a primary factor in ADPKD (Mangos 2010). Transmembrane and ECM proteins such as cadherins and galectins act in cilium retraction and elongation and also play a role in cell-to-cell junction signaling via the ECM (Seeger 2012, Rondanino 2011).

mec-1, mec-5, and *mec-9* encode ECM components that play a role in mechanosensation and degenerin/epithelial sodium channel (DEG/ENaCs) localization in non-ciliated touch receptor neurons (Du 1996, Emtage 2004). *mec-1* and *mec-9* encode proteins with multiple EGF and Kunitz domains; *mec-5* encodes a collagen (Du1996, Emtage 2004). I found that the ECM encoding genes *mec-1, mec-5,* and *mec-9* also regulate polycystin localization in male-specific ciliated sensory neurons. I further show that these proteins regulate mating behaviors and cilia structure. I am currently exploring their roles in localization of other ciliary proteins and determining the mechanisms by which these ECM proteins. This study may provide insight on how extracellular matrix proteins contribute to ciliary localization of sensory receptors like PKD-2, and advance the understanding and expand the options for treatment of ciliopathies such as ADPKD.

W4172A Intermediate filaments EXC-2/IFC-2 and IFA-4 Maintain Tube Structure of the Excretory Canal of the nematode *C. elegans*. *H. Al Hashimi*[1], M. Buechner[2]. 1) The University of Kansas, Lawrence, KS; 2) The University of Kansas, Lawrence, KS.

Seamless single-celled tubules form the excretory canals of the nematode *Caenorhabditis elegans*. These canals are narrow and extend over the whole length of the worm, and are an attractive model to understand how biological tubes maintain diameter and shape. In *C. elegans*, loss-of-function mutations in any of a set of *exc* genes cause the excretory canal lumen to swell into large fluid-filled cysts. The *exc-2* mutant

worm shows one the most severe phenotypes among the *exc* genes. In order to identify the *exc-2* gene, whole-genome sequencing was performed on *exc-2* mutant allele *rh90*. The results suggested a frameshift mutation in the intermediate filament gene *ifa-4*. Knockdown of *ifa-4* gene mimics the Exc-2 cystic-canal phenotype, and the strain RB1483 (Δ*ifa-4*(*ok1734*)) showed a similar cystic phenotype. Unexpectedly, injection of the wild-type *ifa-4* gene did not rescue the *exc-2* mutant; worms and mutants of the two genes genetically complemented each other, which surprisingly confirmed that *ifa-4* and *exc-2* are not the same gene. Next, whole-genome sequencing was performed on three additional *exc-2* alleles (*rh105*, *rh209*, and *rh247*), which provided greater resolution and confidence in identifying the gene. Results showed that the non-essential cytosolic intermediate filament gene *ifc-2* has mutations in all of the four alleles that were sequenced. The *ifc-2* gene has nonsense mutations in the alleles *rh209* and *rh247*, while alleles *rh90* and *rh105* have deletions among coding regions of the gene. *ifc-2* is a relatively large gene (~13kb), with four isoforms: C and D take up the first and second halves of the gene, respectively, while isoforms A and B each cover almost all of the exons. Knocking down any combination of isoforms containing both A and B showed a phenotype similar to Exc-2 cysts, while knockdown of isoform A by itself showed a much milder phenotype. We have rescued the *exc-2* gene by injecting PCR product contains full length of the *ifc-2* gene into *exc-2* mutant worms.

EXC-2/IFC-2 and IFA-4 join the previously discovered role (Labouesse lab) of IFB-1 in regulating the diameter of a model narrow seamless tube. Intermediate filaments could play important roles in other narrow seamless tubes as well. We are now investigating the time and position of expression of *ifa-4* and *ifc-2* isoforms to determine the interactions that form the distinctive shape of the excretory canals.

W4173B Genetic Analysis in NimA-Related Kinase Pathways in *C. elegans*. *D. S. Fay*, Braveen Joseph, Vladimir Lazetic. Univ Wyoming, Laramie, WY.

The family of NimA-related kinases (NEKs) are highly conserved in eukaryotes where they have been reported to regulate a number of diverse processes including mitosis, cytokinesis, DNA repair, ciliogenesis, and inflammation. Correspondingly, misregulation of NEKs have been linked to a variety of diseases including cancer and renal and cardiac disorders. Nevertheless, the targets of NEKs, as well as the components of NEK signaling pathways, remain largely unknown. In contrast to humans, which encode 11 NEK family members, the *C. elegans* genome encodes 4 family members (NEKL-1/NEK9, NEKL-2/NEK8, NEKL-3/NEK6/7, and NEKL-4/NEK10). We recently reported a novel function for NEKL-2 and NEKL-3 in regulating the completion of *C. elegans* molting during larval development. Our published and recent studies indicate that incomplete molting stems, at least in part, from defects in endocytosis in the apical epidermis of *C. elegans* larvae, thus implicating NEKs in process associated with intracellular trafficking. To gain further insight into the molecular, cellular, and developmental functions of NEK kinases, we have undertaken a number of approaches including genetic analysis. Using CRIPSR/Cas9 methods, we have used an iterative approach to generate a range of loss-of-function (LOF) alleles of *nekl* kinases We find that weak hypomorphic alleles in *nekl-2* and *nekl-3*, which display no defects as single mutants, are synthetically lethal, leading to near uniform larval arrest in *nekl-2; nekl-3* double mutants. We reasoned that *nekl-2; nekl-3* may provide an optimal background for identifying suppressors of NEKL-associated molting defects given that the individual *nekl* alleles are weak LOFs and that suppression of lethality could be incurred by mutations that affect either the NEKL-2 or NEKL-3 pathways or both. Using a semi-clonal F1 screen, we were successful in identifying ~12 suppressor mutations of varying strengths and we will describe progress on their molecular identification. We will also describe a non-clonal screen, that makes use of a counter-selectable marker in *C. elegans*, to identify additional *nekl-2; nekl-3* suppressors. This method could be adapted to any suppressor screen where the lethal phenotype of the parental starting strain is incompletely penetrant. Finally, we will discuss focused RNAi approaches to directly identify protein kinases and protein phosphatases that modulate signaling by NEKL kinases.

W4174C O-GlcNAc cycling and mitochondrial oxygen consumption. *M. P. Mahaffey*, J. L. Sacoman, P. M. Berninsone. University of Nevada, Reno, NV.

O-linked-β-N-acetylglucosamine (O-GlcNAc) modified proteins are critical in myriad cellular processes and functions. Abnormal O-GlcNAcylation is associated with a spectrum of diseases, including Alzheimer's disease, cancer, cardiovascular disease, and diabetes. Mitochondrial protein O-GlcNAcylation is emerging as a key regulator of cellular energetic metabolism, redox signaling and cell survival pathways, but the mechanisms involved are largely unknown. O-GlcNAc transferase (OGT) is the enzyme responsible for the addition of O-GlcNAc to target proteins while O-GlcNAcase (OGA) catalyzes the removal of the modification from target proteins. OGT and OGA are encoded by single genes in *C. elegans* (*ogt-1* and *oga-1*, respectively). Null alleles of *ogt-1* and *oga-1* are viable and fertile, unlike knockouts of OGT in mouse that result in embryonic lethality. We hypothesize that O-GlcNAc cycling mediated by OGT and O-GlcNAcase play a role in regulating mitochondrial metabolism and morphology. To that end, we measured oxygen consumption rate (OCR), a proxy for mitochondrial oxidative phosphorylation function, in N2, *ogt-1* and *oga-1* null mutants. OCR was measured using the Seahorse Bioscience XF^{e} 24 Extracellular Flux Analyzer and normalized by number of worms for each individual well. Our results suggest that altered O-GlcNAc cycling reduces oxygen consumption by negatively impacting mitochondrial oxidative metabolism. It is still unknown if these reductions in OCR are caused by reduction in mitochondria content or reduced mitochondrial function. These results will add to the knowledge of how O-GlcNAcylation modulates mitochondrial health and function.

W4175A Male Chemosensory Pathways that Modulate Sperm Navigation Performance. *H. D. Hoang*, M. A. Miller. University of Alabama at Birmingham, Birmingham, AL.

The molecular mechanisms by which environmental cues influence reproductive success are not well understood. In this study, we identify a genomic cluster of serpentine receptor B (SRB) chemosensory receptors that modulate sperm motility performance within the hermaphrodite reproductive tract. SRB chemoreceptors signal via GOA-1 and EGL-30 G protein pathways in male ciliated amphid sensory neurons, including ASI and ASK. SRB signaling is both necessary and sufficient in amphids to modulate sperm motility performance. The neuropeptide ligands FLP-

21 and FLP-18, and their receptor NPR-1, act together with SRB chemoreceptors to transduce signals to the testis. We show that SRB signaling promotes metabolic and cytoskeletal gene expression during spermatogenesis. Changes in sperm mitochondrial respiratory chain complex subunit mRNA levels affect sperm velocity and reversal frequency, impairing their ability to respond to guidance cues called prostaglandins. These results show that chemosensation has a profound impact on sperm function, with each sex manipulating sperm performance to optimize their individual reproductive success. Current efforts aim to better understand the sensory cues that control SRB pathways and evolution of the SRB circuits.

W4176B Three conserved tetraspanin proteins positively modulate BMP signaling in *C. elegans*. *Zhiyu Liu*, Herong Shi, Jun Liu. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853.

The bone morphogenetic protein (BMP) signaling pathway plays essential roles in multiple developmental and homeostatic processes in metazoans. While the core BMP pathway members are well studied, much is needed for understanding the mechanisms involved in the spatiotemporal regulation of BMP signaling in vivo. We recently identified three highly conserved tetraspanin proteins, TSP-12, TSP-14 and TSP-21, as positive modulators of the BMP signaling pathway in *C. elegans* (Liu et al., 2015). *tsp-21(0)* single mutants or *tsp-12(0); tsp-14(0)* double mutants exhibit defects caused by reduced BMP signaling, including a small body size, reduced expression of a BMP signaling reporter, the RAD-SMAD reporter, and suppression of the mesodermal patterning defects of mutants in the *C. elegans* Schnurri homolog SMA-9. While all three tetraspanins are expressed and function in the signal-receiving cells to promote BMP signaling, TSP-21 appears to function in parallel with TSP-12 and TSP-14, because *tsp-12(0); tsp-14(0) tsp-21(0)* triple mutants are smaller than either *tsp-21(0)* single mutant or *tsp-12(0); tsp-14(0)* double mutant. We have found that TSP-21 is plasma-membrane localized and that this localization is not affected in *tsp-12(0); tsp-14(0)* double mutants. We are currently further dissecting the functional relationship among these three tetraspanin proteins. TSP-21 is orthologous to human TSPAN4, TSPAN9 and CD53. TSP-12 and TSP-14 are paralogous to each other and homologous to human TSPAN5, TSPAN10, TSPAN14, TSPAN15, TSPAN17 and TSPAN33. We speculate that these human tetraspanin proteins may also function in modulating BMP signaling.

Liu, Z et al. (2015) Promotion of Bone Morphogenetic Protein Signaling by Tetraspanins and Glycosphingolipids. *PLoS Genetics* 11(5):e1005221.

W4177C AMPK-related kinase UNC-82 has genetic and probable physical interactions with paramyosin. *NT. Schiller*, C. Duchesneau, L. Lane, E. Manzon, P. Hoppe. Western Michigan University, Kalamazoo, MI.

C. elegans striated muscle requires the serine/threonine kinase UNC-82 for proper organization of myosin filaments. Little is known about the targets of UNC-82 kinase, and the mechanism that regulates its activity has yet to be determined. Worms homozygous for *unc-82(0)* exhibit bright amorphous birefringent patches that contain the thick filament proteins myosin and paramyosin (Waterston et al., 1980; Hoppe et al., 2010). Our immunohistochemical analysis of *unc-82* mutants that contain missense mutations in the kinase domain revealed distinctive ectopic birefringent paramyosin accumulations at the ends of muscle cells that are not found in the null mutant. Transgenes in which the missense *unc-82(e1220)* mutation is tagged with GFP/RFP show normal localization to the M-line in a wild-type background, but in the *unc-82* null colocalize with birefringent accumulations at the ends of muscle cells. Driving expression of a heat-shock-inducible paramyosin::GFP in young adults showed that newly made paramyosin forms distinctive accumulations at the ends of cells in the missense mutant, consistent with the hypothesis that newly made paramyosin physically associates with UNC-82 protein. Dose-dependent synthetic lethality was observed between UNC-82 and mutations in paramyosin. Expression of UNC-82::GFP in the paramyosin mutant *unc-15(e73)* revealed that UNC-82 was recruited to the ectopic paramyosin-containing aggregates of this mutant, altering aggregate morphology and reducing motility and viability. UNC-82::GFP is recruited to paramyosin-containing aggregates formed in mutants that affect the M-line proteins UNC-89/obscurin, UNC-98/Zn-finger and UNC-96. We further explored physical interactions between UNC-82 and other M-line or thick-filament proteins by analyzing single and double mutant strains in which myosin, paramyosin or UNC-82 is abnormally localized within muscle cells, and determining which proteins are recruited to the aberrant structures. The phenotype of *unc-82; unc-98* double mutants closely resembles that of unc-*82* single mutants, suggesting that the two proteins act in a single pathway to organize paramyosin. The *unc-89(su75);unc-82(e1220)* double mutant is viable, exhibiting an enhanced phenotype compared to either mutant alone while the *unc-89(su75);unc-82(0)* double is not viable, indicating the two proteins are acting in parallel pathways. Our results suggest that UNC-82 physically interacts, either directly or indirectly, with paramyosin, but not myosin A, UNC-89 or the UNC-98/Zn finger protein. Our data are consistent with the model that UNC-82 kinase is epistatic to UNC-98 and is required for regulation, localization and incorporation of paramyosin into the thick filaments of the contractile apparatus.

W4178A A Tale of Two SNPs: Genetic Analysis of the Dopamine Transporter Structure and Function in DAT-1 Coding Variants Derived from the *C. elegans* Million Mutation Project. *Phyllis Freeman*[1], Emmanuel J. Jackson[1], Peace O. Odiase[1], Bailey Monroe[1], Winston W. Jackson[1], Ciara D. Elie[1], Angeline Eugene[1], Chelsea L. Snarrenburg[2], Jane Wright[2,3], Sarah M. Sturgeon[2], Sarah B. Robinson[2], Randy D. Blakely[2,3]. 1) Fisk University, Nashville, TN; 2) Vanderbilt University, Nashville, TN; 3) Vanderbilt University Medical Center, Nashville, TN.

The dopamine (DA) transporter (DAT) is a polytrophic, membrane protein that utilizes the co-transport of Na^+ and Cl^- ions to energize the rapid re-uptake of DA from the synapse in order to terminate extracellular DA signalling and facilitate re-release. As worm synaptic structure in general, and the expression of DAT in particular, are conserved across phyla, studies with the orthologous *Caenorhabditis elegans* protein model provide an ideal opportunity to probe fundamental questions of transporter structure and function in an *in vivo* setting using powerful forward and reverse genetic screens. The Million Mutation Project (MMP, http://genome.sfu.ca/mmp/) represents a library of ~2,000 mutagenized worm strains where sequencing at a depth of 15X genome coverage reveals the presence of, on average, ~9 new non-synonymous alleles per gene,whose characterization can reveal novel links to protein structure and function. Thirteen such coding variants are

present in the MMP. We have initiated the functional characterization of these variants, focusing initially on five lines that bear amino acid changes (D8N, P9L, M20T, P596S, P609S) at highly conserved locations and/or that are associated with regions suspected to confer/support DAT activity or regulation based on mammalian DAT studies. To date, we have confirmed the behavior of four of these lines consistent with DAT-1 loss of function, assessed by the presence of Swimming-induced paralysis (*S*wip). Results from the successful genetic cross of mutant worms with *cat*-2 tyrosine hydroxylase (TH, cat-2) loss of function mutant lines and the introduction of MMP mutations into a GFP-DAT-1 expression construct via site-directed mutagenesis of the wildtype *dat*-1 will be used to determine the impact of *dat*-1 missense alleles on *in vivo* DAT function and distribution in DA neurons. Supported by NIH award MH095055 (RDB) and NSF award 1505176 (PF).

W4179B The *Caenorhabditis elegans* excreted-secreted protein fraction is enriched in innate immunity related proteins. *P. M. Berninsone*, V. Arinze, J. Sacoman, F. Feyertag, D. Alvarez-Ponce. Univ Nevada, Reno, NV.

We have developed a protocol to isolate an excreted-secreted protein (ESP) fraction by collecting and concentrating the spent media from synchronized large-scale *C. elegans* cultures. Using high-throughput liquid chromatography/ mass spectrometry analysis, we discovered that the *C. elegans* ESP fraction contains C-type lectins domain-containing proteins, proteases, antimicrobial proteins and lysozymes. Many ESP protein components have been previously identified as transcriptionally upregulated upon pathogen exposure, which agrees with the innate immune response role assigned to some of these proteins. In addition, many uncharacterized proteins were found in this preparation, including nematode-specific proteins. Ongoing efforts include the analysis of the evolutionary rate of these excreted-secreted proteins. This study will contribute to our understanding of how pathogen recognition and defense mechanisms evolve and provide insight on proteins that could be key players in host-pathogen interactions.

W4180C Investigating the function of intestinal cell-cell communication in peptide secretion. *Lisa Learman*[1], Amy Vashlishan Murray[2], Maureen Peters[1]. 1) Dept. of Biology, Oberlin College, Oberlin OH; 2) Institute for Liberal Arts and Interdisciplinary Studies, Emerson College, Boston MA.

The *C. elegans* intestine acts as a neurosecretory organ, releasing small molecules that affect multiple aspects of physiology, including dauer formation, locomotion and defecation. Dauer formation is cued by intestinal release of IGF-1 in low food situations. When animals are feeding, the intestine undergoes periodic calcium waves that control the motor steps of the defecation cycle; these waves invoke secretion of signaling molecules that regulate defecation. This calcium spike appears to trigger the release of at least one peptide, NLP-40. Recent findings from the Biron lab suggest that an intestinally-derived signal induces a brief locomotory reversal in synch with the calcium wave. We believe that the intestinal calcium wave induces the release of factors affecting social feeding behavior, the tendency for worms to feed in large clumps rather than individually.

Altering intestinal cell-cell communication via mutation of a gap junctional subunit, INX-16, leads to an increase in social feeding. Worms with mutated *inx-16*, have slow, backward, or failed intestinal calcium waves known to affect defecation steps and the defecation associated locomotory reversals. *inx-16* mutants show a modest increase in social feeding (about 10% higher than N2). To determine whether the social feeding phenotype correlated broadly with defecation defects, we analyzed more defecation mutants: *pbo-4*, *pbo-5* and *aex-2*. None of these exhibited a significant social feeding phenotype suggesting that the social feeding in *inx-16* does not result from abnormal defecation in general.

To determine how *inx-16* mutation interacted with the known social feeding pathways we combined *inx-16* mutation with *npr-1* or *daf-7*. Addition of an *inx-16* mutation to a *daf-7* mutant significantly increased social feeding levels, which suggests that these two pathways are at least partly independent. The *npr-1*;*inx-16* double mutant produced a social feeding score similar to that of *npr-1* alone. This suggests that NPR-1 acts in the same pathway as INX-16 to mediate social feeding. NPR-1 can be activated by the neuropeptide FLP-21, which is reportedly expressed in the intestine. We hypothesize that, in *inx-16* mutants, abnormal intestinal calcium waves lead to defective FLP-21 secretion from the intestine and insufficient activation of NPR-1. Deletion of *flp-21* results in enhanced social feeding similar to that of *inx-16* mutation. Double mutants of *inx-16* and *flp-21* do not result in an additive increase in social feeding compared to single mutants. The intestinal calcium wave, itself dependent on feeding, appears to regulate the release FLP-21, thus modulating feeding behavior.

W4181A Understanding the secretion mechanism of VAPB/ALS8 MSP. *H. Zein-Sabatto*, M. Miller. University of Alabama at Birmingham, Birmingham, AL.

Most eukaryotic, secreted proteins travel through the conventional Golgi-ER secretory pathway. However, some proteins are unconventionally secreted independent of this route. We have shown that the major sperm protein (MSP) domain of VAPB/ALS8 is cleaved from the rest of the protein and secreted. MSP can be detected in human blood and cerebrospinal fluid (CSF), and its levels correlate with amyotrophic lateral sclerosis (ALS) pathogenesis. The goal of this project is to understand how VAPB MSP is proteolytically processed, secreted, and regulated. VAPB is a ubiquitously expressed type II membrane protein found anchored into the endoplasmic reticulum with the N-terminal MSP domain extending into the cytosol. The secreted MSP domain does not harbor a signal peptide characteristic of conventionally secreted proteins. *C. elegans* null mutants for *vpr-1*, the *Vapb* homolog, are sterile and have striated muscle mitochondrial abnormalities. Results from our lab provide evidence that the nervous system, germ line, and intestine are cellular sites of VPR-1 MSP secretion. Based on studies of *C. elegans* sperm MSPs, we hypothesize that VAPB MSP is secreted in a regulated fashion via an unconventional mechanism. In order to test our hypothesis, we developed an RNAi screening method using transgenic *C. elegans* to identify genes required for MSP secretion. *vpr-1(tm1411)* null mutants expressing VPR-1 only in the intestine (*pGES-1::vpr-1*) are fertile and can be grown as transgenic homozygotes, presumably because the MSP is secreted into the pseudocoelom. We predicted that RNAi of genes essential for VPR-1 MSP secretion would cause sterility. Using expression data, I have selected a set of 420 genes for a pilot RNAi screen. I have currently screened 210 RNAi clones and identified

10 candidate clones that cause severely reduced fertility in transgenic *vpr-1* mutants, but not in wild-type worms. Genome-editing techniques will be used to further characterize the role of selected candidates. I am also conducting a structure/function analysis to identify regulatory regions within VPR-1 necessary for MSP secretion. Finally, we have been characterizing multiple mouse *Vapb* mutants with skeletal muscle mitochondrial defects, in collaboration with Jin Chen's lab at Vanderbilt. I plan to use western blots of isolated mouse tissues to help define the mammalian cell types that proteolytically process VAPB to liberate MSP. VAPB MSP injections or drugs that improve VAPB MSP secretion could be therapeutic for ALS patients.

W4182B Identification of Conserved MEL-28/ELYS Domains with Essential Roles in Nuclear Assembly and Chromosome Segregation. *P. Askjaer*[1], G. Gómez-Saldivar[1], A. Fernandez[2,3], D. Ritler[4], Y. Hirano[5], A. Lai[2], M. Mauro[2], C. Ayuso[1], T. Haraguchi[5], Y. Hiraoka[5], F. Piano[3], P. Meister[4]. 1) CSIC-Univ. Pablo de Olavide, Seville, ES; 2) Fairfield Univ., Fairfield, CT, US; 3) NYU, New York, NY, US; 4) Univ. of Bern, Bern, CH; 5) Osaka Univ., Suita, JP.

Nucleoporins are the constituents of nuclear pore complexes (NPCs) and are essential regulators of nucleocytoplasmic transport, gene expression and genome stability. The nucleoporin MEL-28/ELYS plays a critical role in post-mitotic NPC reassembly through recruitment of the NUP107-160 subcomplex and is required for correct segregation of mitotic chromosomes. MEL-28 has a dynamic behavior: it localizes to nuclear pore complexes and chromatin during interphase and shuttles to kinetochores during cell division. However, it is unknown how MEL-28 localization and activity is regulated.

Here we present a systematic functional and structural analysis of MEL-28 in *C. elegans* early development and human ELYS in cultured cells. We have identified functional domains responsible for NPC and kinetochore localization, chromatin binding, mitotic spindle matrix association and chromosome segregation. Surprisingly, we found that perturbations to MEL-28's conserved AT-hook domain do not affect MEL-28 localization although they disrupt MEL-28 function and delay cell cycle progression in a DNA damage checkpoint-dependent manner. Our analyses also uncover a novel meiotic role of MEL-28. Together, these results show that MEL-28 has conserved structural domains that are essential for its fundamental roles in NPC assembly and chromosome segregation during meiosis and mitosis.

To understand the function of MEL-28 chromatin binding we have used DamID to identify the chromatin regions with which MEL-28 associates. Interestingly, MEL-28 is enriched at transcribed genes and correlates positively with active histone marks, suggesting that it may be involved in regulation of gene expression. We compared the MEL-28 chromatin profile with the profile of another nucleoporin, NPP-22, which is permanently anchored to the nuclear pore complex. Surprisingly, we found that the chromatin association profile of NPP-22 was more similar to the profile of the nuclear lamina protein LMN-1 than to MEL-28's profile, suggesting that individual NPPs interact with specific chromatin domains. Finally, GO-term analysis reveals that MEL-28-associated genes are related to larval and reproductive development. This suggests that MEL-28 has postembryonic functions that have not yet been studied.

W4183C LIN-10 promotes LET-23 EGFR signalling independently of LIN-2 and LIN-7. *K. Gauthier*[1,2], O. Skorobogata[1,2], C. Rocheleau[1,2]. 1) McGill Univ., Montreal, QC, CA; 2) The Research Institute of the MUHC, Montreal, QC, CA.

The spatial organization of signal transduction cascades is critical for regulating cell signalling and function. This is particularly evident in the polarized epithelia of *Caenorhabditis elegans* vulva precursor cells (VPCs) that give rise to the hermaphrodite vulva. Epidermal Growth Factor Receptor (LET-23 EGFR) signalling from the basolateral membrane is required for vulva cell fate induction. Basolateral localization of LET-23 EGFR is dependent on a ternary complex composed of LIN-2 CASK, LIN-7 Veli, and LIN-10 Mint1. Disruption of this complex results in exclusive apical mislocalization of LET-23 and a vulvaless (Vul) phenotype. This complex has been well-defined biochemically, however the cellular mechanisms by which it regulates LET-23 localization are not understood. We previously identified a pathway consisting of ARF GTPases, AGEF-1 (a putative Arf guanine exchange factor), and the AP-1 clathrin adaptor complex that antagonizes basolateral localization of LET-23 and negatively regulates signalling. Interestingly, mammalian LIN-10 homologue Mint1 binds Arf GTPases and may serve as an adaptor protein analogous to the AP-1 complex. My primary research objective is to discover how the LIN-2/7/10 complex functions with the AGEF-1/ARF/AP-1 ensemble to regulate LET-23 trafficking and signalling in the *C. elegans* VPCs. I found that GFP::LIN-10 localizes to cytoplasmic foci in the VPCs, which may represent Golgi mini-stacks or recycling endosomes. This localization is independent of LIN-2, LIN-7, and ARF GTPases, and is mediated by the C-terminal PTB and PDZ domains of LIN-10 that also mediate Arf binding in mammalian Mint1. Unexpectedly, I found that overexpression of LIN-10 rescues the *lin-2(e1309)* and *lin-7(e1413)* Vul phenotypes, indicating that LIN-10 promotes signalling independently of its complex. This effect is mediated by the C-terminal domains of LIN-10, and is LET-23 dependent. Going forward, I will test the hypothesis that LIN-10 promotes the EGFR signalling pathway by interacting with ARF. I will find if LIN-10 and ARF colocalize and interact in *C. elegans*, and I will test if this interaction is necessary and sufficient for the rescue of the *lin-2(e1309)* and *lin-7(e1413)* Vul phenotypes. Also, I will test if overexpression of LIN-10 can affect the subcellular localization of LET-23::GFP in the VPCs. This will be crucial towards understanding how LIN-10 overexpression is able to rescue the *lin-2(e1309)* and *lin-7(e1413)* Vul phenotypes. The results will offer new insight into how trafficking and scaffolding components work together to carefully regulate cellular events by controlling the spatial organization of signalling proteins in polarized cells.

W4184A Miro and dynein localize mitochondria in the intestine. *T. Inoue*, H. L. Chua, H. Y. Cheng, Y. Shen. National University of Singapore, Singapore, SG.

We are studying the role of Miro, an atypical small GTPase, in localization of intestinal mitochondria. In the wild-type, intestinal mitochondria are enriched near the apical surface of intestinal cells. We found that a mutation in *miro-1* disrupts this localization such that the distribution is more even across the intestinal cell. Using RNAi, we also found that components of the dynein motor protein are involved in this localization. We are carrying out additional experiments to identify other regulators of intestinal mitochondrial localization.

W4185B The liprin protein SYD-2 regulates synaptic vesicle localization in *C. elegans*. *X. Li*, JZ Qin, M. Ding. Chinese Academy of Sciences, Beijing, CN.

Chemical synapses are specialized structures to mediate communication between neurons and their target cells through neurotransmitter release mechanism. The transport, docking and release of neurotransmitters heavily rely on synaptic vesicles. Therefore the control of synaptic vesicle transport and localization is of great importance in the function, development and maintenance of nervous system. Here, we report that the *C. elegans* liprin protein SYD-2 is required for synaptic vesicle transport and localization. Mutations on *syd-2* lead to ectopic synaptic vesicle distribution, especially to the distal axon. *syd-2* regulated synaptic vesicle localization is dependent on kinesin/UNC-104. Previous study showed SYD-2 can physically interact with UNC-104, and UNC-104::GFP retrograde transport is increased in *syd-2* mutants. However, systematic genetic analysis implies that SYD-2 may recruit synaptic vesicles,which is independent from UNC-104-interaction. Overall, our study reveals an important role of active zone protein SYD-2 in synaptic vesicle localization.

W4186C *In vivo* function of the Kinesin-3 motor, KLP-4. *J. Pieczynski*, M. Magaletta. Rollins College, Winter Park, FL.

Proper neuronal function requires a dynamic microtubule network and the directed movement of ATP dependent, cargo binding motor proteins: the mostly plus-end directed kinesins and minus end directed dyneins. The efficient coordination of both microtubules and motors is required for proper structure-function paradigms of neuronal signaling, the establishment of proper morphology during development and then the movement of vesicular cargo to facilitate proper synapse formation and subsequent signal transduction. Failure of neurons to perform any of these functions can lead to multitudes of disease pathologies, including those characterized as cognitive, degenerative, psychiatric, and developmental. The Kinesin-3 superfamily, consisting of *C. elegans* UNC-104, KLP-6, and KLP-4, are all neuronally expressed motor proteins that are evolutionarily conserved between worms and other higher eukaryotes. *In vitro* studies using mammalian homologues of these motors indicate that Kinesin-3 motors exhibit high affinity for microtubules and that these motors possess relatively long run lengths, thus are highly processive. This high degree of processivitiy has been hypothesized as an evolutionary adaptation for the movement of cargo over long distances, such as neuronal axons. Questions still remain as to the exact roles of these motor proteins *in vivo;* how they contribute to intracellular trafficking, protein stability, neuronal morphology, and signal transduction. To address these questions, we have begun a reverse genetic screen focusing on *klp-4*, the *C. elegans* homologue to human KIF13A and KIF13B. Using a combination of commercially available strains produced via large-scale mutagenesis and bioinformatics, we have identified a number potentially deleterious *klp-4* alleles predicted to severely effect motor function. We are specifically interested in how mutation in KLP-4 may effect the paired AVB interneurons, which contain axons that extend the length the ventral nerve cord and are involved in the animal's forward locomotive circuit and behaviors such as spontaneous reversals, social behavior, and the establishment of long term memory. We have determined that mutation in *klp-4* does not appear to effect the development and gross morphology of the AVB interneuron, however different *klp-4* mutant animals appear to have reduced response to nose touch suggesting role for KLP-4 in the establishment of proper distribution of subcellular components in neurons *in vivo*.

W4187A A Search for Novel Presynaptic Determinants of Dopamine Signaling in *C. elegans*. *O. M. Refai*[1], J. A. Hardaway[2], S. Robinson[3], C. L. Snarrenberg[2], S. L. Hardie[3], P. Freeman[3], R. D. Blakely[1]. 1) Florida Atlantic University, Jupiter, FL; 2) Graduate Neuroscience Program, Vanderbilt University, Nashville, TN; 3) Department of Pharmacology, Vanderbilt University, Nashville, TN.

The catecholamine dopamine (DA) is a catecholamine neurotransmitter found in both vertebrates and invertebrates, where it modulates a wide variety of behaviors including movement, attention and reward. In humans, disruption of DA levels and signaling are associated with multiple brain disorders, including Parkinson's disease, Schizophrenia, Attention Hyperactivity Disorder (ADHD) and addiction. In the nematode *C. elegans*, DA supports motor and sensory function, with excess DA transmission triggering Swimming-Induced Paralysis (Swip), a readily visible phenotype that we have shown to be well-suited to forward genetic efforts to identify DA regulatory genes. Here we describe our efforts to characterize two mutant lines, *vt39* and *vt44*, isolated in an EMS-based screen for animals exhibiting Swip. Similar to DA transporter (*dat-1)* mutants, the Swip exhibited by *vt39* and *vt44* is rescued by either treatment of animals with the vesicular monoamine transporter (*cat-1*) inhibitor reserpine or by crossing these lines to animals that lack expression of the D2-type DA receptor DOP-3. Direct genomic sequencing, and complementation tests, indicate that both lines harbor a normal *dat-1* gene. Using a single nucleotide polymorphism-based mapping approach, we find that *vt39* and *vt44* map to chromosomes I and III, respectively. Fine mapping and whole genome sequencing are underway to identify the genes mutated in these lines, efforts to be followed by genomic and cDNA rescue efforts to validate gene identification and to determine cell autonomy with respect to Swip induction. Supported by NIH Award MH095044 (R.D.B.) and MH093102 (J.A.H.).

W4188B A *C. elegans* model for Human Antigen R. *Zhe Yang*, Matthew Buechner. University of Kansas, Lawrence, KS.

Human antigen (Hu) proteins HuB, HuC, HuD, and HuR are a family of RNA-binding protein that stabilize mRNA by binding to AU-rich elements (ARE) in the 3'UTR. HuR is universally expressed in human cells, and stabilizes many tumor-related genes such as *bcl-2*. HuR is upregulated in many cancer cell types involved in breast and colon cancer. EXC-7 is the sole homologue of the Hu proteins in *C. elegans*. EXC-7 maintains the single-cell tubular canals of the excretory cell by stabilizing *sma-1* mRNA, encoding β_H-spectrin. Mutants in *exc-7* exhibit small fluid-filled cysts throughout shortened excretory canals, as well as a misshapen tailspike. Both EXC-7 and HuR contain 3 RNA-recognition motifs (RRMs), with a region between the 2nd and 3rd RRM that appears to be needed for intracellular transport. We are investigating the functional equivalence of these proteins, by determining whether human HuR can functionally replace EXC-7 in *C. elegans*. If so, then *C. elegans* could be developed as a model for studying HuR *in vivo*, especially for screens for chemicals that interfere with HuR function, using excretory canal phenotype as a measure of HuR activity. I have injected the *hur* coding region driven by various promoters to express HuR within the excretory canals. HuR

cDNA appears to rescue partially both the length and diameter of the excretory canal lumen in *exc-7* mutants. Modification of the structure of the injected HuR gene showed better rescue effects. We will further modify the structure of HuR and seek solutions to fully rescue canal defects, and are making worm/human chimeric proteins to investigate domain activity of these proteins and study the binding partners, the mRNA targets of EXC-7.

W4189C A vesicle-intrinsically regulated pathway for apical polarity. *Nan Zhang*[1,2], Hongjie Zhang[1,3], Liakot Khan[1], Gholamali Jafari[1], Yong Eun[1], Edward Membreno[1], Razan El-Daouk[1], Verena Gobel[1]. 1) Mucosal Immunology and Biology Research Center, MGH/Harvard Medical School, Boston, MA, USA; 2) College of Life Sciences, Jilin University, Changchun, Jilin, China; 3) Faculty of Health Sciences/University of Macau, Taipa, Macau, China.

Polarized epithelial membrane domains are thought to be established by membrane- and junction-associated polarity cues (e.g. partitioning-defective PARs), with directional trafficking subsequently maintaining them. Our prior work showed that glycosphingolipids (GSLs), clathrin and its AP-1 adaptor function as vesicle-associated apical polarity cues in *C. elegans* tubular epithelia. Their loss causes intestinal polarity inversion with a full basolateral-to-apical membrane domain conversion and ectopic lateral lumen formation. These and other findings suggested that trafficking itself may define polarized domain identities, in part by targeting polarity determinants (e.g. PARs), but possibly by targeting multiple membrane components.

Here, the GSL-loss-of-function intestinal polarity inversion phenotype was used to identify components and map trajectories of such a putative vesicle-intrinsically regulated apical polarity pathway. We mined three of our unbiased and targeted multi- and unicellular tubulogenesis screens that had examined the asymmetrical placement and function of the apical/lumenal actin linker ERM-1 for genes with predicted roles in trafficking. 49 trafficking genes with a broad range of vesicle-associated functions were identified as required for apical membrane biogenesis, only few of which previously implicated in directional trafficking. We examined the ability of these genes to modify polarity inversion in the GSL-biosynthetic 3-ketoacyl-CoA reductase deficient mutant *let-767*.

These genetic interaction screens identified eleven enhancers implicating secretory/biosynthetic trafficking routes in this pathway, and five suppressors implicating endocytic/recycling routes. In an attempt to map polarized trajectories, three suppressors, DAB-1/Disabled, RAB-7, and VHA-6 (a V-ATPase component), were selected for further analysis. Imaging and loss-of-function studies of expanding intestines revealed unexpected functions in apical and basolateral membrane biogenesis for these endo- and plasma-membrane-associated molecules and placed them on recycling routes. They thereby position suppressors upstream of GSL sorting sites, while in turn suggesting that GSLs may sort beyond the Golgi on biosynthetic trajectories that receive input from recycling routes during plasma membrane biogenesis.

This study identifies multiple vesicle components for polarized trafficking, including novel vesicle-carrier-associated polarity cues, and proposes trajectories for a vesicle-intrinsically regulated trafficking pathway for apical polarity.

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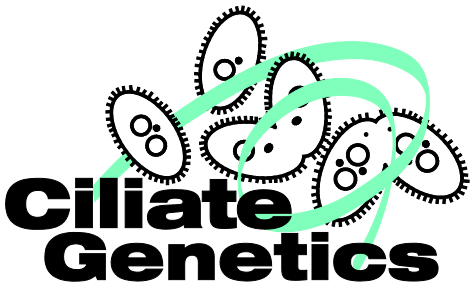

Poster Session Abstracts

CILIATES POSTER SESSION ABSTRACTS

C7001A Evolution of gene families in ciliates. *O. A. Pilling*[1], X. X. Maurer-Alcalá[1,2], L. A. Katz[1,2]. 1) Smith College, Northampton, MA; 2) University of Massachusetts Amherst, Amherst, MA.

We use ciliates as a model to gain insight into how genome structure affects patterns of DNA sequence evolution. Previous research has demonstrated that ciliates, a clade of eukaryotes marked by dimorphic nuclei and cilia in at least one stage of their life cycle, have rapid rates of protein evolution associated with highly processed somatic genomes (Katz et al. 2003; Zufall et al. 2006). We are building on this work by investigating the protein evolution of three species of ciliates in the class Heterotrichea, which has previously been understudied. Our focus is on characterizing patterns of divergenes among paralogs (i.e. genes that differ due to an ancient duplication events). For this project, we focus on the ciliates *Blepharisma americanum*, *Stentor sp.* and *Spirostomum ambiguum*, and six genes (Actin, α-tubulin, β-tubulin, Ef1-α, H3 and H4).

We chose to study *B. americanum* because preliminary data suggest they may have elevated rates of protein evolution (Zufall et al. 2006). Unlike the classes Spirotrichea, Armophorea and Phyllopharyngea where rates of protein evolution are associated with extensively processed somatic genomes (e.g. 'gene-sized' chromosomes), members of the Heterotrichea are not known to extensively modify their macronuclear genomes. We are using PCR based approach to analyze protein evolution and are interpreting the resulting data in a molecular evolution framework.

This project has included both wet work and bioinformatics. While the majority of the wet work is completed, we are now beginning to analyze the data by calculating the dN/dS ratio and performing pairwise comparisons. Through this approach, we can start to better understand the impact genome architecture has on molecular evolution.

Literature Cited:

Katz LA, Bornstein JG, Lasek-Nesselquist E, Muse SV. 2004. Dramatic diversity of ciliate Histone H4 genes revealed by comparisons of patterns of substitutions and paralog divergences among eukaryotes. Mol Biol Evol. 21(3): 555-562.

Zufall RA, McGrath CL, Muse SV, Katz LA. 2006. Genome architecture drives protein evolution in ciliates. Mol Biol Evol. 23(9): 1681-1687.

C7002B Mapping and characterization of DNA replication origins in *Tetrahymena thermophila*. *L. Zhang*, M. Cervantes, G. Kapler. Texas A&M Health Science Center, College Station, TX.

Origins of replication act as sites for the initiation of DNA replication via recruitment of ORC (Origin Recognition Complex). ORC mediates loading of the MCM2-7 helicase to 'license' replication competence. Although ORC is conserved in eukaryotes, the mechanism for origin recognition is not. *S. cerevisiae* ORC binds to a conserved sequence motif present at all origins. *S. pombe* and metazoan ORCs binds non-specifically to AT-rich DNA. Genome-wide studies have uncovered several general features of origins.

We are developing a high throughput DNA sequencing approach to obtain a comprehensive map of replication initiation sites in *Tetrahymena thermophila*. This approach will provide the first comprehensive picture of origin usage in the early diverging Ciliophora lineage and reveal genomic features of replication origins. As part of this project, we have developed a functional assay to validate replication origins. Our data indicate that specific intergenic DNA sequences are required to support DNA replication of episomal plasmids as autonomously replicating sequences (ARSs). ARS assays are being used to map origins and uncover DNA sequences requirements. To identify new origins, nine non-coding segments from a small non-rDNA macronuclear chromosome were introduced into an origin-less vector that confers resistance to paromomycin. These plasmids were introduced into vegetative Tetrahymena by biolistic transformation as a pool. A plasmid containing the well-characterized rDNA origin served as positive control, and an empty vector served as negative control. The non-rDNA pool generated >100 folds more transformants compared to negative controls. After cultured for >100 generations, episomal DNA was recovered by Qiagen columns and re-transformed into *E. coli*. Massive DNA rearrangements were detected in *E. coli* clones. This was not unexpected, as AT-rich Tetrahymena sequence frequently rearrange in *E.coli*. Thus far we have recovered sequences from one 0.8 kb Tetrahymena insert, suggesting that this fragment supports DNA replication in Tetrahymena. We are currently use direct sequencing to identify Tetrahymena origin sequences. As further confirmation, endogenous chromosomal loci are being examined by 2D gel electrophoresis.

Our lab recently discovered an unconventional DNA replication program in Tetrahymena that operates when ORC proteins are dramatically depleted. Known origins are silenced and cryptic origins are activated. This suggests the presence of cryptic origins whose chromosomal replication initiation signal is too weak to be detected in population-based assays. We plan to exploit ARS assays to identify replication determinants under high and low ORC conditions.

C7003C Investigations into the Paramecium iesRNA pathway. *S. Allen*, E. Swart, M. Nowacki. University of Bern, Bern, CH.

The iesRNA pathway is a small RNA pathway involved in the excision of internal eliminated sequences (IESs) during Paramecium development. iesRNAs appear late in development, after IES excision (both scnRNA dependent and independent) has been initiated. Evidence suggests that iesRNAs are formed directly from excised IESs, but the mechanism for this putative transcription is not clear. Many iesRNA-affected IESs are only 27 bp long, and iesRNAs bind perfectly to the ends of the IESs. The transcription of a Dicer cleavage substrate from such a short DNA template seems unlikely.

We wish to investigate the iesRNA pathway in more detail, via a number of approaches. We are examining the robustness of iesRNA-guided IES excision via the introduction of artificial iesRNAs, and by inducing the removal of both endogenous and cryptic IESs via the introduction of IES-like dsDNAs. Of particular interest are iesRNA precursors, the identity of which has proven elusive. We investigate the possibility of IES concatamer formation, which would provide a mechanistic explanation for the formation of iesRNAs from excised IESs.

CILIATES POSTER SESSION ABSTRACTS

C7004A Multiple Layers of Nested Genes in the Complex Genome of *O. trifallax*. *J. Braun*, N. Jonoska, M. Saito. Univ. of South Florida, Tampa, FL.

Massive genome rearrangement processes are known to occur during the sexual reproduction of the ciliate *Oxytricha trifallax* making it an ideal model organism to study gene rearrangements. During these processes a transcriptionally active somatic nucleus (macronucleus) is developed from a copy of its germline nucleus (micronucleus). In the micronucleus, macronuclear genes are found divided into potentially scrambled and inverted segments. Some regions between consecutive macronuclear destined segments of one gene contain multiple layers of segments from other genes. This nesting of gene segments significantly contributes to the complexity of the DNA recombination. Mathematical notions to measure the nesting of gene segments have been developed. Through computational analysis, we have identified all occurrences of interleaving segments and 137 instances of 2 to 4 levels of nested genes have been detected in the recently sequenced genome of *O. trifallax*.

C7005B Environmental temperature and its impact on the process of programmed DNA elimination in *Paramecium*. *F. Catania*, G. Churakov. Institute for Evolution and Biodiversity, Muenster, DE.

In ciliates the process of programmed DNA elimination is crucial for the formation of viable sexual offspring. The elimination of thousands of Internal Eliminated Sequences (IESs) from the germline genome occurs at each sexual event and enables the regeneration of a functional somatic nucleus.

Given how critical it is, one may expect that IES excision is virtually insusceptible to mild environmental stress. The impacts of environmental conditions on the accuracy of IES elimination remain an open question, however. We attempted to address this question by exposing self-fertilizing clonal *Paramecium* lines to different environmental temperatures and then by screening these lines' somatic genome for IES retention. Our observations provide insights on the extent to which the fidelity of genome rearrangements in *Paramecium* varies in response to environmental changes.

C7006C Complex Rearrangements in the Highly Scrambled Genome of O. trifallax. *Lukas Nabergall*, Natasha Jonoska, Masahico Saito. Univ. of South Florida, Tampa, FL.

Certain species of ciliates undergo massive genome rearrangements during the development of a somatic macronucleus from a germline micronucleus, and are used as model organisms to study DNA rearrangement. Recent sequencing of the *O. trifallax* germline genome allows for the detailed study of scrambled patterns genome-wide. We present a mathematical framework to describe the order and orientation of scrambled segments (the macronuclear-destined sequences, or MDSs). For example, we catalog all germline loci with odd/even patterns that partition MDSs (an example would be 1-3-4-2-4-6, etc.) and many elaborate variations. We have observed that occurrences of such patterns can explain approximately 82% of all scrambled loci. We also study the complexity of the remaining highly scrambled cases, some of which contain nested occurrences of such patterns.

C7007A Identification and Characterization of the SIRT4/5 Homologs in *Tetrahymena thermophila*. *Emily Nischwitz*, Joshua Trammell, Joshua Smith. Missouri State University, Springfield, MO.

Tetrahymena thermophila contains 18 putative histone deacetylases (HDACs) homologs. Eleven of these homologs are in the Sirtuins subfamily (NAD-dependent Histone Deacetylases). Sirtuins plays a key role in many functions in the cell such as cellular metabolism, removing acetyl groups on histones as well as other proteins in the cell, and have been show to have a role in cancer, neurodegeneration and cardiovascular disease. The scope of this research is to study the localization and expression levels of Tetrahymena Histone Deacetylases 10, 17, and 18 (*THD10, THD17,* and *THD18*), which are homologs of SIRT4 and SIRT5 in humans. To characterize these genes bioinformatic analysis was conducted and then PCR was performed to amplify the gene. The gene product was cloned into the pENTR-TOPO-D plasmid, transformed into E. coli, and ultimately to study the localization tagged with GFP and other epitope tags to study the function within the cell. The expression of these genes has been analyzed under various damaging agents at various time points through qRT-PCR. The damaging agents used in these studies are hydrogen peroxide, methyl methanesulfonate, ultraviolet light that creates distinctive DNA damage and DNA repair responses. This research will better elucidate if there is a possible function of the Tetrahymena SIRT4 and SIRT5-like homologs (*THD10, THD17*, and *THD18*) within DNA damage repair by studying both the localization and expression levels under different conditions.

C7008B A nuclear RNAi-dependent *Polycomb* repression pathway is required for transcriptional silencing of transposable elements. *L. Feng*[1], J. Xiong[1,2], S. Gao[1,3], W. Dui[1], W. Yang[2], A. Kapusta[4], C. Feschotte[4], R. Coyne[5], W. Miao[2], Y. Liu[1]. 1) University of Michigan, Ann Arbor, MI; 2) Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China; 3) Ocean University of China, Qingdao , China; 4) the University of Utah School of Medicine, Salt Lake City, UT; 5) J. Craig Venter Institute, Rockville, MD.

Studies of developmentally regulated heterochromatin formation and DNA elimination in the unicellular eukaryote *Tetrahymena thermophila* have revealed a pathway requiring both nuclear RNA interference (RNAi) and PcG proteins, providing a unique opportunity to dissect the interaction between them. Internal eliminated sequences (IES) in *Tetrahymena* are derived from transposable elements (TE). A wide array of potential TE are revealed in the recently sequenced *Tetrahymena* MIC genome. Recent transposition in *Tetrahymena* population is supported by TE insertion polymorphism in IES, as well as purifying selection in coding sequences of many potential TE. Nonetheless, the molecular mechanism underlying transcriptional silencing and reactivation of TE remains elusive.

RNAi and *Polycomb* repression play evolutionarily conserved and often coordinated roles in transcriptional silencing. Here we show transcription reactivation of IES in mutants deficient in nuclear RNAi and *Polycomb* repression. Importantly, transcriptional silencing and reactivation of TE-related sequences are contingent upon shunting between the noncoding RNA (ncRNA) and mRNA production pathways,

which can be affected by co-transcriptional processing, nuclear RNAi, and *Polycomb* repression. We propose that interplay between the nuclear RNAi and *Polycomb* repression pathways may be a widespread phenomenon, whose ancestral role is epigenetic silencing of TE.

C7009C Regulation of *Tetrahymena* germline transcription in meiotic prophase by three novel proteins. *M. Tian*[1], K. Woolcock[2], K. Mochizuki[2], J. Loidl[1]. 1) Max F. Perutz Laboratories, Vienna, AT; 2) Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna, AT.

Nuclear dimorphism is a characteristic feature of the ciliated protists. The macronucleus (MAC) is transcriptionally active during vegetative growth, whereas the germline micronucleus (MIC) is completely transcriptionally silent during vegetative growth. However, the active transcription of the MIC genome is essential for sexual reproduction.

During the sexual reproduction of ciliated protist *Tetrahymena*, its MIC undergoes meiosis, at the same time, the RNA polymerase II (RNAPII) is recruited to the MIC, and then double stranded non-coding RNAs are generated by bi-directional transcription of the germline genome. The double stranded non-coding RNAs are further processed into small RNAs (called scnRNAs) by Dicer homolog Dcl1, and are guiding the deletion of one-third of the genome from the developing progeny MAC. Although the molecular mechanisms underlying the small RNA-directed DNA elimination event during the sexual process of conjugation have been intensively studied, the activation and regulation of germline transcription is still a mystery. By deleting genes that are specifically expressed in meiotic prophase of *Tetrahymena*, we have identified three (*RIB1, MIP1, MIP2*) genes that are involved in regulation of germline transcription. Knockout of any of them blocks the accumulation of non-coding RNAs from the germline nucleus and prevents the DNA elimination process, indicating that these three genes are essential for germline transcription. Rib1 and Mip1 proteins are specifically localized in the MIC during meiosis. Interestingly, the localization of Rib1 is similar to that of the RNAPII specific subunit Rpb3 in the meiotic prophase MIC. However, the localization of Rpb3 is not affected by the deletion of *RIB1*, indicating that Rib1 might be critical for activating RNAPII transcription but not required for the localization of RNAPII. The deletion of *MIP1* disrupted the localization of Rib1 and Rpb3 to chromatin, suggesting that Mip1 plays a role in the recruitment of RNAPII and possibly its auxiliary factors, such as Rib1, to the MIC. Also in a *mip2* deletion, Rib1 and Rpb3 are mislocalized. Therefore, the Mip2 protein is possibly required for micronuclear import of Rib1 and the proper distribution of RNAPII within the MIC.

Finally, we noticed that the knockout of *MIP1* and *MIP2* lead to the extension of meiotic prophase, which might suggest delayed repair of meiotic DNA double-strand breaks in *mip1*Δ and *mip2*Δ cells.

C7010A Cas9 localization in the binucleated ciliate *Tetrahymena thermophila*. *K. Fryer*, D. Cassidy-Hanley, D. Kolbin, T. Clark. Cornell University, Ithaca, NY.

CRISPR-Cas9 is quickly becoming a standard genome editing tool and has been used in various model organisms. It has yet to be implemented or optimized for use in *Tetrahymena* or ciliates in general despite the implications it has in facilitating germline modifications in binucleate systems. Cas9 has been shown to localize to the nucleus and gain access to DNA in other organisms without any nuclear localization sequence modifications. While this is standard in most systems, the presence of both a transcriptionally active macronucleus and a transcriptionally silent, germinal micronucleus, which has very different membrane characteristics, presents a unique question for ciliates: is Cas9 targeted to one or both nuclei? To answer this question we transformed *Tetrahymena thermophila* with an expression cassette, provided by Dr. Douglas Chalker and Dr. Robert Coyne, containing Cas9 fused to a micronucleus specific linker histone (MLH) and YFP under the inducible MTT1 promoter. We looked at Cas9 expression in induced transgenic cells during vegetative growth, starvation, early conjugation, and late conjugation. No observable signal was seen in the micronucleus or the macronucleus using either YFP fluorescence or anti-Cas9 antibody immunofluorescence, although some punctate staining was observed in the cytoplasm. Western blots of total cell protein extracted from induced cells probed with anti-Cas9 antibodies also showed no detectable signal corresponding to Cas9. Based on these observations, we hypothesized that the very large fusion protein containing Cas9, MLH, and YFP might be unstable or unable to enter the nuclei and may be rapidly degraded by the cell. To test this hypothesis we constructed two vectors, one containing just Cas9 fused only to YFP and one containing just Cas9. Using these smaller constructs, we intend to determine if Cas9 successfully enters either the micronucleus or the macronucleus during vegetative growth or conjugation. This work will help to establish and optimize conditions for Cas9 localization in *Tetrahymena*, and is an important first step in implementing CRISPR technology in this system.

C7011B Epigenetic control of DNA replication revealed in *Tetrahymena thermophila* TXR1 knockout mutants. *Miguel F. Gonzales*[1], Chunxiao Ge[1], Xianzhou Meng[1], Yin Liu[2], Geoffrey M. Kapler[1]. 1) Texas A&M, College Station, TX; 2) University of Michigan, Ann Harbor, MI.

DNA replication initiates at specific sites in chromosomes termed Origins of Replication (ORI). In eukaryotes, these sites are chosen by the binding of the Origin Recognition Complex (ORC) and subsequent recruitment of the MCM2-7 helicase to generate the pre-replicative complex during the G1 phase of the cell cycle. The relative contributions of the DNA sequence (cis-acting determinants) and chromatin context (histone modifications) to ORI function vary widely in eukaryotes, and are the subject of considerable debate. Previous studies revealed a role for the monomethylation of histone H3 on the 27th lysine residue (H3K27) in the control of DNA replication in the polyploid macronucleus of the model eukaryote, *Tetrahymena thermophila* (Gao et al., Genes Dev. 2013). H3K27 monomethylation appears to be restricted to the ciliate lineage and plants, and is mediated in *Tetrahymena* by TXR1 - a homologue of *Arabidopsis thaliana* ATXR5/ATXR6. TXR1 contains a SET-domain enabling the monomethylation of H3K27, and harbors a PCNA-interacting-protein (PIP) box. Taken together, the features of TXR1 suggest a role in DNA replication and/or DNA damage repair.

Using 2D gel electrophoresis of DNA replication intermediates (RIs), we provide evidence for the role of TXR1 in replication initiation in the ribosomal DNA (rDNA) minichromosome. Aberrantly migrating RIs emanate from the rDNA 5' non-transcribed spacer (5' NTS), but not from the

rRNA coding region which is consistent with TXR1-dependent modification of intergenic histone H3 subunits. The novel properties of these RIs are unprecedented suggesting an unconventional nucleic-acid structure. To unveil the structure and nucleic acid content of aberrant RIs, we are employing different nuclease digestions and using the RNA-DNA hybrid-specific S9.6 monoclonal antibody to assess whether non-coding transcripts are involved. Finally, DNA fiber imaging of non-rDNA chromosomes revealed a global effect on replication initiation illustrated by the firing of fewer origins resulting in increased inter-origin distances in TXR1 knockout strains. The average rate for replication fork elongation in TXR1 mutants is indistinguishable from wild type; however, our data suggests that a subpopulation of forks either stall, collapse, or move slower in a TXR1 knockout background. We surmise two possibilities to explain the data: (1) TXR1-dependent modification of the *Tetrahymena* epigenome determines replication initiation sites, and (2) TXR1 is required to re-start stalled replication forks near origin initiation sites.

C7012C ***Gene expression in Paramecium as a response to DNA damage*****.** *R. Stewart*[1*], T. Doak[1,2], L. Bright[1], M. Lynch[1]. 1) Indiana University Bloomington, Bloomington, IN; 2) NCGAS, Bloomington, IN.

Many environmental assaults damage DNA—an organism's genetic identity—but mechanisms have evolved to repair this damage. Oxidative damage, induced by reactive oxygen species such as hydrogen peroxide, is an example of these assaults. The consequences of DNA damaging agents are directly related to the cell's ability to appropriately respond to and repair DNA damage (Sobol et al 2002), using pathways appropriate to each type of damage. But these processes can't be initiated without the expression of genes coding for the repair enzymes. These processes have yet to be fully studied for ciliated protozoa, single-celled eukaryotes, but a few studies exist for *Tetrahymena thermophila*, using several damage mechanisms. These studies have confirmed that a number of repair genes known from other model systems are also induced in ciliates (Smith et al 2004). But these studies have been limited to candidate genes—homologs to repair enzymes known in other systems. To look for additional genes and to provide complementary data sets, several species of the related ciliate *Paramecium*, including *Paramecium caudatum* and *Paramecium tetraurelia*, were examined for their responses to oxidative damage, using protocols published on the treatments of Tetrahymena. Paramecium were treated to .3% hydrogen peroxide (after verifying that the Tetrahymena dose was appropriate for Paramecium) and RNA were collected after zero, one, two, three and four hours. The next stage is to turn these RNAs into next generation sequencing RNAseq libraries. After these libraries are sequenced at the IU Center for Genomics and Bioinformatics (CGG), I will examine the levels of mRNA transcripts produced for each gene after DNA damage, compared to its normal level of expression. Based on this comparison I should be able to determine which genes are expressed at higher levels after DNA damage. I expect to see a high level of mRNA transcripts for known repair genes, such as those known to be associated with DNA repair in Tetrahymena thermophila. However, it is also possible that I might discover genes that are not yet known to be associated with DNA repair, unique to ciliates.

C7013A Identification and Investigation of the Function of Rad23 in DNA Repair and Proteosomal Degradation in *Tetrahymena thermophila*. *Evan Wilson*, Joshua Smith. Missouri State University, Springfield, MO.

Rad23 is a protein involved in both nucleotide excision repair (NER) and proteasome-mediated degradation, and has been suggested to facilitate interactions between these two pathways. Until recently, Rad23-related research has been conducted using *Saccharomyces cerevisiae*. By instead using *Tetrahymena thermophila,* which has a transcriptionally silent micronucleus, the role of Rad23 in global genome NER (ggNER) can be better understood. The *T. thermophila* homolog for RAD23 was identified through bioinformatics analysis. Expression levels and ubiquitination of Rad23 before and after multiple genotoxic stressors was assessed using qRT-PCR and western blot analysis, respectively. Additionally, interacting partners of Rad23 in both the proteasome and the NER pathway will be analyzed using fluorescent co-localization and co-immunoprecipitation. Finally, survival assays of knockdown strains will be used to determine the roles of Rad23 and other interacting partners. An advantage of this research is that tagging and knockdown constructs will be created by using an endogenous tagging vector. Inserting a tagged gene into its natural locus under its own promoter prevents aberrant expression and phenotypes from an inducible promoter that can result from certain experimental conditions.

C7014B The investigation of *Caedibacter taeniospiralis* Reb-related genetic elements in paramecia using fluorescent and phylogentic methodologies. *David Johnson*. Samford University, Birmingham, AL.

The paramecium bacterial endosymbiont *Caedibacter taeniospiralis* produces a protein R-body with four Reb subunits. Recently, a homolog of the gene responsible for the RebB subunit has been discovered as a trichocyst gene in the nuclear genome of a cryptomonad. Here we present our progress on developing a novel *in situ* methodology for detection of the RebB gene and a preliminary phylogenetic analysis of cryptomonad trichocysts genes found in freshwater and marine water samples.

C7015C On the evolution of a family of cis-acting elements for programmed somatic chromosome fragmentation. *E. Orias*. UC Santa Barbara, Santa Barbara, CA.

Ciliates segregate germline and somatic function to two separate nuclei within a single cell. During the development of a new somatic nucleus in *Tetrahymena thermophila*, site-specific fragmentation of the 5 chromosomes derived from the germline (micronucleus or MIC) generates ~200 somatic (macronuclear or MAC) chromosomes. The 15-bp Cbs (Chromosome breakage sequence) family is the necessary and sufficient cis-acting DNA element for programmed chromosome fragmentation. Roughly half of the Cbs's in the MIC genome (109/225) have the consensus sequence, TAAACCAACCTCTTT, with T at each of the only 5 variable positions. No Cbs known to be functional contains substitutions at >2 variable positions. Only at variable positions 1 & 11 have the nucleotide frequencies achieved the maximum diversity expected if all isoforms are functionally equivalent. *Cbs-mediated* chromosome breakage is limited to Tetrahymenine ciliates, raising the possibility that not enough evolutionary time has elapsed to achieve maximal diversity at Cbs positions 13-15.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

To better understand how the current *T. thermophila* Cbs family evolved, a computer simulation has been undertaken. Random mutations at natural rates are allowed in DNA segments that contain the 15-bp Cbs, while 15-bp segments of unconstrained sequence flanking the Cbs on either side act as controls. Simulation results include the following:

1) Simulations starting with the current Cbs complement have reproduced the finding, by calculation, that diversity is currently maximal at positions 1 & 11, but not at positions 13-15.

2) In simulations starting with an all-consensus full Cbs complement, the consensus Cbs frequency decreases from 100% to its current value (~50%) well before maximal diversity is approached at positions 1 & 11 – seemingly inconsistent with the maximum diversity currently observed as these 2 positions.

3) A and T are the only nucleotides allowed at positions 1 & 15. The observed asymmetry in the A:T frequency ratio at positions 1 (32% A) and 15 (9% A) is not reproduced by these simulations, which generate equal relative frequencies at both positions.

The results are informative, as they suggest refinement of particular null assumptions made in the initial simulations. Additional results and hypotheses will be discussed. Sequencing additional Tetrahymenine germline genomes should generate valuable relevant data and additional evolutionary insights.

C7016A Hemp seed extract enhances excystation and survival across genetically diverse ciliates. *S. Phadke*. J Craig Venter Institute, La Jolla, CA.

Starvation triggers various morphological and physiological responses in ciliates. One of the common responses is the formation of dormant structures such as cysts, which have highly reduced metabolic activity that allows survival of the individuals until optimum environmental conditions return. Dormancy represents an adaptive strategy for the ciliates and is also ecologically important for maintenance of microbial diversity. We investigated the diversity of cyst forming ciliates in 42 dried soil samples collected across the continental United States. Together, the samples represent 11 soil types and 5 climate zones. The soils were hydrated with or without hemp seed extract and ~10-50 single cells representing various ciliate morphospecies were isolated from each sample at ~48 hours after hydration. We analyzed the species diversity using 18s rRNA sequences of the isolates and found significant differences in species composition across the samples collected in different soil types. Also, hydration in the presence of hemp seed extract allowed excystation of a higher diversity of species and also allowed longer survival of the ciliates. It is unclear if addition of hemp seed extract simply provided higher nutrients allowing denser growth of bacteria that in turn supported the higher diversity of ciliates. Alternatively, hemp seed extract may directly provide complex nutrients required for excystation of certain ciliate species without significantly affecting bacterial growth. Our results emphasize the need to investigate how nutrient sensing pathways have diversified across cyst-forming ciliates.

C7017B Phylogenetic framework of the systematically confused *Anteholosticha-Holosticha* complex (Ciliophora, Hypotricha) based on multigene analysis. *X. Zhao*[1,2], S. Gao[2], Y. Fan[2], M. Strueder-Kypke[3], J. Huang[1,2]. 1) Key Laboratory of Aquatic Biodiversity and Conservation of Chinese Academy of Sciences, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China; 2) Laboratory of Protozoology, Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao 266003, China; 3) Department of Zoology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada.

The *Anteholosticha–Holosticha* complex is an extremely divergent group within the urostylids, especially because the genus characterization lacks suitable synapomorphies. Previous studies have shown that morphological classification of species within this group often conflicts with SSU-rDNA data, that is this complex is not recovered as a monophyletic group and *Anteholosticha* spp. are widely dispersed throughout the urostylid assemblage in SSU-rDNA trees. In this study, we provided 38 new sequences (including the type species of *Anteholosticha*) of SSU-rDNA, ITS1-5.8S-ITS2 and LSU-rDNA genes to infer molecular phylogenies of all available taxa in the *Anteholosticha–Holosticha* complex. The results show that: (1) *Holosticha* is monophyletic in all trees, suggesting it is a well-defined genus; (2) *Anteholosticha* is polyphyletic and distinctly separated from *Holosticha* in all single-gene based and concatenated phylogenies; (3) the monophyly of *Arcuseries*, a recently established genus split from *Anteholosticha*, is strongly supported by all molecular data; (4) *Anteholosticha multicirrata*, *Anteholosticha manca*, *Anteholosticha paramanca* and *Bakuella subtropica* may share a most recent common ancestor; (5) multi-gene analyses receive higher support values than the single-gene analyses.

C7018C Genetic analysis of the molecular properties underlying centriole stability. *N. E. DeVaul*, K. F. O'Connell. National Institutes of Health , Bethesda, MD.

Centrioles are barrel shaped organelles that play a central role in microtubule organization; in dividing cells, centrioles direct the formation of the mitotic spindle, and in differentiated cells, centrioles organize the formation of cilia and flagella. Centrioles typically exist in pairs, and to properly execute centriole-dependent processes, cells must possess one or two pairs of centrioles. Three properties of centrioles contribute to the strict copy number control: a precise duplication event that occurs once per cell cycle, equal partitioning of centriole pairs to daughter cells at division, and the long–term stability of centrioles once formed. While much is known about centriole duplication and segregation, relatively little is understood about the molecular basis of centriole stability. Five highly conserved factors required for centriole assembly have been identified in the nematode *C. elegans*. This includes SAS-6, a coiled-coil containing protein that forms a central scaffold for centriole assembly. Down regulation of any of the centriole assembly factors blocks formation of new centrioles but does not affect existing centrioles. We have recently identified a new mutant allele of the *sas-6* gene that contains a missense mutation (D9V) affecting the globular N-terminal domain. This domain directs the formation of higher order oligomers of SAS-6 and the assembly of a centriole scaffold. Animals homozygous for this mutation exhibit an embryonic lethal phenotype marked by the presence of monopolar spindles, a phenotype consistent

with a defect in centriole assembly. However, these mutants also exhibit an additional unexpected phenotype. In the male germ line of such mutants, centrioles duplicate normally during the early stages of spermatogenesis but appear to disassemble following the meiotic divisions. As a result, sperm lack detectable levels of centriole proteins including SAS-6, SAS-4 and SPD-2. Thus in the male germ line, centrioles appear to duplicate normally but the long-term stability of these structures is compromised. Our results thus indicate that SAS-6 does not just serve as a scaffold for centriole assembly but also plays a key role in maintaining the structural integrity of these organelles. Future studies will be directed toward identifying how this mutant version of SAS-6 affects the critical protein-protein contacts that underlie centriole longevity.

C7019A Characterization of the Striated Rootlet Proteins of the *Paramecium* Basal Body. *A. Nabi*[1], T. Picariello[2], MS Valentine[1], J. Yano[1], J. Van Houten[1]. 1) University of Vermont, Burlington, VT; 2) UMass Medical School,Worcester, MA.

In *Paramecium,* the ciliary basal body is connected to three rootlets (Post ciliary rootlet (PCR), Transverse rootlet (TR) and Striated rootlet (SR)). The *Paramecium* cell surface is divided into units each of which has one or two basal bodies. The SR projects from the basal body toward the anterior in straight rows for several units. In *Tetrahymena*, a full-length SR is required to resist ciliary beating forces that cause the basal bodies to move out of alignment (Galati *et al*., 2014). We previously found that the depletion of the transition zone protein Meckelin results in the misaligned SR and disorganized basal bodies, reminiscent of the phenotype of the *Tetrahymena* mutant *DisA*. This mutant shows shortened SR and disorganized basal body rows. We wished to study the SR in *Paramecium* to determine its connection to Meckelin. To do so, we identified the genes for potential SR proteins. Through the expression of epitope tagged proteins, we found 9 proteins located in the SR. All of these proteins have the predicted domains of SF-assemblin protein which form the SR of *Chlamydomonas*. Proteins without this domain are not found in the *Paramecium* SR.

Immunoprecipitation (IP) of epitope tagged SR proteins followed by LC-MS/MS showed that SR family members co-IP. We purified the intact SRs and found that the epitope tagged proteins co-purify with the intact SR. LC-MS/MS analyses of the fraction of density gradients in which the SR resides show that all SR family member (SR-D, -E, -F and –G) are found together. Other proteins that co-purify are Centrin, Actin, α-, β-, and g- tubulin, Rab/Ras small GTPase.

SR-B and SR-C family members by homology but lacking the domains of SF-assemblin proteins are not located in the SR as shown by immunofluorescence, but both of are identified in the purified SR sample by LC-MS/MS.

RNAi for one of the SR family members (SR-F) showed a phenotype similar to that of the *Tetrahymena* mutant *DisA:* shortening of the SR and misaligned basal bodies rows all over the cells from the posterior to the anterior pole as compared to control cells. However, this phenotype is not found for RNAi depletion of a second SR protein SR-D4.

Acknowledgement:

LC-MS/MS analysis was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103449.

C7020B A NIMA-related kinase CNK4 regulates ciliary stability and length. *J. Pan*[1,2], Dan Meng[1]. 1) Tsinghua University, Beijing, Beijing, CN; 2) Qingdao National Laboratory for Marine Science and Technology, Qingdao, CN.

NIMA-related kinases (Nrks or Neks) have emerged as key regulators of ciliogenesis. In human, mutations in *Nek1* and *Nek8* cause cilia-related disorders. The ciliary functions of Nrks are mostly revealed by genetic studies, however, the underlying mechanisms are not well understood. Here we show that a *Chlamydomonas* Nrk, CNK4 that is closely related to Nek1, regulates ciliary stability and length. CNK4 is localized to the basal body region and the flagella. *cnk4* null mutant exhibited long flagella with formation of flagellar bulges. The flagella gradually became curled at the bulge formation site leading to flagellar loss. Electron microscopy shows that the curled flagella involved curling and degeneration of axonemal microtubules. *cnk4* mutation resulted in flagellar increases of IFT trains as well as its accumulation at the flagellar bulges. IFT speeds were not affected, however, IFT trains frequently stalled leading to reduced IFT frequencies. These data are consistent with a model that CNK regulates microtubule dynamics and IFT to control flagellar stability and length.

C7021C Plasma Membrane Calcium ATPase Regulates Ciliary Calcium in *Paramecium tetraurelia*. *J. Yano*, J. Van Houten. University of Vermont, Burlington, VT.

Paramecium tetraurelia, which is a cell covered in cilia*,* responds to sensory stimuli with changes in swimming behavior. Membrane potential controls beat frequency, hence speed, and graded Ca^{2+} action potentials cause the cell to turn. The physiology of the action potentials caused by activation of ciliary voltage-gated Ca^{2+} (Ca_v) channels has been known for 50 or more years. It has also been known that the Ca_v channels which are necessary for the action potential are found exclusively in the cilia. The Ca^{2+} entering the cilium through these Ca_v channels affects the ciliary beating, reversing the power stroke and transiently sending the cell swimming backward. As ciliary Ca^{2+} levels return to normal, the cell pivots in place and then swims forward in a new direction. Thus, the activation of the ciliary Ca_v channels causes cells to swim backward transiently and make a turn in their swimming paths. The pore-forming subunit proteins ($Ca_v\alpha1$) were not identified until recently when we determined the *P. tetraurelia* ciliary membrane proteome. Three $Ca_v\alpha1$ subunits were subsequently cloned and confirmed to be expressed in the cilia. We also demonstrated using interference RNA (RNAi) that these channels function as the ciliary Ca_v channels that are responsible for the reversal of ciliary beating. The ciliary membrane proteome included plasma membrane Ca^{2+} ATPases (PMCAs) that are in high abundance. RNAi for these PMCAs (PMCA 18/19) increased the duration of backward swimming, suggesting that these calcium pumps play a role in controlling the ciliary Ca^{2+} levels following the activation of the Ca_v channels. These PMCAs can be reciprocally co-precipitated with the $Ca_v\alpha1$, and can be found in the same lipid raft fractions as the $Ca_v\alpha1$. Therefore, we postulate that an important mechanism for the immediate

removal of Ca^{2+} upon activation of the Ca_v channels is the physical proximity and interaction of the ciliary calcium pumps with the channels.
Acknowledge P20GM103449.

C7022A Depletion of SUMO-conjugating enzyme Ubc9p causes nuclear defects during the vegetative and sexual life cycles of *Tetrahymena thermophila*. . *J. Forney*[1], Q. Yang[1], A. Nasir[1], R. Coyne[2]. 1) Purdue University, West Lafayette, IN; 2) J. Craig Venter Institute, Rockville, MD.

Ubc9p is the sole E2-conjugating enzyme for SUMOylation and its proper function is required for regulating key nuclear events such as transcription, DNA repair and mitosis. In *Tetrahymena thermophila* the genome is separated into a diploid germline micronucleus (MIC) that divides by mitosis and a polyploid somatic macronucleus (MAC) that divides amitotically. This unusual nuclear organization provides novel opportunities for the study of SUMOylation and Ubc9p function. We identified the *UBC9* gene and demonstrated that its complete deletion from both the MIC and MAC genome is lethal. Rescue of the lethal phenotype with a *GFP-UBC9* fusion gene driven by a metallothionein promoter generated a cell line with $CdCl_2$-dependent expression of GFP-Ubc9p. Depletion of Ubc9p in vegetative cells resulted in the loss of MICs but MACs continued to divide. Unequal partitioning of MIC DNA was observed in cells undergoing mitosis. Interestingly, expression of a catalytically inactive Ubc9p resulted in the accumulation of multiple MICs but no defect in cytokinesis was observed. During sexual reproduction endogenously tagged Ubc9p localized in the parental MAC at early stages of conjugation and later accumulated in the developing MAC. The timing and localization is consistent with our previous observations that a large increase in SUMOylation occurs in the developing MAC between 7-10 hours post mixing. Similar to Uba2p (SUMO-activating enzyme) depleted cell lines, Ubc9p depleted cell lines cannot form cell pairs after nutrient starvation and fail to mate. To analyze the function of Ubc9p during sexual reproduction we expressed the catalytically inactive Ubc9p in mating cells. A large fraction of mating pairs (42%) arrested at an early stage of macronuclear development prior to the elimination of the parental MAC. This arrest was dependent on induced expression of the mutant Ubc9p and is consistent with our observation that Ubc9p is localized in the developing macronucleus. The results demonstrate the importance of Ubc9p and the SUMO pathway in the sexual life cycle of *Tetrahymena*.

C7023B Proteinases and phagocytosis in *Tetrahymena thermophila*. *J.William. Straus*[1], Fiona Chen[1], Neila Kline[1]. 1) Vassar College, Poughkeepsie, NY; 2) Vassar College, Poughkeepsie, NY; 3) Vassar College, Poughkeepsie, NY.

Papain family cysteine proteinases have long been associated with lysosomal and phagosomal compartments. Aqueous extracts of *Tetrahymena* contain a variety of proteinases, predominantly papain family cysteine proteinases sensitive to E64 and other broad spectrum cysteine proteinase inhibitors. While there are 114 candidate cysteine proteinase genes in the *Tetrahymena* genome, few have been characterized with regard to expression, localization, and function. Membrane permeable and non-permeable fluorogenic proteinase substrates and fluorescent inhibitors localize primarily to small (≤1 μm) vesicles, usually on the surface or interior of phagosomes. Non-permeable fluorogenic substrates are taken up through the oral apparatus and incorporated into vesicles that initially align with the postoral microtubule system (detected by antibody staining), and subsequently with discrete phagosomes in transit toward the cell posterior. Zymographic analyses of aqueous cell extracts reveal 11 - 16 discrete cysteine proteinases ranging in size from about 25 kDa to over 150 kDa. A proteomic approach was used to determine which of the 114 candidate proteinases were present in aqueous and detergent homogenates. Briefly, homogenates were partially purified by affinity chromatography against immobilized inhibitor, then subjected to reduction, alkylation, and trypsin digestion.. Resulting peptides were further purified by HPLC coupled to tandem mass spectrometry and SpectrumMill analytical software. We identified 12 valid papain family cysteine proteinase sequences were detected. Future efforts will focus on additional proteomics as well as determining cellular localization, function, and gene structure of these enzymes.

57TH ANNUAL DROSOPHILA RESEARCH CONFERENCE

Poster Session Abstracts

D1001A Quantitative analysis of myosin-driven apical constriction in delaminating neuroblasts. *Yanru AN*[1,2], Guosheng XUE[1], Toyotaka ISHIBASHI[1], Chris DOE[3], Yan YAN[1,2]. 1) HKUST, Division of Life Science, Hong Kong, China; 2) HKUST, Center for Systems Biology and Human Health, Hong Kong, China; 3) University of Oregon, Institute of Neuroscience, Eugene, OR, United States.

The epithelial to mesenchymal transition (EMT) is a process important for organ formation, tissue homeostasis and tumor metastasis. We use *Drosophila* embryonic ventral nerve cord neural stem cells, the neuroblasts, as a model to study EMT events. In the neuroectoderm, for each proneural cluster of cells, one cell undergoes EMT, delaminates from the epithelium and becomes the neuroblast, while the surrounding cells remain as epithelial cells. Apical constriction is one of the key events during EMT, and in our project we aim at dissecting how the actin-myosin network drives apical constriction in a single cell delamination event.

Through imaging live embryos, we noticed that dynamic myosin loci and fibers are present across the apical surface of the delaminating neuroblasts as well as the neighboring non-delaminating cells. Quantitative analysis showed that medial myosin contractions correlate with apical cell area changes. Although the medial myosin contractile pulses are present in both delaminating neuroblasts and their non-delaminating neighbors, the medial pulses exhibit higher amplitudes and appear at higher frequency in the delaminating cells than their non-delaminating neighbors. Interestingly, when the embryos are injected with low-dose CytoD to deplete the medial myosin pulses, we noticed that the single presumptive neuroblasts still undergo pulsatile apical constriction but fail to maintain the cell area, reflex back and fail to effectively decrease their apical area over time. In these cells, the junctional myosin intensity increase precedes apical area decrease in one round of apical constriction, indicating that the junctional myosin plays a role in initiating apical constriction and the medial myosin might function to stabilize the cell shape in the delaminating neuroblasts.

D1002B An acentrosomal perinuclear microtubule-organizing center in *Drosophila* fat body cells maintains cell shape and organelle positioning. *R. Buchwalter*, Y. Zheng, J. Chen, B. Dietrick, L. R. Kao, T. Megraw. Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL 32306, USA.

While the centrosome is generally considered the main microtubule-organizing center (MTOC) in animal cells, acentrosomal MTOCs are found in diverse contexts in differentiated cells. Acentrosomal MTOCs can assemble in the cytoplasm, at the plasma membrane, nuclear periphery, adherens junction, golgi, and mitochondrion. These varied MTOCs serve roles such as nuclear positioning, establishing planar cell polarity, sperm morphogenesis, and neuron dendrite branching, depending on the cell types where they assemble. However, little is known about the assembly and molecular makeup of acentrosomal MTOCs. We have discovered an MTOC located at the nuclear periphery in larval fat body cells. Similar to adipose tissue and liver in mammals, *Drosophila* fat bodies have high metabolic activity and mobilize lipid stores and other sources to provide energy for the organism during development and under stress conditions such as starvation. We show that the fat body MTOC is comprised of at least 9 proteins that are also components of the centrosome. Specifically, a subset of centrosomal proteins that are components of the pericentriolar material (PCM) of the centrosome constitute the fat body perinuclear MTOC. We will present these components and their requirements for MTOC function and their link to the intranuclear cytoskeleton through the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex. Mutations in at least two components impair nuclear position and cell shape. We also show that the MTs at this MTOC are rich in posttranslational modifications and may be unusually stable. Finally, we aim to connect the function of the fat body MTOC to the regulation of autophagy: a function we show belongs to several of the PCM proteins at the MTOC and likely a key function of the fat body to regulate metabolism (see poster by Y. Zheng *et al.* for study of the centrosome's role in autophagy).

D1003C A tissue-specific regulation of Myosin II dynamics during tube formation. *S. Chung*, S. Kim, C. Hanlon, D. Andrew. Johns Hopkins Univ, Baltimore, MD.

During embryogenesis, the salivary gland (SG) primordia change geometry from two-dimensional epithelial sheets on the embryo surface into fully internalized, three-dimensional tubes. Invagination of the SG absolutely depends on the FoxA transcription factor Fork Head (Fkh). Here, we show the temporal and spatial regulation of myosin cytoskeleton during SG invagination and reveal the tissue-specific signaling pathway that regulates it. Myosin re-localizes from cortical to apicomedial region of the SG cells prior to invagination, which is dependent on Fkh. Using both live and fixed sample imaging, we revealed that these apicomedial myosin cables are pulsatile and that they provide motive forces for cell deformation and subsequent apical constriction. We also show similar temporal and spatial re-distribution of Rho kinase (Rok), which also requires the Fkh function. We further discovered that Fkh regulates the SG expression of a secreted ligand *folded gastrulation* (*fog*) and that apicomedial localization of Rok and myosin is defected in *fog* mutant SGs, which have a range of invagination defects. Surprisingly, unlike mesodermal tissue invagination, blocking apical constriction does not prevent the SG cells from internalizing. Instead, SGs with genetic manipulation to block apical constriction still invaginate but show abnormal tissue geometry. We also provide evidence on the putative G protein coupled receptor(s) that appear to mediate the Fog signals to the Rok and myosin.

D1004A Characterization of Garz Function during Epithelial Morphogenesis. *J. Gates*, S. Andrews, R. Franz, J. Korn. Bucknell Univ, Lewisburg, PA.

Regulation of actin dynamics is essential during epithelial morphogenesis to allow cells to change shape, rearrange and migrate. Numerous proteins have been identified that influence actin dynamics including the Abelson (Abl) non-receptor tyrosine kinase and one of its targets Enabled (Ena). Previous work has shown that both Abl and Ena are required during epithelial morphogenesis in the embryo, including during the processes of dorsal closure and head involution. Furthermore, Abl appears to negatively regulate Ena during dorsal closure by reducing the levels of Ena available to assist with the formation of filopodia by the leading edge epithelial cells. To identify additional proteins that may function with Abl and/or Ena during epithelial morphogenesis, we are collaborating with Traci Stevens' lab at Randolph-Macon College. The

Stevens lab has carried out a genetic screen to identify mutations in genes that dominantly enhance or suppress the epithelial defects caused by overexpressing a constitutively active form of Abl called BCR-Abl. One of the genes they identified is *gartenzwerg* (*garz*), an ARF-GEF. We have found that mutations in *garz* dominantly enhance the head involution phenotype of *ena* homozygous mutants, but appear to have essentially no effect on the dorsal closure phenotype of these mutants. Interestingly, examination of the levels and localization of Ena and actin in *garz* mutant embryos has not yet revealed head involution defects, but has revealed dorsal closure defects that are consistent with a potential role for Garz in regulating contraction of the leading edge actin/myosin cable. To examine whether Garz may be functioning as a canonical ARF-GEF during epithelial morphogenesis we are currently testing whether mutations in *ArfGAP1* or *Arf79F* also dominantly modify the *ena* homozygous mutant phenotype. The continued examination of the roles of these proteins during dorsal closure may reveal novel roles for the Arf family of small GTPases during epithelial morphogenesis.

D1005B Characterization of a novel actin regulator, HtsRC. *J. Gerdes*, A. Hudson, K. Mannix, L. Cooley. Yale University, New Haven, CT.

Cell division of germline cells throughout the animal kingdom is characterized by incomplete cytokinesis resulting in clusters of sibling cells connected by intercellular bridges called ring canals (RC). In the *Drosophila melanogaster* female germline, ring canals acquire a robust actin cytoskeleton that supports the dramatic increase in ring canal lumen diameter over the course of egg chamber development. Genetic studies show that the *hts* gene is involved in the accumulation of ring canal F-actin, and a product of this gene, HtsRC, could be the substrate of the Kelch-Cullin3 RING ubiquitin ligase, whose function is also essential for ring canal growth. We demonstrate that HtsRC, produced by a germline-specific *hts* transcript, is necessary for actin accumulation. Using CRISPR/Cas9 directed mutagenesis of HtsRC, we obtained a series of alleles in the HtsRC coding region that block accumulation of F-actin to RCs and cause a dramatic reduction in RC size. Homozygous mutant females have severely reduced fertility. Ectopic expression of HtsRC in somatic follicle cells results in accumulation of filamentous actin aggregates. These results support the conclusion that HtsRC is necessary and sufficient for recruiting F-actin assemblies. We are now carrying out biochemical and functional analyses of the HtsRC protein in order to determine how it affects actin organization. The predicted protein contains no known functional domains and is conserved only in the Drosophila family. Therefore studying HtsRC should enhance our understanding of existing actin regulatory mechanisms, and provide new insights into the regulation of F-actin structures.

D1006C *Drosophila* myosin 7a in Phagocytosis and Eye Development. *Amy Hong*, James Sellers. NIH, Bethesda, MD, USA.

Drosophila myosin 7a (Dm7a) is an unconventional myosin required for the maintenance of the Johnston's organ (the auditory center in *Drosophila*) and the morphology of the bristle structures on the thorax. Both of these structures are formed by bundled arrays of actin. Here we show that Dm7a is involved in the process of engulfment of bacteria particles in the *Drosophila* larval hemocytes. Using the UAS/GAL4 system, we express GFP-Dm7a in the larval hemocytes where it localizes to the cortical regions of the cell and induces the formation of filopodia that are also enriched in Dm7a. We imaged the phagocytosis of fluorescently-tagged bacteria by hemocytes expressing GFP-Dm7a . When a bacterium contacts the cell surface of a hemocyte, the latter sends out a filopodium which wraps around the bacterium followed by engulfment. Occasionally an extended filopodia was seen to contact a bacterium and transport it back to the cell surface. Phagocytosis assays revealed that hemocytes from Dm7a mutant flies are defective in their ability to engulf bacteria compared to wildtype hemocytes, although bacteria do bind to their cell surface. Additionally, a lethality assay whereby larvae were challenged with bacteria show that 21% of wildtype larvae eclosed into adult flies, but less than 10% of Dm7a mutant larvae eclosed under these conditions. These results suggest a role for Dm7a in phagocytosis and in the defense mechanism of the fly. Additionally, we found that in electron microscopy sections of the adult eyes of Dm7a mutants showed missing pigment granules around the primary pigment cells and at the base of the rhabdomeres of the photoreceptor cells.

We have also identified a binding partner of Dm7a using the MyTH7 as a bait in a yeast two hybrid system and named it *Myosin 7a Binding Protein* (M7BP). M7BP protein has a potential Rab-binding domain in the N-terminus. Immunoprecipitation experiments using M7BP antibody pulled down several *Drosophila* Rab proteins. Dm7a, M7BP and Rab11 are expressed in the larval eye disc in the lens-secreting cone cells. We are in the process of investigating the functions of Dm7a and M7BP in the development of the eye.

D1007A Zasp52 is a core Z-disc scaffold protein mediating myofibril assembly by dimerizing and binding F-actin. *K. Liao*, F. Schöck. Department of Biology, McGill University, Montreal, QC, Canada.

Zasp family proteins Zasp52 and Zasp66 directly interact with alpha-actinin (Actn), colocalize at the Z-disc, and play a crucial role in myofibril assembly, the last step of muscle development. In live imaging, GFP-Zasp52 forms steadily growing Z-bodies initially evenly dispersed in myotubes, which sort out to future Z-disc locations and eventually fuse to form a contiguous Z-disc. Here we show the molecular mechanism of Zasp function. Genetic experiments demonstrate that *Zasp52* and *Actn* are both haploinsufficient genes whose gene products are required at similar stoichiometry at the Z-disc. Next, pull-down and chemical crosslinking assays show that Zasp52 dimerizes and heterodimerizes with Zasp66, likely explaining the cooperation of Zasp52 and Zasp66 and the steady growth of Z-bodies during myofibril assembly. Finally, we demonstrate through pull-down and cosedimentation assays as well as direct visualization that Zasp52 binds and bundles F-actin. Genetic interaction data indicate that binding of Zasp52 to actin thin filaments is crucial for indirect flight muscle development.

D1008B Remodeling the actin cytoskeleton by ubiquitin-dependent proteolysis. *Katelynn M. Mannix,* Juli Gerdes, Andrew Hudson, Ronit Kaufman, Lynn Cooley. Yale University, New Haven, CT.

In order for cells to perform their various functions, they must assemble and continually regulate specialized actin-based structures and networks. Remodeling of actin networks is critical to allow cells to respond to external stimuli and carry out important processes during development. In the *Drosophila* germline, ring canals (RCs) are specialized actin-based structures that function as intercellular bridges and

connect germline cells to support growth of the oocyte. RCs grow from 1 µm to 10 µm in diameter during development of the egg chamber, and their growth is driven by a robust and dynamic actin cytoskeleton. We previously found that mutations in – or inhibition of – components of a Cullin3-RING E3 ubiquitin ligase complex ($CRL3^{Kelch}$) caused aberrant accumulation of F-actin in the lumen of the RC – referred to as a *kelch*-like phenotype. Inhibition of the proteasome in the female germline also caused a *kelch*-like RC phenotype, and loss of one copy of *kelch* (the substrate recognition subunit of the ubiquitin ligase complex) enhanced the *kelch*-like phenotype, suggesting that Kelch and the proteasome function in a common pathway. We hypothesize that $CRL3^{Kelch}$ functions to target a ring canal-specific substrate for ubiquitination and proteasomal degradation, a process required for proper regulation and remodeling of the RC actin cytoskeleton during development. We are currently investigating HtsRC, a novel ring canal protein, as the substrate based on the following genetic evidence: (1) overexpression of HtsRC induces *kelch*-like RCs; (2) downregulation of HtsRC suppresses the *kelch*-like phenotype; and (3) HtsRC protein levels are dependent on Kelch protein levels. We are currently performing biochemical assays to validate HtsRC as the substrate. Specifically, we are testing if HtsRC is ubiquitinated, and if its ubiquitination is dependent on $CRL3^{Kelch}$. Further, we have made a non-degradable HtsRC by mutating all 18 lysine residues (sites of ubiquitination) to arginine, and we are testing if expression of non-degradable HtsRC induces *kelch*-like RCs. Lastly, we are performing co-IP binding assays to see if HtsRC and Kelch physically interact. Given the role HtsRC may be playing in the crucial remodeling and organization of the RC actin cytoskeleton during development, we are now interested in identifying its binding partners. To this end, we fused HtsRC to the APEX enzyme to achieve proximity-dependent biotinylation of HtsRC-interacting proteins in egg chambers. These biotinylated proteins can be captured by streptavidin beads and identified by mass spectrometry (MS). Excitingly, HtsRC::APEX MS hits include known ring canal proteins and are unique compared to Pav::APEX (another RC-specific fusion) hits, indicating that this technique can be used to identify interacting proteins with high spatial specificity.

D1009C A splice variant of Centrosomin converts mitochondria to MTOCs to facilitate sperm tail elongation in *Drosophila*. *T. L. Megraw*, J. V. Chen, L. R. Kao. Florida State University, Tallahassee, FL.

In *Drosophila* spermatids, the giant mitochondrion (nebenkern) provides a structural platform for microtubule (MT) organization to support sperm tail elongation. *centrosomin* (*cnn*) expresses several variants that fall into two major forms: a ubiquitously expressed centrosomal form (CnnC) and a non-centrosomal form in testes (CnnT). CnnC is essential for functional centrosomes, the major microtubule-organizing centers (MTOCs) in animal cells. We found that CnnT is expressed exclusively in testes and localizes to nebenkerns in spermatids. When expressed ectopically in flies or in mammalian cell culture, CnnT localizes to mitochondria in those cells too. We show that CnnT targets to the mitochondrial surface and recruits the γ-TuRC MT-nucleating complex to assemble MTs on mitochondria, converting mitochondria to MTOCs. We have mapped two separate domains on CnnT that are necessary and sufficient to 1) target it to mitochondria, and 2) to recruit the γ-TuRC and nucleate MTs, respectively. Disrupting the conserved CM1 domain in CnnT (which is shared with CnnC) abolishes the MT-nucleating function but does not block γ-TuRC recruitment, indicating that CM1 is essential for the activation of bound γ-TuRCs independent from their recruitment to MTOCs. *In vivo*, CnnT forms speckles on the surface of the nebenkern in spermatids, where it is required to recruit γ-tubulin. c*nnT* mutant males have significantly smaller seminal vesicles, shorter mature sperm tails, and reduced fertility. CnnT therefore assembles unique non-centrosomal MTOCs on nebenkerns to facilitate the morphogenesis of the extremely long sperm that are found in Drosophila.

D1010A The *Drosophila* Ninein homolog *bsg25D* cooperates with *ensconsin* in myonuclear positioning. *J. N. Rosen*, M. M. Azevedo, D. Soffar, M. K. Baylies. Sloan Kettering Institute, New York, NY.

In fruit flies as in humans, skeletal muscle cells are multinucleated with nuclei evenly spaced throughout the cell. The mechanisms by which myonuclei are correctly positioned and the consequences of mispositioning are poorly understood. In *Drosophila*, myonuclei are initially grouped together in immature muscle cells, prior to moving to the locations that they occupy in fully differentiated muscle. Our group has previously demonstrated that the conserved microtubule (MT) associated protein Ensconsin (Ens) is essential for myonuclear movement in the embryo. Recently, through a yeast two-hybrid screen, we identified Bsg25D (human ortholog: Ninein), a MT-anchoring protein, as an Ens binding partner. Muscle-specific overexpression of Bsg25D causes a complete block in myonuclear movement in the embryo, recapitulating the *ens* null phenotype. Genetic interaction and rescue experiments combined with colocalization studies suggest that exogenous Bsg25D exerts its effect by sequestering Ens away from MTs where it normally functions. Later in development, Bsg25D-overexpressing larval muscles exhibit severely mispositioned nuclei and dramatic alterations in MT organization; larval motility is reduced, demonstrating a loss of muscle activity. To complement our overexpression studies, we generated a *bsg25D* null mutant. Even though *bsg25D* mutant muscles are normal, loss of *bsg25D* enhances myonuclear positioning defects in *ens* heterozygotes. Similarly, *bsg25D* mutant flies have nearly wildtype viability, but loss of *bsg25D* in an *ens*-compromised background causes embryonic lethality. We are currently investigating the hypothesis that an Ens-Bsg25D interaction regulates MT dynamics at the myonuclear envelope, a critical MT organizing center in muscle cells (which lack centrosomes). The functions of centrosomal proteins in muscle are unknown, and this is the first evidence for a centrosomal protein participating in myonuclear positioning. As certain muscular dystrophies characteristically feature mispositioned myonuclei, our results will shed light on human disease.

D1011B Regulation Of Actomyosin Network By Homophilic Cell Adhesion Molecule Echinoid During Epithelial Morphogenesis. *Rahul Rote*, Arsida Noçka, Laura Nilson. Department of Biology, McGill University, Montreal, Quebec, CA.

Cell shape changes during epithelial morphogenesis are driven by differential distribution of cell adhesion molecules, cytoskeletal components and polarity factors. Echinoid (Ed) is a homophilic cell adhesion molecule required in normal epithelial morphogenesis during embryonic dorsal closure and ovarian follicular epithelium development. Both of these processes are characterized by loss of Ed from a defined group of cells, which leads to the formation of a supracellular actomyosin cable and smooth contour at the interface of Ed-positive (Ed) and Ed-

negative (no-Ed) cells. Ed is also lost from the Ed-positive cells at this interface, due to the absence of a homophilic binding partner in the adjacent cells, resulting in a planar polarized distribution of Ed in these cells. We are testing two alternate hypotheses to explain this phenotype. The first is that planar polarized distribution of Ed guides the formation of actomyosin cable at the interface devoid of Ed. The second hypothesis is that Ed simply functions as a negative regulator of the actomyosin network and absence of Ed at an interface leads to actomyosin enrichment at that particular interface.

Quantification of actin using fluorescently-labeled phalloidin, and myosin using fluorescently labeled heavy or light chain of Myosin II, revealed that significant enrichment of actin and myosin occurs at interfaces between Ed/no-Ed cells as well as no-Ed/no-Ed cells. Cells lacking Ed are also apically constricted, consistent with higher actomyosin contractility. These observations seem to support the negative regulator hypothesis. However, Ed/no-Ed interfaces exclusively display a smooth border, loss/disruption of the polarity protein Par3/Bazooka and enrichment of the actomyosin contractility regulator Rho-Kinase. We also studied distribution of myosin tagged with GFP and RFP–in adjacent wild type (WT) and ed mutant cells respectively. We observed that myosin polarization is stronger in the WT cells at the ed clone border than in the adjacent ed mutant cells, indicating that polarization of Ed has a stronger effect on myosin than the global absence of it. These observations are consistent with the planar polarization hypothesis. Thus, the regulation of actomyosin network by Ed may encompass a variety of interactions between downstream effector molecules involved in cell polarity and cytoskeletal dynamics.

D1012C Novel mechanisms of phosphoregulation of Moesin by Clic in *Drosophila* rhabodomere formation. *S. Tanda*, R. Hikida, M. Berryman. Ohio Univ, Athens, OH.

Moesin (Moe) is a key component to link the actin cytoskeleton to the plasma membrane for stable surface structures such as microvilli. Moe function depends on phosphorylation in its actin binding domain. Phosphorylated Moe (p-Moe) is an active form that binds to both actin filaments and membrane proteins to tether them together. Levels of p-Moe are regulated by a kinase, Slik, and a phosphatase, Pp1-87B, which work antagonistically. Clic (Chloride Intracellular Channel) is proposed to interact with Moe and regulate levels of p-Moe. For example, loss of Clic function results in increased levels of p-Moe in the larval salivary gland. To elucidate how Clic regulates p-Moe levels in cell types rich in actin-based surface structures, we chose the rhabdomeres of photoreceptors in the compound eyes of *Drosophila melanogaster*. The rhabdomeres are a cylinder like surface structure consisting of ~60,000 microvilli. As we observed in the larval salivary gland, p-Moe levels were elevated in Clic-deficient clones in the pupal retina. The rhabodomeres are extremely sensitive to levels of Moe phosphoregulators like Slik. We examined rhabodomere structures in different genetic backgrounds using transmission electron microscopy. We changed expression of Slik and Pp1-87B in a wild-type as well as Clic-deficient background. We also examined rhabdomere structures in loss-of-function as well as gain-of-function of Moe in the presence or absence of Clic. We found that Clic suppresses Slik and Pp1-87B functions. Effects of Clic on these Moe phosphoregulators also depend on the level of Moe. Clic showed more pronounced effects on Slik than Pp1-87B when less Moe was available in photoreceptors, while Pp1-87B activity was more suppressed by Clic when Moe is more abundant. Based on these findings, we propose that Clic plays a critical role in maintaining a proper level of p-Moe in photoreceptors in the compound eye.

D1013A Investigating patterns of cell interactions during epithelial folding. *Hannah G. Yevick*[1], Norbert Stoop[2], Jörn Dunkel[2], Adam Martin[1]. 1) MIT Biology, Cambridge, MA; 2) MIT Mathematics, Cambridge, MA.

Correct tissue shape is essential for proper tissue function. How groups of hundreds or even thousands of cells coordinate to yield stereotypic shape change through large-scale movements is still poorly understood. One way for cells to interact is through direct mechanical coupling. In fact, largescale networks of actomyosin connections, linking across neighboring cells are present in a wide range of developing tissues across various model organisms. The *Drosophila* ventral furrow represents one such system for studying supracellular networks. During furrow formation cells coordinate constrictions to yield tissue-wide bending. The tissue possesses a dynamic myosin network which fully forms prior to folding. Little is known, however, how mechanical information in the network guides reproducible tissue constriction. We have developed a novel approach, integrating mathematical concepts from topological feature analysis and network theory to map the previously unquantifiable myosin network across hundreds of cells in the developing ventral furrow tissue. Our approach aims to identify a novel unit of cooperation between the cell and the tissue scale over which cells synchronize. We have identified an initial growth phase and a subsequent contractile phase in the network. Our framework allows us to explore how geometric and topological patterns in the myosin network inform tissue-level constriction.

D1014B Syncytial embryo cleavage through an actomyosin Goldilocks effect set by Rho kinase and myosin phosphatase. *Y. Zhang*, T. Jiang, C. Yu, T. Harris. University of Toronto, Toronto, Ontario, CA.

For cytokinesis, the physical division of two nascent cells, actomyosin networks drive cleavage furrow ingression. During this process, network contractility is promoted through myosin phosphorylation mediated by Rho kinase (Rok) and reversed by myosin phosphatase. Cleavage of the early *Drosophila* embryo involves incomplete cytokinesis to maintain syncytial organization. Furrows ingress as lateral membranes, but actomyosin networks at their basal tips do not constrict to close the base of the cells. How myosin activity affects membrane ingression remains unclear. Previous work suggested that myosin is not essential for furrow ingression, whereas other studies indicated that actomyosin networks are actively inhibited to prevent full cell division. To investigate whether myosin is involved in furrow ingression, we perturbed Rok by RNAi. We found that myosin is lost from furrow membranes and furrows failed to ingress when Rok was severely depleted. Myosin regulatory light chain (MRLC) RNAi mimics the defects seen with Rok-RNAi, indicating myosin is required for proper furrow organization. To test the role of myosin de-phosphorylation, we targeted the myosin binding subunit of myosin phosphatase (Mbs) by RNAi. Loss of Mbs increased myosin accumulation at the base of furrows and induced constriction at these sites. As a result, basal membranes closed abnormally and impinged on

nuclei. To confirm the effects were due to excessive myosin activity, we also overexpressed Rok or phosphomimetic-MRLC and observed similar basal membrane closure, indicating Mbs keeps myosin activity in check during furrow ingression. To dissect how myosin activity is controlled by this phosphor-regulation, we compared the furrow localization of myosin, Rok, and the catalytic subunit of myosin phosphatase, Flapwing (Flw). The cortical level of all three proteins changed over the cell cycle. As new cleavage furrows formed, levels of all three protein gradually increased on the furrow membrane, with enrichment at their basal tips Together, these data suggest that syncytial embryo cleavage depends on myosin activity that is not too low or too high, a Goldilocks effect, and that the proper range of activity is set locally by the counteracting activities of Rok and myosin phosphatase.

D1015C The Role of Retromer-Dependant Recycling in Epithelial Polarity and Morphogenesis. *K. Al Kakouni*[1], K. Harris[2], U. Tepass[1]. 1) University of Toronto, Toronto, ON, CA; 2) MIT, Cambridge, MA, USA.

Epithelial sheets are barriers that subdivide the body into physiologically distinct compartments. To achieve this, epithelial cells acquire apical-basal polarity. Polarity regulators associated with the apical membrane include atypical protein kinase C (aPKC), Cdc42, and the transmembrane protein Crumbs. Moreover, the vesicle trafficking machinery contributes to epithelial polarization by facilitating the transport of proteins and lipids to apical and basolateral domains. The mechanisms by which polarity proteins interact with the trafficking machinery remain unclear. Our previous work showed that the regulation of Crumbs endocytosis or recycling by Cdc42 and its downstream effector aPKC is important for epithelial integrity This led us to hypothesize that aPKC has phosphorylation targets in the vesicle trafficking machinery.

Bioinformatic analysis suggests that aPKC may have several phosphorylation targets in the trafficking machinery, including the retromer component Vps26. The retromer retrieves proteins from endosomes and transports them back to the Golgi or to the plasma membrane. Our data suggest that aPKC, when activated by Cdc42, phosphorylates Vps26, and as a result increases the activity of the retromer complex. We generated Vps26 mutations and found that *Vps26* maternal/zygotic mutants show loss of epithelial integrity and defects that resemble those observed in *crumbs* or other polarity mutants. Normal, phosphomimic and non-phosphorylatable isoforms of Vps26 form a complex with aPKC *in vivo*, and, expression of phosphomimic Vps26 suppresses the Cdc42 loss-of-function phenotype. Furthermore, Cdc42 or aPKC compromised embryos show loss of Crumbs from the plasma membrane, and its accumulation in Vps26 positive endosomes. These findings suggest that the retromer is recruited to endosomes in order to recycle Crumbs but is unable to do so in the absence of aPKC phosphorylation of Vps26. We propose therefore that aPKC phosphorylation of the retromer is essential mechanism for maintaining epithelial polarity.

D1016A Investigating the Role of Rab Proteins in Drosophila Photoreceptor Apical Vesicle Trafficking. *A. Laffafian*, U. Tepass. University of Toronto, Toronto, Canada.

Protein trafficking is an essential cellular process and required, for example, for cell signaling and membrane polarization. Problems in protein trafficking have been linked to many human diseases such as cancer and Alzheimer's disease. We investigate the mechanisms of vesicle trafficking of apical proteins in Drosophila photoreceptor cells (PRCs). Individual ommatidia in the Drosophila compound eye house 8 PRCs that surround a matrix filled lumen. Our goal is to investigate the role of Rab proteins in vesicle trafficking of apical proteins including the photopigment Rhodopsin (Rh), which localizes to the rhabdomere, the apical determinant Crumbs (Crb), which is enriched in the stalk membrane, and the proteoglycan Eyes shut (Eys), which is secreted into the interrhabdomeral space. Rab proteins are universal regulators of vesicle transport and more than 30 Rab proteins are found in Drosophila. Rab11, for example, is important in the delivery of rhabdomere bound proteins like Rh (Satoh et al., 2005, Development. 132: 1487-1497). We have examined the localization of Eys, Crb, and Rh, in animals with compromised Rab proteins using available RNAi and dominant negative (DN) lines. One example is expression of RabX5-DN, which leads to the cytoplasmic accumulation of Rh. We are currently in the process of determining the subcellular localization and producing CRISPR mutants for select Rabs that show interesting trafficking defects.

D1017B Cell chirality is a novel and evolutionarily conserved cell polarity that drives left-right asymmetric morphogenesis. *K. Matsuno*, A. Okubo, S. Utsunomiya, M. Inaki, T. Sasamura. Osaka University, Toyonaka, Osaka, JP.

Most macromolecules found in cells are chiral (an object is chiral if it is distinguishable from its mirror image). However, chirality of cells has not been noticed until very recently. In our attempt to understand the mechanisms of left-right (LR) asymmetric development in *Drosophila*, we revealed that cells have intrinsic chirality in their structure.

Mechanisms of LR asymmetric development are evolutionarily diverged and remain elusive in invertebrates. *Drosophila* has various organs, such as the gut, testes, and male genitalia, which show stereotypic LR asymmetry. Our research focused on the LR asymmetric development of the embryonic gut that is first organ to show LR asymmetry during *Drosophila* development. The embryonic hindgut rotates counterclockwise 90 degree during its LR asymmetric morphogenesis. The active force driving this rotation originates from hindgut epithelium without any contribution of cell proliferation or apoptosis. Our three dimensional analysis of hindgut epithelium structure revealed that epithelial cells show chirality in their shape, which is the first demonstration of cell chirality *in vivo*. Our computer simulation analysis suggested that cell chirality drives the LR asymmetric rotation of the hindgut, so demonstrating the biological function of cell chirality for the first time. Similar cell chirality was found in other fly organs showing LR asymmetry, and its contributions to the LR asymmetric morphogenesis of these organs were similarly indicated.

To identify genes involved in LR asymmetric development, we have conducted genetic screens covering most of fly genome. We identified *Myosin31DF* (*Myo31DF*), encoding *Drosophila* Myosin ID, as mutants in which LR asymmetry becomes sinistral (mirror image of wild-type counterpart). These results suggest that the default state of handedness is sinistral in *Drosophila*, and the default handedness is reversed by Myo31DF in wild type. Interestingly, cell chirality also becomes mirror image in these mutants. Our mosaic analyses involving *Myo31DF*

mutant revealed that cell chirality is intrinsically formed in individual cells.

Recently, cell chirality was reported in various vertebrate cultured cells, and these analyses suggested evolutionarily conservation of cell chirality. However, biological roles of cell chirality remain unknown in vertebrates. Thus, the *Drosophila* system may provide important insights into the functions of cell chirality and the genetic mechanisms of its formation.

D1018C Tension-dependent junction remodeling repositions polarity proteins and coordinates EMT progression. *M. Weng*[1,2], E. Wieschaus[1,2]. 1) Princeton University, Princeton, NJ; 2) The Howard Hughes Medical Institute.

Although Snail (Sna) is the driving factor of epithelial-mesenchymal-transition (EMT), loss of adherens junctions and polarity in *Drosophila* gastrula is delayed until mesoderm is internalized, despite the early expression of Sna in that primordium. By combining live imaging and quantitative image analysis, we show that contractile myosin on the apical surface of the epithelia that triggers the internalization of the tissue, also strengthens and repositions the adherens junctions, which in turn repositions and delays the complete loss of the polarity proteins until the completion of tissue morphogenesis. We found that before the initiation of mesoderm internalization, expression of Sna in mesodermal epithelia leads to a series of changes that resembles partial EMT: decreased localization of polarity proteins such as Bazooka (Baz) and aPKC, downregulation of cortical actin, and disassembly of adherens junctions. Similar phenotypes can be induced by ectopic expression of Sna in ectoderm. Such Snail-dependent junction loss appears to be mediated by loss of Baz, as Baz knockdown leads to a similar loss of adherens junctions whereas Baz localization maintains in the absence of junctions. The decrease in junction level however is reversed upon the activation of apical myosin despite the continued Sna expression. Adherens junctions then are shifted from subapical position to apical position and strengthened in a myosin-dependent manner. Although Baz is not recovered as much as the junctions and results in decreased ratio between Baz and junctions, its level is maintained until tissue internalization. By tracking individual junction and Baz clusters, we found that as the junction clusters shift apically, Baz is often left behind to diminish but is quickly restored in the newly positioned junction clusters. This junction-directed polarity redistribution can override the polarity protein redistribution mechanism that drives basal junction shift during dorsal folds formation, as ectopic activation of myosin in those cells can induce apical repositioning of junctions and Baz and abolishes the dorsal folds. In summary, to prepare timely EMT of mesodermal tissue early expression of Snail induces partial EMT meanwhile to ensure proper folding of the tissue preceding EMT, the contractile actomyosin, the same machinery that drives the morphogenic event, is used to pause the partial EMT by reorganizing adherens junctions followed by the redistribution of polarity proteins.

D1019A Differential Subcellular Trafficking of Membrane Proteins in Secondary Cells of the *Drosophila* male Accessory Glands. *Felix Castellanos*, Benjamin Kroeger, Shih-Jung Fan, Mark Wainwright, Siamak Redhai, Carina Gandy, Clive Wilson. University of Oxford, Oxford, GB.

The male accessory glands of *D. melanogaster* are paired structures whose secretory products constitute an essential fraction of the seminal fluid and play a critical role in reproduction. Two morphologically different populations of secretory cells form a monolayered epithelium in the accessory glands. The less abundant (~5%) and larger spherical cells, collectively known as secondary cells (SCs), localise at the distal ends of each lobe.

A number of different subcellular compartments can be identified in SCs by assessing acidity and other biochemical properties such as the differential Rab GTPase localisation. For example, Rab7 localises to the surface of large acidic late endosomes and lysosomes, while Rab11 localises to the limiting membrane of non-acidic secretory vacuoles that contain dense-core granules (DCGs).

We are currently investigating how secretory compartments and different populations of intraluminal vesicles (ILVs) are formed in adult SCs. Many cells secrete vesicles that are thought to be formed in the late endosomal multivesicular body (MVB) and are ultimately released by compartment fusion to the cell surface. These so-called exosomes can fuse with target cells, transferring a broad range of biomolecules.

Using state-of-the art imaging and pulse-chase analysis of membrane proteins tagged with fluorescent markers, we have characterised the selective trafficking routes taken by different fusion proteins to reach their final subcellular destinations. These proteins localise to the plasma membrane, in addition to non-acidic vacuolar limiting membranes, their DCGs and ILVs. Using a fusion protein that traffics to DCGs, we are currently testing several molecules for putative roles in DCG biogenesis (e.g. lipid modifying enzymes, as well as the ESCRT genes). Based on these experiments, we have started to develop a mechanistic model to explain cargo trafficking to DCGs, which unexpectedly implicates ILVs. Our results contrast with other data using a previously characterised human CD63-GFP exosome marker, which traffics to other compartments and is loaded on to a different subset of ILVs.

This work sheds new light on the mechanisms regulating DCG compartment biogenesis and the different trafficking routes taken by cargos to reach these compartments. Ultimately, it may inform our understanding of DCG regulation in processes controlling homeostasis, such as glucose-dependent insulin secretion by pancreatic beta cells.

D1020B RPTPs mediate the temporal control of *Drosophila* airway maturation through an interaction with the non-receptor tyrosine kinase Btk29A and its effector WASH. *V. Tsarouhas*[1], D. Liu[1], G. Tsikala[1], K. Zinn[2], C. Samakovlis[1]. 1) Stockholm University, Stockholm, Sweden; 2) California Institute of Technology, Pasadena, CA, USA.

The respiratory tubes of mammalian lungs and the *Drosophila* tracheal system undergo a series of maturation events at the end of embryogenesis. An important transition during *Drosophila* airway maturation is the clearance of the solid luminal material before the replacement of luminal liquid by gas. We had shown earlier that luminal protein clearance depends on a wave of apical endocytosis but its regulation is largely unknown. Here we show that *Drosophila* type III receptor tyrosine phosphatases (RPTPs), Ptp4E and Ptp10D restrict tube growth and apical endocytosis during embryonic airway maturation. Live imaging of mutant embryos lacking both RPTP genes revealed an earlier clearance of GFP-tagged luminal proteins compared to wild type embryos. Genetic alterations in endocytosis by the generation of

ptp4E10D and *rab5* or *vps45* triple mutants could ameliorate luminal clearance phenotypes suggesting elevated endocytic levels in *ptp4E10D* mutants. Quantifications of the intercellular vesicles through an established endocytic Dextran assay in live embryos revealed a direct role of RPTPs in the temporal control of luminal endocytosis. Endo-lysosomal analysis showed an enhanced endocytic trafficking oriented towards the degradation pathway in *ptp4E10D* mutants. Following a short scale genetic screen we identified the non-receptor tyrosine kinase Btk29A as a downstream target of RPTPs with *ptp4E10D;btk29A* triple mutants showing rescue in tube size and premature luminal clearance. Immuno-precipitation experiments from embryo extracts confirmed a physical interaction between Ptp10D and Btk29A and revealed binding of Btk29A to actin nucleator protein WASH. Single mutant analysis showed that both Btk29A and WASH proteins are required for the endocytic clearance of luminal material. Interestingly, loss of *wash* in *ptp4E10D* mutants suppressed the tube size defects suggesting an antagonistic role of RPTPs in Btk29A/WASH pathway. In summary, we show that RPTPs and Btk29A have antagonistic functions on WASH mediated actin polymerization to control the timing of luminal protein clearance in *Drosophila* airways.

D1021C Fat2 and Lar define a planar signaling system controlling collective cell migration. *Kari Barlan*, Maureen Cetera, Sally Horne-Badovinac. THE UNIVERSITY OF CHICAGO, CHICAGO, IL.

Collective migration of cells in an epithelial sheet underlies tissue remodeling events associated with morphogenesis, wound repair, and the metastatic cascade. Similar to individually migrating cells, each epithelial cell forms actin-based protrusions at its leading edge that form new adhesions to the extracellular matrix (ECM). Each cell also releases cell-ECM adhesions at its rear to allow the trailing edge to retract and cell body to advance. These migratory behaviors must be coordinated with outstanding precision to allow sheet migration to occur. Each cell's protrusions extend beneath the trailing edges of the cells in front, similar to overlapping shingles on a roof. Thus trailing edge retraction in the leading cell must be coordinated with protrusion formation in the trailing cell so that the two cells don't compete to adhere to the same region of ECM. Studying the migration of the follicular epithelium, we find that the cadherin Fat2 and the receptor phosphatase Lar form the core of a planar signaling system that coordinates leading edge protrusion and trailing edge retraction between neighboring cells. Fat2/Lar signaling is similar to the Frizzled/VanGogh and Fat/Dachsous planar cell polarity (PCP) signaling that is used to organize many epithelial tissues, in that Fat2 and Lar localize to opposite sides of each cell and mediate communication across cell-cell boundaries. However, whereas the PCP systems operate near the apical surface to transmit long-range information across the tissue, the Fat2/Lar system operates near the basal surface to transmit short-range information between adjacent cells. Previously, Fat2 was shown to localize to the trailing edge of each migrating cell. Our analysis in mosaic epithelia reveals that Fat2 signals from the trailing edge to non-cell-autonomously induce leading edge protrusions in the cell directly behind. Conversely, we find that Lar localizes to the leading edge, and is cell-autonomously required for protrusions. We also observe a trailing edge retraction defect in both *Lar* and *fat2* mosaics. In this process, however, Fat2 plays a cell-autonomous role, while Lar plays a non-cell-autonomous signaling role. Further, we provide evidence suggesting that Fat2 and Lar participate in a protein complex that spans cell-cell boundaries. Together our data suggest that bidirectional signaling between Fat2 and Lar across cell-cell interfaces coordinates leading and trailing edge behaviors between neighboring cells to promote collective cell migration.

D1022A Analysis of chiral cellular dynamics in left-right asymmetric rotation of *Drosophila* hindgut. *M. Inaki*[1], H. Honda[2], K. Matsuno[1]. 1) Osaka Univ., Toyonaka, Osaka, JP; 2) Kobe Univ., Kobe, Hyogo, JP.

Although most of organisms appear bilaterally symmetric in their body shapes, many of their internal organs show left-right (LR) asymmetry. To understand mechanisms underlying LR asymmetry formation of internal organs, we are using *Drosophila* embryonic hindgut as a model system. The *Drosophila* hindgut is first formed as a bilaterally symmetric structure consisting of columnar epithelial cells, rotates 90 degree in counter clockwise direction, and eventually exhibits an LR asymmetric morphology. It has been shown that before rotation, hindgut cells have chirality with LR asymmetric apical surfaces and that the cell chirality is involved in the LR asymmetric rotation of the hindgut. However, it is unclear how cellular chirality contributes to whole organ rotation. The cellular dynamics during rotation is also unknown since previous studies were based on observations of fixed hindgut samples. In this study, we tried to clarify cellular dynamics during LR asymmetric rotation and relationships between the dynamics and the cell chirality. First, we performed computer simulations of hindgut epithelium using a vertex model. Based on predictions from the simulations, we carried out live-imaging of hindgut epithelial cells during rotation. As results, we found that hindgut cells change their relative positions to their subjacent cells, as sliding leftwards (in rotation direction) against subjacent cells. In *Myosin31DF* mutants, in which the cell chirality and the direction of rotation are inverted, cells slide in opposite direction. These results suggested that "cell-sliding" contributes to the LR asymmetric rotation of the hindgut and the cell chirality is reflected by the direction of the cell-sliding. In this study, we found a novel cellular dynamics, chiral cell-sliding, which drives LR asymmetric morphogenesis.

D1023B Out-of-Step is a novel serine/threonine kinase that directs myotube pathfinding. *Aaron N. Johnson*, Brenna Clay. Univ Colorado Denver, Denver, CO.

Skeletal muscles attach to the skeleton via tendons, and skeletal movement requires a tight physical interaction between skeletal muscles and tendon cells. Myotubes are the skeletal muscle precursors, and nascent myotubes are guided to tendon cells through largely unknown mechanisms. In a genetic screen for regulators of embryonic body wall muscle development, we identified the Out-of-step (*ofs*) phenotype in which nascent myotubes failed to extend toward tendon cells in a synchronized manner. The *ofs* allele mapped to a previously uncharacterized serine/threonine kinase that is orthologous to mammalian Vaccinia Related Kinase (VRK), and neither VRK nor Ofs have been studied in the context of myogenesis. Using LifeAct.GFP, we found that *ofs* myotubes failed to extend filopodia during pathfinding. The leading edges of *ofs* myotubes initiate outgrowth but are unable to reach and attach to tendon cells. By RNA-seq, we found that Notch target genes in the Enhancer of Split Complex [E(Spl)-C] are enriched in *ofs* embryos. We are testing the hypothesis that contact dependent Notch signals inhibit

myotube outgrowth, and that Ofs modulates the intracellular response to Notch. This study is the first to identify a protein kinase that directs myotube pathfinding, and will provide essential insights into the mechanisms by which filopodia probe the extracellular environment during cell migration.

D1024C Twinstar/cofilin is required for regulation of epithelial integrity and tissue growth in *Drosophila*. *C. Ko*, Y. G. Kim, T. P. Le, K. W. Choi. KAIST, Yuseong-gu, Daejeon, KR.

Regulation of actin assembly and depolymerization is important for the organization of epithelia. Recent studies have shown that the actin-capping proteins are required to prevent cell extrusion and inappropriate activation of Yorkie (Yki) activity in *Drosophila*, implicating the importance of actin regulation for epithelial integrity and Yki-dependent tissue growth. However, the role of Twinstar (Tsr), the *Drosophila* homolog for cofilin/actin depolymerization factor (ADF), in epithelial integrity and Hippo signaling is unknown. Both capping proteins and Tsr are crucial components of actin depolymerization process. Therefore, like capping proteins, Tsr may participate in epithelial integrity and tissue growth. Hence we investigated the consequence of Tsr reduction on epithelial integrity and development in wing imaginal disc, using GAL4-UAS and FRT-FLP methods. We show that reduction of Tsr in wing imaginal disc induces not only F-actin accumulation but also ectopic expression of Wingless (Wg) and Yki target gene expanded (ex). Knockdown of Yki in Tsr-depleted cells reduced the level of ectopic Wg expression. Reduced Tsr also led to downregulation of cell junction proteins and extrusion of affected cells from the basal part of the epithelium. Rho GTPase 1, a known actin modifier protein, was upregulated in Tsr-depleted tissue, supporting the Tsr function in the inhibition of cell extrusion from the epithelium. Tsr is also required for blocking cell death and c-Jun N-terminal kinase (JNK) signaling, as loss of Tsr induced both apoptotic features and upregulation of JNK downstream target genes. Ectopic JNK activation induced caspase activation but did not cause cell extrusion in this case, hence JNK activation is not sufficient condition for invasive cell movement. Further, the invasiveness of Tsr-depleted cells was not suppressed by inhibition of cell death or JNK signaling, and it was enhanced in Tsr knockdown cells with cell death inhibitor P35, indicating a critical role of Tsr in cell migration control. In contrast, Yki upregulation was significantly suppressed by cell death inhibition. Taken together, our data suggest that Tsr is required for cell survival and tissue growth by regulating JNK and Yki signaling while maintaining the epithelial integrity by controlling cell junctions. This study provides an insight into potential roles of ADF/cofilin in invasive cell migration and tumor suppression in higher animals.

D1025A Inhibition of Protein Phosphatase 1 activity switches border cells from a collective to single cell mode of migration. *J. A. McDonald*[1], D. Ramel[2], G. Aranjuez[3], A. Burtscher[3], K. Sawant[1], X. Wang[2]. 1) Division of Biology, Kansas State University, Manhattan, KS; 2) LBCMCP, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, France; 3) Lerner Research Institute, Cleveland Clinic, Cleveland, OH.

Cells often migrate in distinctly organized groups, or 'collectives', to form, remodel and sculpt tissues and organs. The mechanisms underlying how cells in collectives stay together and coordinate their movement rather than migrate as individual cells are still poorly understood. *Drosophila* border cells undergo collective cell migration during oogenesis. Border cells travel as a cohesive cluster of 6-10 cells between the large nurse cells to reach the oocyte. While several serine-threonine kinases and their target proteins have known roles in border cell migration, the functions of serine-threonine phosphatases are unknown. Here we show that Protein Phosphatase 1 (PP1) maintains the collective cohesion and migration of border cells. Inhibition of PP1 activity, either through the endogenous inhibitor NiPP1 or by knockdown of multiple PP1 catalytic subunits, causes border cells to round up and completely dissociate from the cluster while they are migrating. These individual border cells have altered motility, with protrusion formation between cells, and slower movement. Rac activity is still enriched in the leading border cell, showing that guidance signaling and directionality are not affected. However, levels of E-cadherin between cells are strongly reduced. Moreover, myosin localization is altered and F-actin becomes enriched around individual border cells rather than at the periphery of the entire border cell cluster. Together, these cellular alterations contribute to the inability of PP1-inhibited border cells to adhere tightly to each other and move as a group. Thus, PP1 activity promotes a collective rather than individual cell migration mode.

D1026B The vesicle fusion regulator α-SNAP is required for STAT pathway activation and induction of cell motility. *A. Saadin*, M. Starz-Gaiano. University of Maryland Baltimore County, Baltimore, MD.

Cell migration is a fundamental and precisely regulated event during animal development, immune response and wound healing. It can also lead to detrimental outcomes such as tumor metastasis and atherosclerosis. Hence, unraveling the detailed mechanism of this process is crucial to advance our understanding of development and to improve therapeutics. A cluster of cells termed the border cells in Drosophila egg chambers provides a well-founded and genetically tractable system to study cell migration *in vivo*. The border cell cluster, originating from the follicular epithelium, is composed of four to six migratory border cells and a pair of non-motile polar cells. Polar cells induce the specification of border cells by secreting the Unpaired (Upd) ligand, which activates the well-conserved Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway in the neighboring follicle cells. Border cells then wrap around polar cells and migrate between nurse cells toward the developing oocyte. In an RNAi screen to identify novel regulators of cell migration, we uncovered a requirement for *α-Soluble NSF Attachment Protein* (*α-SNAP*) in border cell cluster specification. α-SNAP is known to function in synaptic transmission, membrane fusion, and vesicle trafficking by facilitating association of N-ethylmalemide-Sensitive Factor (NSF) and SNAP Receptor (SNARE) during vesicle fusion. RNAi-mediated depletion of *α-SNAP* in the anterior follicle cells, including the polar cells, results in egg chambers that lack both polar

and border cells. Mosaic mutant analysis for *α-SNAP* supports these results, and over-expression of *α-SNAP* rescues the knockdown phenotype, confirming the specificity of the RNAi reagents. Immunofluorescent labeling reveals the localization of α-SNAP at the periphery of both the polar and border cells. Over-expression of viral anti-apoptotic gene, *p35*, in the *α-SNAP* depleted egg chamber verifies that the lack of a border cell cluster is due to impaired signaling, not apoptosis. Through suppression/enhancement assays with various regulators of the STAT signaling pathway and *α-SNAP* RNAi, we found that *α-SNAP* functions upstream of *upd* in the polar cells to regulate the JAK/STAT signaling pathway during border cell cluster formation. Among several SNAREs we examined, only *syntaxin 1A* recapitulates the lack of border cell cluster phenotype when depleted in the anterior follicle cells, indicating that it likely functions with *α-SNAP* during border cell cluster formation. Therefore our study identified a specific new role for *α-SNAP* in STAT-mediated motile cell specification.

D1027C *Drosophila* Fondue is a critical extracellular matrix organizer during muscle attachment and coagulation. *Nicole M. Green*[1], Nadia Odell[2], Cheryl Clark[1], Mitch Dushay[3], Erika R. Geisbrecht[1]. 1) Kansas State University, Manhattan, KS; 2) University of Missouri-Kansas City, Kansas City, MO; 3) Cornell University, Ithaca, NY.

The orchestration of cells to form and maintain larger structures is a complex and dynamic process reiterated throughout organismal development. Connective extracellular matrix (ECM) provides structural integrity and flexibility to allow for stresses associated with movement and mechanical force transmission in epithelial and muscle tissues. In *Drosophila*, perturbation of ECM results in weakened or malformed muscle attachment sites (MASs) that detach, making the MAS an excellent model for studying this phenomenon. We have identified a new role for a secreted hemolymph protein called Fondue (Fon) in *Drosophila* muscle attachment. Previous studies of Fon focused on its role in clot stability and innate immune response. However, early pupal lethality and abnormal pupal morphology in unchallenged individuals indicated that Fon had a developmental role. Transgenic flies containing a tagged Fon construct revealed that Fon-GFP accumulates at both indirect and direct attachment sites in L3 larvae. Both *fon* mutants and *fon* RNAi fillets contained body wall muscles that detach with large gaps between subsets of muscles across hemisegments. Using TEM to analyze MAS ultrastructure, we found that *fon* mutants had disrupted cuticle and tendon architectures, a lack of muscle-tendon interdigitation, and a loss of electron-dense matrix accumulation. Together, the TEM data and abnormal muscle morphologies observed in L3 fillets indicate that Fon is a critical regulator of ECM organization and overall MAS integrity. In a *fon* mutant sensitized background, we identified both *tig* and *tsp* as candidate interactors of Fon at the MAS. RNAi knockdown of either transcript increased detachment phenotypes compared to *fon* heterozygotes or candidate RNAi fillets alone. Tig has also been identified as a component of the hemolymph clot. We were able to identify one other secreted hemolymph protein, Lsp1γ, that results in muscle detachment upon RNAi knockdown. Using an Lsp1γ-GFP construct, we observed Lsp1γ-GFP localizing in a tendon-associated pattern at the MAS. From this data we can conclude that the analogous structures of the hemolymph clot and the muscle attachment site require a unique and overlapping set of secreted proteins in *Drosophila melanogaster*, identifying a connection between innate immune and muscle development processes.

D1028A implication of the basement membrane and adhesion complexes during leg morphogenesis in drosophila. *A. ARNAUD*, B. Monier, T. Mangeat, M. Suzanne. LBCMCP, TOULOUSE, FR.

Mechanical signals are key players in morphogenetic events.

Indeed mechanical signaling has been implicated in numerous cellular processes, such as migration or differentiation and number of studies, over the past years, had also pointed out the role of forces during morphogenesis.

Recently the team unveiled the implication of mechanical forces as initiators of fold formation, in the morphogenesis of the distal part of the drosophila leg. Furthermore they have shown that, fold -inducing forces are generated initially by apoptotic cells. Following this exiting discovery, the team now wants to focus on the basal consequences of fold formation. Indeed, ours model allow clear observation of the apical and the basal side of epithelial cells.

This offers the possibilities to study the role of basal attachment and basement membrane in the fold formation process which is particularly interesting since until now the folding process has been mainly described from an apical view.

Using confocal imaging on live and fixed tissue with fusion proteins, we manage to follow the dynamic of the basal side of the leg epithelium (ECM and adhesions). We were also able to access the role of these basal side components during leg morphogenesis thanks to the genetic tools available in drosophila.

Our results shown that the basal components of the leg disc epithelium are highly dynamic and that they are required during the fold formation process.

The aim of my work is to get one step further in the comprehension of mechanical forces and their influence during morphogenetic events such as fold formation and also get a “new” basal side view of this phenomenon.

D1029B Investigating the regulation of the imaginal disc epithelial barrier during regeneration. *Danielle DaCrema*, Ryunosuke Yano, Rajan Bhandari, Adrian Halme. University of Virginia, Charlottesville, VA.

The epithelial barrier, formed by septate junctions in invertebrates, compartmentalizes the body and provides a barrier to pathogens. To sustain distinct compartment environments, the epithelial barrier must be stably maintained. Therefore, we are interested in how epithelia regulate barrier function during tissue regeneration. To address this, we have been examining the dynamics of the epithelial barrier during regeneration in the *Drosophila* wing imaginal disc. Following X-irradiation, we observed a temporary depletion and/or mislocalization of septate junction components in wing discs. Normal localization of these septate junction components is recovered prior to pupation. To determine if the epithelial barrier is also functionally impaired following damage, we examined whether the imaginal disc epithelia of irradiated discs is permeable to a fluorescently-labeled dextran. We observed infiltration of labeled dextran into the lumen of irradiated discs, but not

control discs, indicating that the epithelial barrier is compromised following damage. To determine if this disruption of barrier function in irradiated discs is a physical response to apoptotic damage or a regulated process during regeneration, we irradiated larvae late in larval development after a point when they can no longer initiate regenerative repair on imaginal discs, and examined the localization of septate junction components. Following late irradiation, we observed that septate junction components of imaginal discs are not disrupted or mislocalized; suggesting that septate junction disruption following damage is a regulated process, and dependent on the regenerative activity of the imaginal disc.

We also observed that septate junction disruption causes a synergistic increase in *Drosophila* insulin-like peptide 8 (*dilp8*)-mediated developmental delay. This suggests that the epithelial barrier limits *dilp8* signaling. Dilp8 is upregulated following damage and accumulates in the imaginal disc lumen following expression, but acts on the brain and ring-gland. Therefore, we hypothesize that damage-induced Dilp8 is produced and apically secreted into the lumen of wing imaginal discs, then leaks through disrupted septate junctions to enter the hemolymph and travel to the brain and ring-gland where it signals to regulate developmental timing and growth. It is possible that the subsequent recovery of the epithelial barrier following damage serves as a method to covey the time course of regeneration to the brain and other organs by controlling the release of Dilp8.

D1030C Assembly of septate junctions and midbody displacement during epithelial cytokinesis. *Z. Wang*[1,2], F. Bosveld[1], Y. Bellaiche[1]. 1) Institut Curie, Paris, FR; 2) Sorbonne Universités, UPMC, Paris, FR.

Epithelial cell cytokinesis is coupled to the formation of a new septate junction between the daughter cells, thereby preserving the integrity of the permeability barrier function during proliferation. Here, using live confocal imaging, we studied the formation of bicellular and tricelluar septate junctions during cytokinesis in the notum of Drosophila pupa. We found that the septate junctions are maintained during cytokinesis, and the assembly of the new septate junctions relies on neighboring epithelial cells ingressing in between the two daughter cells, binding and following the movement of the midbody. The detachment of the neighboring cells from the midbody coincides with the maturation of the bicellular and tricellular septate junctions. Our studies reveal how epithelial cells establish new septate junctions during cytokinesis while maintaining septate junctional seals.

D1031A Polo kinase mediates the phosphorylation and cellular localization of Nuf/FIP3, a Rab11 effector. *L. Brose*[1], J. Crest[1,2], L. Tao[1,3], W. Sullivan[1]. 1) University of California, Santa Cruz, Santa Cruz, CA; 2) University of California, Berkeley, Berkeley, CA; 3) University of Hawai'i at Hilo, Hilo, HI.

To understand the mechanisms underlying the cell cycle regulation of vesicle-mediated membrane addition to the cytokinesis furrow, this study follows the localization and regulation of Nuf, a Rab11 effector, in the early *Drosophila* embryo. Nuf, and its mammalian homolog FIP3, is required for activating recycling endosome-based membrane delivery to the cytokinesis furrow. We used molecular genetic, biochemical, cell biological, and fluorescent confocal imaging techniques to determine that Nuf/FIP3 is a phosphoprotein whose pericentrosomal localization is regulated throughout the cell cycle. We show that Polo kinase directly phosphorylates two residues of Nuf, Ser225 and Thr227, and that phosphorylation of these sites correlates with Nuf being driven off the centrosome and into the cytoplasm during prophase, after furrow formation is complete. Further, reduced *polo* expression results in prolonged maintenance of Nuf at the centrosome. Conversely, increased *polo* expression limits Nuf association at the centrosome during interphase.

D1032B A Genetic Dissection of the *Drosophila* Larval Salivary Gland: A Model for Exocrine Gland Physiology. *K. M. Lantz*, M. Al-Karawi, V. Zhitny, A. J. Andres. University of Nevada, Las Vegas, LAS VEGAS, NV.

Exocrine glands are important secretory organs that store specialized cargoes in a limited luminal space until they are released later to the exterior of the animal. The bilobed salivary gland of the *Drosophila* larva is an excellent model for exocrine tissue physiology because each lobe secretes a mix of highly glycosylated glue proteins into a lumen that is connected to a centralized duct. The glue will later be expelled from the animal at puparium formation so that the prepupa can cement itself to a solid surface above the food as it completes it metamorphic stages of development. The stimulus for secretion is a high titer of the insect steroid molting hormone, 20 hydroxyecdysone (20E). Thus, the hormone sets in motion multiple molecular pathways that connect steroid signaling to calcium signaling and cargo activation. This results in a polarized coordinated movement and exocytosis of glue granules at the apical membrane as the tissue undergoes dramatic changes in cell shape. While it is understood that 20E is the cause of cargo exocytosis, little is known about these processes at the molecular level. Using live imaging techniques on cultured dissected tissues compromised for specific genes in the salivary gland, we have begun to identify key components that are 20E regulated. As expected, these proteins are needed to change intracellular Ca^{2+} levels and reorganize the cytoskeleton for long- and short-range movement of glue granules during this important aspect of exocrine physiology.

D1033C Fascin regulates nuclear actin during *Drosophila* oogenesis. *D. J. Kelpsch*[1], C. M. Groen[1,2], T. N. Fagan[1], S. Sudhir[1], T. L. Tootle[1]. 1) University of Iowa, Iowa City, IA; 2) Mayo Clinic, Rochester, MN.

Drosophila oogenesis provides a developmental system to study nuclear actin. During Stages 5-9, nuclear actin levels are high in the oocyte and exhibit dynamic variation within the nurse cells. Cofilin and Profilin, which regulate the nuclear import and export of actin, also localize to the nuclei. Expression of GFP-tagged Actin results in nuclear actin filaments. These findings indicate that nuclear actin must be tightly regulated during oogenesis. One factor mediating this regulation is Fascin. Overexpression of Fascin enhances nuclear GFP-Actin filament formation, and Fascin colocalizes with the filaments. Loss of Fascin reduces, while overexpression of Fascin increases, the frequency of nurse cells with high levels of nuclear actin, but neither alters the overall nuclear level of actin. These data suggest that Fascin regulates nuclear actin dynamics,

allowing specific cells to accumulate nuclear actin. Evidence indicates Fascin positively regulates nuclear actin through Cofilin. Loss of Fascin results in decreased nuclear Cofilin. Additionally, Fascin and Cofilin genetically interact, as double heterozygotes exhibit a reduction in the number of nurse cells with high nuclear actin levels. These findings are likely applicable beyond *Drosophila* follicle development, as the localization and functions of Fascin, and the mechanisms regulating nuclear actin, are widely conserved.

D1034A Hedgehog promotes the production and release of PI(4)P to interact and activate Smoothened. *K. Jiang*, Y. Liu, J. Jia. Univ of Kentucky, Lexington, KY.

The Hedgehog (Hh) signaling pathway plays important roles in both embryonic development and adult tissue regeneration and homeostasis. Inappropriate activation of the Hh pathway has been linked to malignancies such as basal cell carcinoma and medulloblastoma. In *Drosophila*, the Hh signal is transduced through a receptor system at the plasma membrane, which includes the receptor complex Patched-Interference Hh (Ptc-Ihog) and the signal transducer Smo. Binding of Hh to Ptc-Ihog relieves the Ptc-mediated inhibition of Smo, which allows Smo to activate the cubitus interruptus (Ci)/Gli family of zinc finger transcription factors and thereby induce the expression of Hh target genes such as *decapentaplegic* (*dpp*), *patched* (*ptc*), *collier* (*col*) and *engrailed* (*en*). Though Hh signal transduction has been widely studied, it is still unclear how Ptc inhibits Smo to block the activation of the Hh pathway and how Ptc inhibition of Smo is relieved by Hh stimulation. We report here that Hh elevates the production of phosphatidylinositol 4-phosphate (PI(4)P), a specific phospholipid that directly interacts with Smo through an arginine motif in the Smo C-terminal tail and promotes Smo phosphorylation, activation, and ciliary localization. Ptc also interacts with PI(4)P, which is inhibited by Hh stimulation, indicating that Hh triggers the release of PI(4)P from Ptc. We further uncover that Hh induces the production of PI(4)P, likely by regulating PI(4)P kinase and phosphatase. Finally, in addition to the direct role in regulating Smo phosphorylation, G protein-coupled receptor kinase 2 (Gprk2) facilitates PI(4)P interaction with Smo. This study suggest that PI(4)P acts as a special small molecule shuttling between Ptc and Smo to modulate Hh responses.

D1035B Regulation of Smo phosphorylation and high-level Hh signaling activity by a plasma membrane associated kinase. *Shuangxi Li*[1,2], Shuang Li[1,2], Yuhong Han[1,2], Chao Tong[2,5], Bing Wang[1,2], Yongbin Chen[2,4], Jin Jiang[1,2,3]. 1) Department of Molecular Biology,University of Texas Southwestern Medical Center at Dallas, Dallas, TX; 2) Department of Developmental Biology,University of Texas Southwestern Medical Center at Dallas, Dallas, TX; 3) Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX; 4) Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China; 5) Life Sciences Institute and Innovation Center for Cell Biology, Zhejiang University, Hangzhou, China.

Hedgehog (Hh) signaling controls embryonic development and adult tissue homeostasis through the GPCR-family protein Smoothened (Smo). Upon stimulation, Smo accumulates on the cell surface in *Drosophila* or primary cilia in vertebrates, which is thought to be essential for its activation and function, but the underlying mechanisms remain poorly understood. Here we show that Hh stimulates the binding of Smo to a plasma membrane-associated kinase Gilgamesh (Gish)/CK1γ, and that Gish promotes high level Hh pathway activity by phosphorylating a Ser/Thr cluster (CL-II) in the juxtamembrane region of Smo carboxyl-terminal intracellular tail (C-tail). We find that CL-II phosphorylation is promoted by PKA-mediated phosphorylation of Smo C-tail and depends on cell surface localization of both Gish and Smo. Consistent with CL-II being critical for high threshold Hh target gene expression, its phosphorylation appears to require higher levels of Hh or longer exposure to the same level of Hh than PKA-site phosphorylation on Smo. Furthermore, we find that vertebrate CK1γ is localized at the primary cilium to promote Smo phosphorylation and Shh pathway activation. Our study reveals a conserved mechanism whereby Hh induces a change in Smo subcellular localization to promote its association with and activation by a plasma membrane localized kinase, and provides new insight into how Hh morphogen progressively activates Smo.

D1036C The PARP enzyme Tankyrase antagonizes activity of the βcatenin destruction complex through ADP-ribosylation of Axin and APC2. *H. An*, C. Powers, J. Tran, H. Croy, C. Fuller, J. Giannotti, P. Robinson, A. Foley, R. Yamulla, S. Cosgriff, B. Greaves, R. von Kleeck, A. Tocker, K. Jacob, B. Davis, D. Roberts. Franklin and Marshall College, Lancaster, PA.

Most colon cancer cases are initiated by truncating mutations in the tumor suppressor, Adenomatous Polyposis Coli (APC). APC is a critical negative regulator of the Wnt signaling pathway that participates in a multi-protein "destruction complex" to target the key effector protein βcatenin for ubiquitin-mediated proteolysis. Prior work has established that the Poly ADP ribose Polymerase (PARP) enzyme Tankyrase (TNKS) antagonizes destruction complex activity by promoting degradation of the scaffold protein Axin, and recent work suggests that TNKS inhibition is a promising cancer therapy. We performed a yeast two-hybrid (Y2H) screen and uncovered TNKS as a putative binding partner of Drosophila APC2, suggesting that TNKS may play multiple roles in destruction complex regulation. We find that TNKS binds a C-terminal RPQPSG motif in Drosophila APC2, and that this motif is conserved in human APC2, but not human APC1. In addition, we find that APC2 can recruit TNKS into the βcatenin destruction complex, placing the APC2/TNKS interaction at the correct intracellular location to regulate βcatenin proteolysis. We further show that TNKS directly PARylates both Drosophila Axin and APC2, but that PARylation does not globally regulate APC2 protein levels as it does for Axin. Moreover, TNKS inhibition in colon cancer cells decreases βcatenin signaling, which we find cannot be explained solely through Axin stabilization. Instead, our findings suggest that TNKS regulates destruction complex activity at the level of both Axin and APC2, providing further mechanistic insight into TNKS inhibition as a potential Wnt pathway cancer therapy.

D1037A Proteomic analysis reveals APC-dependent post-translational modifications and identifies a novel regulator of β-catenin. *M. A. Blundon*, D. R. Schlesinger, A. Parthasarathy, S. L. Smith, H. M. Kolev, D. A. Vinson, E. Kunttas-Tatli, B. M. McCartney, J. S. Minden. Carnegie Mellon University, Pittsburgh, PA.

Wnt signaling generates patterns in all embryos, from flies to humans, and controls cell fate, proliferation, and metabolic homeostasis. Inappropriate Wnt pathway activation results in diseases, including colorectal cancer. The *Adenomatous polyposis coli* (*APC*) tumor suppressor gene encodes a multifunctional protein that is an essential regulator of Wnt signaling and cytoskeletal organization. While progress has been made in defining the role of APC in a normal cellular context, there are still significant gaps in our understanding of APC-dependent cellular function and dysfunction. We expanded the APC-associated protein network using a combination of genetics and a proteomic technique called Two-dimensional Difference Gel Electrophoresis (2D-DIGE). We show that loss of *APC2* causes protein isoform changes reflecting misregulation of post-translational modifications (PTMs), which are not dependent on β-catenin transcriptional activity. Mass spectrometry revealed that proteins involved in metabolic and biosynthetic pathways, protein synthesis and degradation, and cell signaling are affected by the loss of *APC2*. We demonstrate that changes in phosphorylation partially account for the altered PTMs in *APC* mutants, suggesting that *APC* mutants also affect other types of PTM. Finally, through this approach Aminopeptidase P was identified as a new regulator of β-catenin abundance in *Drosophila* embryos. This study provides new perspectives on APC's cellular effects that may lead to a richer understanding of APC's role in development and disease.

D1038B Activation of Sona requires furin cleavage and autocatalysis. *S.-S. Lee*, J.-H. Won, G.-W. Kim, K.-O. Cho. KAIST, DAEJEON, KR.

ADAMTS (a disintegrin and metalloproteases with thrombospondin motif) family consists of secreted proteases, and is shown to cleave extracellular matrix proteins. Their malfunctions result in cancers and disorders in connective tissues. We have previously reported that a *Drosophila* ADAMTS named Sol narae (Sona) promotes Wnt/Wingless (Wg) signaling by modifying the activity and diffusion of Wg. Thus, spatiotemporal regulation of Wg signaling may be linked to the regulation of Sona activity. To become an active Sona, the prodomain of Sona has to be cleaved by furin, a proprotein convertase. To understand which processes are important for Sona activation, we generated mutations in five putative furin cleavage sites, three N-glycosylation sites, and carboxyl terminal region, and then examined which mutations affect the activation of Sona. We found that one of the five furin sites is essential for the activation of Sona, and N-glycosylation sites are not important for activation of Sona but for targeting Sona to the destined place. Interestingly, Sona has an autocatalytic site in the prodomain whose cleavage is prerequisite to the cleavage at the furin cleavage site. In addition, the carboxyl terminal region was also important for Sona processing. Taken together, the generation of active Sona requires sequential processing at the autocatalytic site and the furin site.

D1039C The extracellular protease *AdamTS-B* negatively regulates wing vein patterning through BMP signaling. *Afshan Ismat*[1], Minh Pham[2], Mark Schuweiler[1]. 1) University of St. Thomas, St. Paul, MN; 2) Franklin and Marshall College, Lancaster, PA.

Vein patterning in the Drosophila wing provides a powerful tool to study regulation of BMP signaling. Altering levels of BMP effectors can induce major defects in the cross veins and longitudinal veins of wings. The extracellular protease *AdamTS-B* (*CG4096*) encodes an ADAMTS protease expressed in the embryonic trachea and wing imaginal disc precursor cells. The absence of *AdamTS-B* displayed an extra PCV vein, and a branched or incomplete distal tip of the longitudinal L5 vein. These defects were similar to over-expression of BMP agonist *crossveinless* (*cv*), and down-regulation of the BMP inhibitor *short gastrulation* (*sog*). The defects observed in over-expression of *AdamTS-B* were also similar to those of *sog* up-regulation and *cv* knock-down mutations; PCV were not formed completely. Furthermore, the downstream effector of BMP signaling, pMad, showed altered expression levels consistent with the PCV defects seen in the loss and over-expression of *AdamTS-B*. These findings suggest that *AdamTS-B* might be a BMP antagonist, modifying BMP agonists or receptors to alter BMP signals. Further study needs to be undertaken to fully unravel interactions between *AdamTS-B* and BMP signaling.

D1040A Interplay of BMP and JAK/STAT in Developmentally Related Apoptosis. *A. R. Mascaro*, A. Borensztejn, K. A. Wharton. Brown University, Providence, RI.

In the *Drosophila melanogaster* ovary, somatic follicle cells are critical to multiple aspects of oocyte development. The specification of different sets of follicle cells establish signaling centers that not only impact the formation of the micropyle, vitelline envelope, and specific chorion structures, but also play important roles in initiation of the future A/P and D/V axes. A crucial somatic cell type, the stalk cells, form a linear structure that separates consecutive egg chambers as they are produced and continue to develop through each stage of oogenesis. Without stalk cells, egg chambers, composed of a highly organized arrangement of 16 germline cells surrounded by hundreds of specialized somatic follicle cells, can merge. Such merged or 'fused' egg chambers lack organization required for proper patterning and fail to produce a viable oocyte. Thus, the maintenance of stalk cells is critical for *Drosophila* oogenesis. We found that during normal oogenesis the stalk cells comprising a single stalk, or the connection between two egg chambers, are produced in excess and reduce in number as oogenesis proceeds and they bridge increasingly developed egg chambers. We find that this reduction arises as a result of apoptosis, a process critical in many developmental events requiring defined numbers of cells or involved in sculpting organs. Interestingly, while establishing stalks during normal development involves the eliminating of some stalk cells through apoptosis, mechanisms are clearly in place to prevent excessive loss of these cells. We have found that excessive apoptosis is prevented by JAK/STAT signaling, distinct from its proapototic role in defining exactly two polar cells at the anterior and posterior poles of each egg chamber. In addition to JAK/STAT signaling, we find that BMP signaling is also required to inhibit abnormal loss of stalk cells through apoptosis. If either JAK/STAT signaling or BMP signaling is specifically reduced in the stalk cells, we observe a more significant loss of stalk cells, such that the complete fusion of egg chambers may occur. The converse is true when JAK/STAT signaling or BMP signaling is increased. The reduced numbers of stalk cells observed when knocking down JAK/STAT signaling can be rescued through the overexpression of the BMP 5/6/7 ortholog Gbb. Similarly, the 'long' stalks produced by overactivation of JAK/STAT signaling can be suppressed by downregulation of gbb, supporting an intimate relationship between these two signaling pathways in defining the precise number of stalk cells that make up interfollicular stalks.

D1041B The Notch-mediated hyperplasia circuitry in *Drosophila*. *D. M. Ho*[1], S. K. Pallavi[2], S. Artavanis-Tsakonas[1,3]. 1) Harvard Medical School, Boston, MA; 2) Translational Health Science and Technology Institute, Faridabad, India; 3) Biogen Inc., Cambridge, MA.

Notch signaling controls a wide range of cell fate decisions during development and disease via synergistic interactions with other signaling pathways. We performed a genome-wide genetic screen in *Drosophila* and uncovered a highly complex Notch-dependent genetic circuitry that profoundly affects proliferation and consequently hyperplasia. We further examined two novel synergistic relationships, between Notch and either of the non-receptor tyrosine kinases Src42A and Src64B, and between Notch and the transcription factor Mef2. Both interactions promote hyperplasia and tissue disorganization, largely via activation of the JNK pathway, indicating that there are commonalities within the Notch-dependent proliferation circuitry; however, they differ in how they access and interpret the JNK signal. Most notably, whereas Notch-Src causes high levels of apoptosis, Notch-Mef2 does not display this phenotype, thus resulting in a comparatively higher degree of hyperplasia. We examined differential targets of the two synergies to identify downstream anti- or pro-apoptotic factors in Notch-Mef2 or Notch-Src respectively, and identified the transcription factor disco as a potential suppressor of apoptosis in Notch-Mef2 tissues. These findings underscore the complexity and specificity of the Notch signaling network and have potential implications for Notch-related cancers.

D1042C CG9650: A novel regulator of patterning of the Indirect Flight Muscles of *Drosophila melanogaster*. *S. Jawkar*[1], U. Nongthomba[1]. 1) Indian Institute of Science, Bangalore, Bangalore,560012- INDIA; 2) Indian Institute of Science, Bangalore, Bangalore,560012-INDIA.

Myoblasts housed on the notum region of the wing disc undergo cell-lineage dependant specification, proliferation, migration and differentiation to finally generate the two groups of Indirect Flight Muscles (IFMs), i.e. the Dorsal Longitudinal Muscles (DLMs) and Dorsal Ventral Muscles (DVMs). Defects in any of these highly synchronised processes lead to abnormal patterning of the IFMs. CG9650, a gene predicted to be involved in neural development, was found to be highly expressed in proliferating and migrating myoblasts. Tissue specific knockdown of CG9650 led to a defect in the pattern of the IFMs. The function of CG9650 was found to be important in the founder cell population of the myoblasts. Immunostaining for known regulators of IFM patterning indicated a role of CG9650 in regulating the expression of Notch, and subsequently Twist in proliferating and migrating myoblasts. Knockdown of CG9650 also led to an accumulation of Wingless levels. In this study, we provide evidence implicating CG9650 as an important regulator of Notch expression during early IFM development.

D1043A Functional investigation of a late-onset Alzheimer's disease associated variant in TM2D3. *J. L. Salazar*[1], D. Li-Kroeger[1], H. J. Bellen[1,2,3], J. M. Shulman[1,3], J. Jakobsdottir[4], S. Yamamoto[1,3], CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium. 1) Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Houston, TX; 3) Texas Children's Hospital, Houston, TX; 4) The Icelandic Heart Association, Kopavogur, Iceland.

Alzheimer's disease (AD) is a major cause of dementia that affects more than 44 million people worldwide. Mutations in *APP* and catalytic subunits of the γ-secretase complex (*PSEN1*, *PSEN2*) have been identified as major causes of familial early-onset AD. However, genetic factors that contribute to late-onset AD (LOAD) are mostly unknown. Through an exome-wide genotyping microarray approach, the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium identified a novel LOAD-associated risk variant in the *TM2D3* locus within an Icelandic population. Interestingly in *Drosophila*, mutations in *almondex* (*amx*), the fly homolog of *TM2D3*, cause a maternal effect neurogenic phenotype that phenocopy loss-of-function phenotypes of *Notch* and *Presenillin*. However, multiple bioinformatics programs (PolyPhen, SIFT, CADD) predicted that this LOAD associated variant in *TM2D3* is not damaging, raising the question of whether or not this is a true functional variant.

To determine if the LOAD-associated variant in *TM2D3* is functional, we "humanized" the *amx* gene by rescuing the *Drosophila amx* mutants with a genomic rescue construct that encodes human *TM2D3*. Interestingly, while a genomic rescue construct of *amx* that expresses wild-type human *TM2D3* can significantly suppress the maternal effect neurogenic phenotype, the LOAD-variant failed to do so. These results suggest that the LOAD-associated variant in *TM2D3* acts as a loss-of-function mutation in the context of embryonic Notch signaling. Considering that *amx* has been previously implicated to function at the γ-secretase mediated activation step of Notch, we hypothesize that *TM2D3* plays a role in AD through modulation of the β-amyloid pathway.

D1044B The cell-type specific functions of an ER modulating factor, Pecanex in Notch and Wnt signaling pathways. *T. Yamakawa*, P. Das, A. Yamagishi, N. Liu, K. Matsuno. Osaka Univ, Osaka, JP.

Notch (N) signaling is an evolutionarily conserved mechanism that regulates a broad spectrum of cell-specification through local cell-cell interaction. *Drosophila pecanex* (*pcx*) encodes an evolutionarily conserved multi-pass transmembrane protein that is a component of N signaling. We previously suggested that Pcx localizes to the endoplasmic reticulum (ER) and is required for the maturation of N receptor in there. However, the functions of *pcx* in N signaling appears to be context-dependent. Therefore, in this study, we examined the tissue-specific roles of *pcx* in N and other signaling pathways.

First, we examined various cell-fate decision that requires N signaling activity in *pcx* mutant embryos. We found that *pcx* mutant embryos showed abnormalities in the tracheal system, as found in *N* mutant embryos, suggesting that *pcx* is required for N signaling in tracheal development. However, *pcx* was not required for N signaling in the border cells specification of the hindgut and the invagination of the esophagus to the proventriculus.

Unexpectedly, however, the visceral mesodermal cells were missing in *pcx* mutant embryos, whereas *N* mutant embryos showed an increase in them as compared with wild-type. These results suggested that *pcx* is required for a different signaling pathway, besides N signaling. Because mutant embryos of *wingless*, which encodes a ligand of Wnt signaling, showed the missing of visceral mesodermal cells, it is likely that *pcx* may

be required for Wnt signaling in the visceral mesoderm. In addition, we found that the ER of phagocyte was enlarged in *pcx* mutant embryos. However, N signaling is not required for the phagocytic development during embryogenesis, and *N* mutant embryos did not show the ER enlargement phenotype. Thus, we speculated that *pcx* may function independently of N signaling in phagocytic development. From these results, we concluded that *pcx* plays cell-type specific functions in N and Wnt signaling pathways.

D1045C An *in vivo* screen for novel small molecule inhibitors of PLCγ. *Chitra Naidu*, Michelle Latino, Claire Rosenwasser, Todd Rosenberg, Justin Thackeray. Clark University, Worcester, MA.

PLCγ is a key signaling molecule that regulates pathways required for cell proliferation, differentiation and apoptosis. Various studies have reported PLCγ overexpression to be a key factor in transforming primary tumors to metastatic by affecting these very pathways. A PLCγ-specific inhibitor could therefore be an invaluable tool not only for basic research but also for anti-cancer studies.

Small wing (*sl*), the *Drosophila* homolog of *PLCγ*, plays a dual role. It negatively regulates the EGFR pathway controlling photoreceptor and wing vein differentiation while positively regulating the Insulin pathway affecting growth. An *sl* null mutant (sl^9/sl^9) shows a reduced wing size, ectopic veins and rough eyes as a result of extra R7 photoreceptors in ~60% ommatidia. Our objective is to identify a novel small molecule inhibitor of PLCγ using *Drosophila* as a model system.

In a primary screen, we looked at vein differentiation patterns to identify molecules that alter EGFR signaling. Argos, an inhibitor of EGFR, when overexpressed in wings causes severe loss of venation which is significantly rescued in L3 by a partial loss of Sl function. Thus, drug fed MS1096>Aos flies were used as a sensitive model system to detect potential Sl inhibition by looking for L3 vein recovery. 37 of the 1,596 small molecules, provided by the NCI, showed significant results.

We are currently in the process of performing secondary screens to confirm EGFR inhibition by looking at photoreceptor differentiation in the eye in an sl^7 mutant. sl^7 is a missense mutation that results in 5-10% ommatidia with extra R7 photoreceptors. Further inhibition of Sl or the EGFR pathway would result in a higher percentage of R7 recruitment. So far, we have identified 7 small molecules as potential inhibitors. In conjunction, these molecules are also being tested on homozygous *rolled* (rl^1) mutants. rl^1 flies have a disrupted EGFR pathway and exhibit ~22% ommatidia without R7's. Amplification of the EGFR pathway, potentially through Sl inhibition would result in a recovery of photoreceptor numbers. Subsequent experiments will try to determine whether any of the small molecules identified do in fact inhibit Sl.

D1046A The COP9 signalosome regulates EGFR signaling by stabilizing Capicua. *A. Y. T. Suisse*, D. Q. He, K. Legent, J. E. Treisman. Skirball Institute of Biomolecular Medicine, NYU, New York, NY.

Post-translational modifications that alter protein activity are essential for signal transduction in many signaling pathways. The Epidermal Growth Factor Receptor (EGFR) pathway uses a kinase cascade to activate Mitogen-Activated Protein Kinase (MAPK) by phosphorylation. Phosphorylated MAPK can translocate into the nucleus and activate the transcription of target genes. In the *Drosophila* wing disc, the effect of MAPK on its target gene *argos* (*aos*) is mediated by phosphorylation and inactivation of the transcriptional repressor Capicua (Cic). Phosphorylated Cic can be exported from the nucleus and/or degraded.

In a genetic screen for regulators of EGFR signaling, we found two mutations in the *csn1b* gene. Loss of *CSN1b* leads to ectopic activation of the EGFR target gene *aos* in the wing disc. *csn1b* encodes a subunit of the COP9 signalosome (CSN), a complex that removes Nedd8 (a Ubiquitin-like protein) from substrates. The best characterized substrates for the CSN are Cullins (scaffold subunits of E3 ubiquitin ligases), which are active in their neddylated state. *aos* is also misexpressed in the absence of other CSN subunits, and this misexpression requires a basal level of EGFR signaling.

We have traced the effect of the CSN on *aos* to its positive regulation of Cic stability. Cic levels are reduced in cells lacking CSN subunits, and the expression of other Cic target genes, including *cyclin E* and an artificial reporter, is increased. Interestingly, removing the MAPK docking site from Cic protects it from degradation in *csn5* mutant cells, but not in *csn1b* mutant cells, suggesting the existence of two separate degradation mechanisms.

We are now investigating the mechanisms by which the CSN regulates Cic levels. The activity of Cullin-based ubiquitin ligases appears to be increased in *CSN* mutant cells, suggesting that a Cullin complex could ubiquitinate Cic and send it to 26S proteasome-mediated degradation. Alternatively, another type of ubiquitin ligase, or Cic itself, could be a substrate for deneddylation by CSN. As human Cic is a tumor suppressor gene, these results may shed light on the role of the CSN in cancer.

D1047B Yki interacts with the JNK pathway to regulate epidermal wound healing in *Drosophila* larvae. *C. Tsai*[1,2], A. Anderson[2], S. Burra[2], M. Galko[2]. 1) Baylor College of Medicine, Houston, TX; 2) MD Anderson Cancer Center, Houston, TX.

To cope with inevitable injury, organisms possess efficient wound healing mechanisms to maintain tissue integrity and guard against infection. However, the cellular and molecular details by which wound healing is accomplished remain poorly defined. *Drosophila* melanogaster serves as a great model organism to study wound healing because of the versatile genetic tools available and the simple anatomy of the epidermal barrier. Upon larval epidermal wounding, epidermal cells around the wound elongate and migrate in the absence of cell division to ultimately close the wound. Using the wound closure assay we established, we found the Hippo downstream transcriptional regulator, Yorkie (Yki) and its TEAD binding partner Scalloped (Sd) to be required for epidermal wound healing. Intriguingly, unlike in other regenerative contexts, Yki does not regulate the balance of mitosis or apoptosis in the healing larval epidermis. Rather, it seems to regulate actin polymerization in the migrating wound-edge epidermal cells. Moreover, with a series of genetic analyses, we found that Yki has a strong genetic interaction with another wound closure signaling, the Jun N-terminal Kinase (JNK) pathway. Our results suggest that there is a positive feedback loop between these two pathways to facilitate proper epidermal wound healing.

D1048C Identification and characterization of novel epidermal growth factor receptor target genes implicated in *Drosophila* development. *Michael Warkala*, John Nawrocki, Sergey Svintozelskiy, Sean Thomas, Lisa Kadlec. Wilkes University, Wilkes-Barre, PA.

Signaling by the *Drosophila* epidermal growth factor receptor (Egfr) plays an important role in many aspects of development, including oogenesis, embryogenesis and proper development of both the eye and the wing. In the ovary, the Egfr pathway is known to play a key role in the establishment of the body axes during oogenesis. In the wing, Egfr signaling plays an important role in vein tissue specification, and in the eye it has been found to be important for photoreceptor cell differentiation. Microarray screens by our lab and others have been used to identify potential downstream transcriptional targets of the Egf receptor using the *Drosophila* ovary as a model system. Our initial work compared gene expression using fly ovaries in which the activity of the Egfr-pathway was reduced (*grk* mutant), normal (OreR), or constitutively active (CY2/λTop). We have employed a number of approaches to further investigate the expression, biological function, and mechanism of action of an initial set of putative genes of interest, focusing primarily on genes of previously unknown function. Several of these targets exhibit developmentally regulated expression in the ovary. UAS-RNAi was used to knock down expression of various target genes in either specific tissues or ubiquitously via a tubulin driver. Gene knockdown phenotypes include abnormal wing morphology, decreased eggshell integrity, and, in at least one case, lethality. We are currently extending our studies by utilizing the UAS/Gal4 system to perform a functional screen of previously identified, but untested, target genes whose expression is highly upregulated in response to constitutive Egf receptor activity. In particular, the available libraries of UAS-RNAi transgenic flies are being used in concert with ovarian and wing disc Gal4 driver lines to identify additional genes with roles in *Drosophila* developmental events. We have identified several additional genes which may have roles in normal eggshell formation. RT-PCR has confirmed the up-regulation of some of our newly identified targets, and we are further investigating gene expression patterns via *in situ* hybridization.

D1049A The Rap Guanine Nucleotide Exchange Factor (GEF) C3G is required for nephrocyte function in *Drosophila melanogaster*. *C. Picciotto*[1], C. P. Dlugos[1,2], A. Jeibmann[3], M. Krahn[4], R. Wedlich-Söldner[2], H. Pavenstädt[1], C. Klämbt[5], B. George[1]. 1) Medizinische Klinik D, University Hospital Münster, Germany; 2) Institute of Cell Dynamics and Imaging, University of Münster, Germany; 3) Institute of Neuropathology, University Hospital Münster, Germany; 4) Institute of Molecular and Cellular Anatomy, University of Regensburg, Germany; 5) Institute of Neurobiology, Westfälische-Wilhelms University Münster, Germany.

Introduction and Aim: Glomerular epithelial cells (podocytes) are essential for kidney filter function. Podocyte defects are central to many chronic glomerular diseases and are characterized by aberrant intercellular junctions (slit diaphragms) and actin cytoskeletal dynamics. Patients expressing mutated variants of the slit diaphragm protein Nephrin fail to develop functional slit diaphragms, due to signaling defects to the podocyte actin cytoskeleton. *Drosophila* nephrocytes form a filtration barrier, express the Nephrin orthologue Sticks and stones (Sns), and serve as a genetically tractable model for mammalian podocytes. The aim of our study is to analyze Nephrin signal transduction.

Methods: An assay was established to analyze the role of genes of interest in *Drosophila* nephrocytes. In the experimental strain, the secreted protein Atrial Natriuretic Protein (ANP)-GFP-GFP was ectopically expressed by the *ubiquitin* promoter (*ubi::ANP-GFP-GFP*). This line also contained a *sns-Gal4* transgene. Nephrocytes filter and then take up proteins of defined size, here ANP-GFP-GFP. This allows testing the impact of genes of interest on nephrocyte filter function by crossing in respective *UAS-dsRNA* strains. Standard immunofluorescence and co-immunoprecipitation assays were employed to establish a novel protein interaction.

Results: Immunohistochemistry indicates that Nephrin and the Rap GEF C3G co-localized in human podocytes. The interaction of the two proteins was confirmed by co-immunoprecipitating Nephrin and C3G in HEK cell lysates. To test the functional relevance of these proteins we used the *Drosophila* system where cell type specific gene silencing experiments can be performed. Nephrocyte specific knockdown of the Nephrin orthologue *sns* as well as *C3G* compromised filtration and protein uptake as shown by defective ANP-GFP-GFP uptake. We demonstrated that the C3G effector Rap1 was necessary for *Drosophila* nephrocyte function.

Discussion and conclusion: *Drosophila melanogaster* is a powerful model system to study nephrocyte function and Nephrin signaling. C3G interacts with Nephrin and is required for nephrocyte function.

D1050B Piragua, a ZAD and zinc finger transcription factor, genetically interacts with the membrane protein Flower in the embryo. *Juan Riesgo-Escovar*. Instituto de Neurobiologia, UNAM, Queretaro, Queretaro, MX.

The *piragua* (*prg*) locus encodes a protein with an amino terminal ZAD (zinc associated domain) and nine C2H2 zinc fingers. It is expressed throughout the life cycle, and is required for embryogenesis and early larval life. A fraction of mutant embryos die during embryogenesis, either with an early phenotype (no cuticle formed), or at the end of embryogenesis with defective cuticles (dorsal closure and head involution defects). Surviving embryos die as first instar larvae. I also found that *flower*, that codes for a membrane protein, is required for embryogenesis. Two mutants *flower* alleles examined display late embryonic developmental cuticular phenotypes, of which most are head involution defects. Surviving embryos die as first instar larvae, as do surviving *piragua* embryos. In a published gain-of-function screen in wing imaginal discs, both genes were isolated by virtue of expression in dying, outcompeted cells, and for both being required for cell death in these cells (Rhiner, et al, 2010). We wondered whether the two loci might genetically interact in the embryo. Genetic experiments show that indeed the two loci interact, as homozygous mutants for one allele while heterozygous for the other show a significant augmentation of embryonic phenotypes. In contrast, mutant eye phenotypes for *piragua* are at variance from those of mutant *flower* alleles. *piragua* mutant eye clones show extensive cellular disarray, picnotic figures, lack of eye bristles, and aberrant cone morphology. This compares to a much less dramatic phenotype of lack of supernumerary cell culling at the eye borders during eye formation, a process that requires *flower* (Merino, et al 2013). This implies that *piragua* and *flower* might have both common and distinct functions.

Funded by CONACYT # 177962.

Bibliography:

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

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Merino, et al (2013). *Curr. Biol.* **23(14)**: 1300--1309.

D1051C Identification and characterization of Sugar-free frosting/SAD kinase substrates that regulate neural glycosylation in the *Drosophila* embryo. *Sarah Baas Robinson*, Varshika Kotu, Peng Zhao, Debora Witkoski, Lance Wells, Michael Tiemeyer. University of Georgia, Athens, GA.

Spatio-temporal regulation of protein glycosylation is critical for normal development and is altered in various human pathologies. However, the signaling pathways that confer tissue and cell specific glycan expression patterns are largely unknown. We previously characterized a *Drosophila* mutation which we named *sugar-free frosting* (*sff*), that impacts the expression of a subset of N-linked glycans known as HRP-epitopes in the embryonic nervous system. The gene affected in the *sff* mutant is the *Drosophila* homologue of the *C.elegans* and mammalian SAD kinases. In *Drosophila*, we demonstrated that the *sff* mutation affects glycan processing through reorganization of Golgi compartments. However, the downstream effectors through which Sff kinase acts to bring about these changes are currently unknown. To identify the molecular targets of Sff kinase, we undertook differential phosphoproteomic analysis of wildtype and *sff* mutant embryos collected at the developmental stages at which HRP epitopes first appear. Using immunohistochemistry, genetic analysis, and confocal microscopy, Bifocal (Bif), Rasputin (Rin), and Liprin-alpha (Lip-α) were identified as downstream effectors of Sff signaling in the regulation of glycan processing. In combination with *bif* or *liprin-α* mutations, *sff* double mutants exhibit altered overlap between the trans-Golgi network and cis-Golgi compartment. We also detected Rasputin co-localization with Golgi compartmental markers and this association was altered in *sff* mutant embryos. Taken together, Bif as a putative cytoskeletal regulator, Lip- α as a coiled coil protein, and Rin as the ortholog of RasGAP SH3 binding protein, highlight downstream pathways through which Sff kinase activity fine tunes protein glycosylation in response to external stimuli.

D1052A The UPR Pathway Activates the TOR Signaling through Atf6. *J. Seo*, D. Allen, S. Petty. Rogers State University, Claremore, OK.

Multiple cellular signaling pathways are interconnected to maintain homeostasis in a cell. The target of rapamycin (TOR) signaling pathway is an essential regulator of cell growth and survival; the unfolded protein response (UPR) pathway is a central mechanism to counteract endoplasmic reticulum (ER) stress instigated by the accumulation of misfolded proteins in ER. Although the two pathways regulate similar cellular processes, they have been until recently studied independently. Here, we show that the UPR pathway activates the TOR signaling pathway through activating transcription factor 6 (Atf6).

Atf6 is a transmembrane protein resides in the ER membrane. Upon ER stress, cytoplasmic domain of Atf6 is processed, is translocated to the nucleus, and activates its target genes to abate the stress. We demonstrated that knocking-down of Atf6 has decreased the TOR activity in S2R+ *Drosophila* cells by assessing the phosphorylation state of ribosomal protein S6 kinase (S6K), a well-characterized TOR target. Conversely, overexpression of Atf6 has increased the TOR activity. Supporting the notion, knocking-down of enzymes processing Atf6 has reduced the TOR activity. Taken together, our data demonstrate that Atf6 is a key molecule to connect the two signaling pathways.

D1053B Characterizing the role of the Fat cadherin family in the mitochondria using CRISPR. *N. Yau*[1,2], A. Sing[1], Y. Tsatskis[1], Y. Qu[1], H. McNeill[1,2]. 1) Lunenfeld-Tanenbaum Research Institute, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Proper development of multicellular organisms requires a coordination of conserved developmental pathways involved in proper tissue growth and patterning. Genetic analyses have identified Fat (Ft), an atypical cadherin that functions to regulate planar cell polarity (PCP) tissue organization and the Hippo pathway. Mutations of Ft cadherins have been implicated in polarity defects, tissue overgrowth, and spindle orientation defects.

The intracellular domain (ICD) of Ft has been previously dissected using structure-function analyses, and distinct regions affecting Ft's function in PCP and Hippo signaling have been identified. We recently have identified a novel signaling role for Ft in regulating mitochondrial function that maps to previously identified regions in the ICD. We showed that Ft is processed intracellularly to generate Ft^{mito}, and is required for regulating the stability and/or ability of Complexes I and/or V. Our analysis of the ICD of Ft identified two predicted mitochondrial targeting sequences (MTS), and a highly conserved region that is necessary for Ndufv2 binding. Subsequent experiments will help understand the mechanism of how Ft regulates mitochondrial function. We are currently conducting CRISPR mutagenesis and genetic analyses to ablate the two MTS sites as well as other conserved regions in the ICD to understand how their loss affects mitochondrial function. I am also deleting a conserved potential cleavage site in the ICD to examine if the intracellular processing of Ft is lost and whether the mitochondrial localization of Ft is affected. I will conduct tissue-specific RNAi knockdown of Ft to determine whether the mitochondrial activity of Ft is ubiquitous throughout the *Drosophila* larvae. These experiments will allow us to examine the role of Ft in the mitochondria endogenously, and can be applied to understand how endogenous deletions within the ICD affect Ft's other functions in PCP and Hippo signaling.

D1054C Transcriptome analysis to identify genes responding to mechanical force in developing *Drosophila* embryos. *T. Ishibashi*[1], K. Yamaguchi[2], S. Shigenobu[2], K. Matsuno[1]. 1) Osaka University, Toyonaka, Osaka, JP; 2) National Institute for Basic Biology, Okazaki, Aichi, JP.

Various types of mechanical force are involved in biological processes. Especially during development, cells are subjected to dynamic mechanical force. Cells growing in limited area push and pull their neighboring cells, which may change the shape of the tissues encompassing or surrounding these cells. Recently, it has been reported that mechanical force also modulates cell behaviors, such as polarization, cell migration, cell growth, and differentiation. However, most of these studies rely on cultured cells. Therefore, it is largely unknown what molecules are involved in generation and reception of mechanical force, and how they work in developmental events.

In order to reveal these issues, we planed to perform RNA-seq transcriptome analysis to identify genes induced by mechanical force in *D. melanogaster* embryos. First, we constructed the mechanical force application system. We confirmed that the compression during the whole embryogenesis perturbed development, however the compression for 30 minutes did not affect the hatching rate. Next, we checked the global stress response to the compression. We confirmed that ER-stress response was not affected by the compression for 30 minutes.
Here, we will describe our experimental system and discuss our future directions.

D1055A Functional characterization of *creld* in *Drosophila melanogaster*. *M. Paradis*, B. Stümpges, M. Hoch, R. Bauer. University of Bonn, Limes Insitute, Bonn, DE.

cysteine-rich with EGF-like Domains (creld) genes encode evolutionary conserved proteins. Two paralogues are identified in mammals: *Creld1* was shown to act as a regulator of the calcineurin/NFATc signaling pathway, whereas c*reld2* is involved in ER stress response.
The Drosophila genome encodes only one *creld* gene yielding two splice variant proteins (46% similarty to mammalian CRELD1 and 49% similarty to CRELD2). In order to elucidate the function of *Drosophila* Creld proteins, we generated a *Drosophila* null mutant for c*reld* and analyzed the ensuing phenotypes.
As the mammalian orthologues, Creld is localized to the ER membrane. However, we could not confirm an upregulation of gene expression under ER stress of either Creld isoforms, neither in wild type flies nor Schneider cells. Addtionally, c*reld* mutants are able to survive under ER stress (induced by feeding tunicamycin and thapsigargin), indicating that the knock out of Creld is not provoking ER stress sensitivity.
Creld mutant flies suffer from extensive neurodegeneration, accompagnied by severe locomotor and flight defects and reduced viability, but fertile. q-RT-PCR analysis of adult flies could identify a decreased relative mitochondrial DNA content in *creld* mutants compared to wild type flies, suggesting problems with mitochondrial biogenesis or quality control. Additonally, a decreased number of mitochondria was confirmed in larval tissue using immunofluorescent techniques. The observed phenotypes could significantly be rescued by complementing the food with mitochondrial activity inducing drugs. We performed climbing assays with mutant flies fed with these drugs and were able to see significantly increased climbing capabilites of those flies compared to mutants fed with normal yeast food without any addtives. But no indication of oxidative stress or increased ROS production was observable in *creld* mutant flies.
We hypothesize that the observed neurodegeneration is at least partly induced by energy deprivation that goes along with reduced mitochondrial mass, and which was evident by increased AMPK phosphorylation.
We conclude from our data that Creld might be involved in mitochondrial biogenesis and/or quality control. Due to its localization to the ER membrane, it might pose a link between the ER compartment and mitochondria, which are closely connectet in order to regulate Ca^{2+} signaling, regulating intracellular trafficking and controlling mitochondrial biogenesis. Future research will adress the function of Creld in this context.

D1056B The Tumor Microenvironment And Mechanisms Governing Ras Tumor Overgrowth. *Chiswili Yves Chabu*[1,2], Tian Xu[1,2]. 1) Yale University School Of Medicine, New Haven, CT; 2) Howard Hughes Medical Institute.

We have been using *Drosophila* genetics to dissect the regulation of growth and metastasis, and have discovered conserved cell intrinsic and cell-cell signaling mechanisms within the tumor microenvironment. Here, I will discuss our recent findings of tumor-host and tumor-tumor signaling mechanisms promoting oncogenic Ras-mediated tumor overgrowth. First, we found that oncogenic Ras cells elevate the exocytosis of TNF to the surrounding wild-type cells, which consequently activate JNK signaling. This causes surrounding wild-type cells to secrete JAK-STAT ligands to activate JAK/STAT signaling in tumor cells and result in tumor overgrowth. Second, we discovered that oncogenic Ras signaling triggers the secretion of EGFR ligands. In turn, EGFR stimulates tumor overgrowth independent of the canonical Sos/Ras signaling pathway, but via ARF6. EGFR promotes ARF6 to control Hedgehog cellular transport in order to activate Hh signaling, which cooperates with oncogenic Ras to drive overgrowth. Consistent with these studies, blocking secretion or ARF6 function inhibits the growth of oncogenic Ras-driven tumors in mammals. Collectively, these data highlight important cell-cell interactions within the tumor microenvironment and also explain both, the puzzling requirement of EGFR in some Ras cancers and the oncogenic cooperation between EGFR and Hh signaling.

D1057C The "gatekeeper" function of *Drosophila* Seven-IN-Absentia (SINA) E3 ligase and its human homologs, SIAH1 and SIAH2, is highly conserved for proper RAS signal transduction in *Drosophila* eye development. *Robert E. Van Sciver*[1], Yajun Cao[1], Atique U. Ahmed[2], Amy H. Tang[1,2]. 1) Eastern Virginia Medical School, Norfolk, VA; 2) Mayo Clinic, Rochester, MN 55905.

Seven-IN-Absentia (SINA) is an evolutionarily conserved E3 ubiquitin ligase that is the most downstream signaling module identified in the RAS pathway. Underscoring the importance of SINA is its high evolutionary conservation with over 83% amino acid identity shared between *Drosophila* SINA and human SINA homologs (SIAHs). As a major signaling "gatekeeper" in the RAS pathway, we have shown that SIAH is required for oncogenic K-RAS-driven tumorigenesis and metastasis in human pancreatic, lung and breast cancer. Since SIAHs appear to be the ideal drug target to inhibit "undruggable" K-RAS activation, it is important to delineate the activity, regulation, and substrate targeting mechanism(s) of this highly conserved family of SINA/SIAH E3 ligases by deploying this elegant and well-established *Drosophila* photoreceptor development system to study RAS activation. In the developing eye, photoreceptor cells are recruited sequentially and acquire their distinctive cell fates through a series of local inductive events. The 800x cell arrays allowed us to dissect the role of SINA/SIAH downstream of RAS activation in photoreceptor cell development. To delineate SINA function, we have isolated multiple EMS-induced *sina* point mutant alleles that have shown much stronger mutant phenotypes than those of the previously published *sina*^{2} and *sina*^{3} alleles, suggesting that the *sina*^{2} and *sina*^{3} alleles are hypomorphic alleles. Here, we have an opportunity to characterize the null and stronger *sina*^{mutant} alleles by sequencing the EMS-induced *sina* mutant collection. To define SINA/SIAH functional conservation, we have generated a complete panel of transgenic fly models that express either wild-type (WT) or proteolysis-deficient (PD) SINA/SIAH. The corresponding *UAS-sina/siah*$^{GOF/LOF}$ phenotypes have

been characterized using *sev*-, *GMR*-, *dpp*- and *salivary gland*-GAL4 drivers to elucidate the PNS developmental outcome of altered SINA/SIAH expression upon RAS activation. Ectopic expression of $sina^{WT/PD}$/$siah^{WT/PD}$ in neurons resulted in dramatic changes in neuronal cell fate in the developing eye, and PNS neurodegenerative phenotypes. Our results show that the biological functions of fly SINA and human SIAH1/SIAH2 are evolutionarily conserved and functionally interchangeable. Mechanistic insights and regulatory principles learned from *D. melanogaster* can be directly applied to cancer biology to develop and validate next-generation anti-SIAH-based anti-K-RAS and anticancer therapy in the future.

D1058A Calcium dynamics can be used to reveal mechanisms of epithelial wound detection. *Erica Shannon*, Monica E. Lacy, M. Shane Hutson, Andrea Page-McCaw. Vanderbilt University, Nashville, TN.

When an epithelial tissue is wounded, the cells undergo a set of coordinated behaviors to close the wound and repair the tissue. Cells across the epithelium participate in this response, not just the cells bordering the wounds. We want to know how cells, including those away from the wound site, get information that a wound has occurred. The earliest observed wound response is a calcium wave, previously reported to originate in cells on the wound margin and expand outward several cells in diameter. This calcium wave is highly conserved and is important for wound healing. Normally, calcium concentrations inside cells are maintained at low levels by pumping calcium out of the cell or into the ER. When the cytoplasmic concentrations rise, calcium can act as a second messenger and can affect multiple signaling pathways. However, the mechanism of wave initiation remains unknown. Based on the calcium wave kinetics we have observed, we hypothesize that the calcium wave upon wounding is a result of changes in tissue mechanics.

We have analyzed the kinetics of the calcium wave using *in vivo* live imaging of the *Drosophila* pupae notum, an epithelial monolayer of diploid cells that expresses the GFP-based GCaMP calcium reporter. Our data reveals two distinct stages of calcium dynamics upon wounding; an initial release and stochastic flares. During the initial release, calcium flows simultaneously into multiple cells (at varying distances from the wound margin), only milliseconds after wounding. The second stage of calcium release occurs ~30-60 seconds after wounding and is characterized by random flashes of calcium that propagate around the wound for up to 30 minutes. These flashes are consistent with calcium induced calcium release.

The spread of the initial release from cells proximal to the wound toward cells distal to the wound is on the order of 1-10 μm/ms. Preliminary analysis suggests that diffusion cannot account for such a rate of spread. Thus we are testing the role of mechanotransduction in wound detection. We will use genetic tools to manipulate tissue mechanics and then assess how the calcium wave is affected by the mechanical perturbations.

D1059B Involvement of the histone demethylase KDM5 in the control of apoptosis. *C. Drelon*, X. Liu, J. Secombe. Albert Einstein College of Medicine, Bronx, NY.

KDM5 proteins are highly conserved histone demethylases with several conserved domains. In addition of well-described JmJC domain, the catalytic domain, KDM5 proteins contain a JmJN domain, an ARID DNA binding domain, a C5HC2 zinc finger of unknown function and two or three histone binding PHD motifs. Whereas mammalian cells encode four KDM5 paralogs KDM5A, KDM5B, KDM5C and KDM5D, Drosophila has a single KDM5 ortholog, making of flies a good model to study the biology of KDM5 family proteins. KDM5 proteins are involved in the control of gene transcription, acting as an activator or a repressor in a context-dependent manner. Because knockout of mouse KDM5B and Drosophila kdm5 result in lethality, KDM5 proteins play an essential role in transcriptional regulation. Moreover alterations of KDM5 expression have been described in several human cancers. For example overexpression of KDM5A or KDM5B are implicated in prostate, breast and gastric cancers. However the roles of KDM5 proteins in physiological contexts and in tumor development remain poorly understood.

In order to study the biology of KDM5, our lab carried out RNA sequencing experiments of *kdm5* hypomorphic mutant adult flies that have 70% less KDM5 protein than wildtype. These analyses highlighted alterations in genes required for apoptosis. Additionally anti-KDM5 ChIP sequencing experiments conducted by our lab shown that KDM5 is bound to the promoter region of several of cell death genes. Our qPCR analyses confirmed the down-regulation of many pro-apoptotic genes in *kdm5* mutant flies. Moreover to verify the ChIP-seq data, we conducted ChIP qPCR experiments and confirmed the binding of KDM5 to the promoter of the *Dark* gene. Dark is required for the activation of Dronc initiator caspase and is essential for most of cell death. We therefore propose that kdm5 could directly participate at the induction of apoptosis.

We continue to investigate the role of KDM5 in apoptosis and to define the precise mechanism by which KDM5 controls the expression of apoptosis genes. Because KDM5 interacts with several transcription factors involved in stimulating cell death such as Myc and Foxo, these are good candidates for recruiting KDM5 to its targets. In parallel we are identifying which domains of KDM5 are required for this function. Although KDM5 is most well known for its histone demethylase activity, previous work from our lab and others have demonstrated critical KDM5 functions are independent of its well-described enzymatic activity.

D1060C A Role for Histone Deacetylases in Regulating Sensitivity to Apoptotic Stimuli in *Drosophila melanogaster*. *K. Marischuk*, Y. Kang, A. Bashirullah. University of Wisconsin-Madison, Madison, WI.

A cell's sensitivity to apoptotic stimuli is highly variable, making it possible for the same stimuli to trigger a death response in one tissue and not in another. Previous research in our lab has shown that differences in sensitivity to apoptotic stimuli during Drosophila development are established by regulating levels of pro-apoptotic proteins—cells with lower levels being less sensitive to apoptotic stimuli. We hypothesized that histone deacetylases (HDACs) could play a major role in repressing transcription of pro-apoptotic genes in tissues with low sensitivity. By using HDAC inhibitory drugs, we showed that reducing the activity of Class I HDACs is sufficient to increase expression of pro-apoptotic genes and thereby elevate apoptotic sensitivity. We then used knockdown of individual HDACs to identify Rpd3 as the HDAC most responsible for

these changes in responsiveness to apoptotic stimuli in whole animals as well as in specific tissues. HDAC inhibitors are used widely in drug cocktails to treat cancer patients, but the mechanism by which they act is not wholly clarified. Our results suggest that these drugs may act, at least in part, by priming both normal and malignant cells for a death response.

D1061A Argonaute-1 regulates developmental apoptotic process through JNK signaling pathway in *Drosophila*. *T. Mondal*[1], U. Bhadra[2], M. Pal Bhadra[1]. 1) CSIR-Indian Institute of Chemical Technology, Hyderabad, TS, INDIA; 2) CSIR-Centre for Cellular & Molecular Biology, Hyderabad, TS, INDIA.

Argonaute family proteins are not only conserved in higher organisms but also found in archaea and bacteria (*Aquifex aeolicus*). The name Argonaute (Ago) came from the phenotypic resemblance of *Arabidopsis thaliana* mutants with mollusk 'Argonaut'. PAZ and PIWI domain containing argonaute family proteins are well known for their role in RISC complex of RNAi pathway. Role of Ago-1 in micro RNA biogenesis has been thoroughly studied, but its role in mitotic cell cycle progression is not studied much. In our earlier observation we have established the role of Ago-1 in cell cycle control in association with G2/M cyclin. We have further extended our study in understanding the relationship of Ago-1 in the regulation of apoptosis during *Drosophila* development. Apoptotic cell death plays a very critical role during development of multi cellular organism. Apoptotic process is controlled by multiple regulatory pathways. In this study we have used *Drosophila* eye as the phenotypic marker in producing overexpression of Ago-1 by employing the unique UAS-GAL4 system under the expression of eye specific promoter. Overexpression of Ago-1 in the eye resulted in smooth-eye phenotype with reduced number of ommatidia along with fused ones and loss of bristles in eye area. By combining a single copy of the dominant negative *basket* (*Drosophila* homolog of JNK) gene mutation, we found a drastic reversal to the normal phenotype indicating an active involvement of the basket with Ago-1 induced developmental apoptotic process. Pro-apoptotic genes hid, grim & rpr and *Drosophila* caspase genes, dronc, ice & Dcp-1 are also involved in this machinery. Thus Ago-1 regulated developmental apoptotic process in *Drosophila* development is JNK dependent.

D1062B Signaling mechanisms between apoptotic cells and non-professional phagocytes. *Sandy Serizier*[1], Iker Etchegaray[2], Kim McCall[1]. 1) Boston University, Boston, MA; 2) University of California San Francisco, San Francisco, CA.

Cells death is a key process that is necessary for the maintenance of tissues, and the removal of dangerous or unwanted cells. Apoptosis is one of the best-characterized types of programmed cell death. Membrane blebbing, caspase activation, chromatin condensation, and DNA fragmentation are all hallmarks of apoptosis. The Drosophila ovary is a powerful system to study programmed cell death because this system uses both apoptotic and non-apoptotic mechanisms. During mid-oogenesis, starvation cues reproducibly induce an apoptotic program and in late oogenesis, non-apoptotic programmed cell death occurs developmentally. Upon apoptotic cell death, follicle cells act as non-professional phagocytes to clear the debris. The molecular mechanisms that govern the communication between apoptotic cells and non-professional phagocytes in this system, however, are understudied. We have found that Draper, an engulfment receptor, is required for engulfment by follicle cells in the ovary. Interestingly, when Draper is overexpressed in follicle cells, the germline undergoes programmed cell death. In an effort to understand the underpinnings of engulfment receptor-induced cell death, we sought to characterize the type of cell death and signaling pathways involved in Draper-mediated germline cell death in the Drosophila ovary. Because Draper loss of function mutants elicit defects during cell death in late oogenesis, some of the key regulators of engulfment receptor mediated cell death may function normally during non-apoptotic cell death.

D1063C Drosophila Wnt and STAT Define Apoptosis-Resistant Epithelial Cells for Tissue Regeneration after Irradiation. *S. Verghese*, T. T. Su. University of Colorado, Boulder, CO.

Drosophila larvae irradiated with doses of ionizing radiation (IR) that kill about half of the cells in larval imaginal discs still develop into viable adults. How surviving cells compensate for IR-induced cell death to produce organs of normal size and appearance remains an active area of investigation. We have identified a subpopulation of cells within the continuous epithelium of *Drosophila* larval wing discs that shows intrinsic resistance to ionizing radiation (IR)- and drug-induced apoptosis. These cells reside in domains of high Wingless (Wg, Drosophila Wnt-1) and STAT92E (sole *Drosophila* STAT homolog) activity, and would normally form the hinge in the adult fly. Resistance to IR-induced apoptosis requires STAT and Wg, and is mediated by transcriptional repression of pro-apoptotic gene *reaper*. Lineage tracing experiments show that, following irradiation, apoptosis-resistant cells lose their identity and translocate to areas of the wing disc that suffered abundant cell death where they assume appropriate new identities. Our findings provide a new paradigm for regeneration in which it is unnecessary to invoke special damage-resistant cell types such as stem cells. Instead, differences in gene expression within a population of genetically identical epithelial cells can create a subpopulation with greater resistance, which, following damage, survive, alter their fate, and help regenerate the tissue.

D1064A BAF phosphorylation regulates necrotic pyknosis. *L. Hou*[1,2], K. Liu[1], Y. Li[2], X. Ji[2], L. Liu[2]. 1) State Key Laboratory of Membrane Biology, School of Life Sciences, Peking University, Beijing, 100871, China; 2) Aging and Disease Lab of Xuanwu Hospital and Center of Stroke, Beijing Institute for Brain Disorders, Capital Medical University, Youanmen, Beijing, 100069, China.

Necrotic cell death (necrosis) widely occurs in human pathologies, however, the therapeutic strategies to suppress it are still lacking due to our inadequate knowledge on its molecular mechanisms. One of the morphological markers of necrosis is nuclear condensation (pyknosis), which has been used widely to distinct from apoptosis. However, the molecular mechanism of necrotic pyknosis and its functional role in necrosis propagation are still unclear. To address these questions, we generated a genetic model in *Drosophila* to temporally follow the progression of necrotic pyknosis. Surprisingly, we observed an intermediate state of chromatin detachment from the nuclear envelope (NE),

followed with the NE completely collapsed onto chromatin. This phenomenon leads us to discover that phosphorylation of barrier-to-autointegration factor (BAF) mediates this initial separation of NE from chromatin. Functionally, inhibition of BAF phosphorylation suppressed the necrosis in both *Drosophila* and human cells. This suggests that necrotic pyknosis is an essential and evolutionally conserved step for the propagation of necrosis. Therefore, BAF phosphorylation may represent a biochemical marker of necrosis and potentially serve as a therapeutic target for necrosis-related diseases.

D1065B Signaling and mechanisms regulating Germ Cell Death (GCD), an alternative cell death pathway in *drosophila*. *K. Yacobi-Sharon*, E. Arama. Weizmann Institute, Rehovot, IL.

Apoptosis is the most abundant form of programmed cell death (PCD) in metazoans, but some cells seem to utilize different cell death pathways independent of caspases, the executioners of the apoptotic program. To date, very little is known about the signaling and molecules underlying such alternative cell death pathways. In a previous work (Yacobi-Sharon et al., *Dev Cell*, 2013), we discovered and delineated a cell death pathway which is utilized to normally and constantly eliminate about one quarter of the emerging (premeiotic) spermatogonial cells in the adult *Drosophila* testis. This new cell death pathway, also termed Germ Cell Death (GCD), displays distinct morphological features reminiscent of both apoptosis and necrosis, requires lysosomal and mitochondrial proteases, and is independent of the main apoptotic effector caspases. Whereas this study uncovered some of the GCD executioners, nothing is known about the signaling pathway which triggers this cell death pathway.

Similar to the male germ cells in mammals, which require constant contact with their somatic supporting (Sertoli) cells for proper development, the *Drosophila* male germ cells are enveloped by a pair of somatic supporting (cyst) cells throughout maturation. Interestingly, we found that following GCD, the cyst cells activate caspases and undergo apoptosis, thus suggesting that GCD may be regulated extrinsically by signals originating in these somatic supporting. Indeed, by knocking down different signaling pathways in the cyst cells we identified the Hippo/Warts pathway as an important regulator of GCD. The Hippo/Warts signaling pathway is known to limit organ/cell growth and promote apoptosis by inhibiting the anti-apoptotic and pro-proliferative activity of the transcriptional coactivator Yorkie. Cyst cell-specific knockdown of either the main kinase in this pathway, Warts, or its coactivator, Mats, significantly attenuated GCD levels, whereas knockdown of Yorkie resulted in the enhancement of GCD levels. Further genetic and electron microscopy experiments support a model in which Hippo/Warts signaling in the supporting cells triggers the alternative cell death of the germ cells, which is followed by apoptosis of the supporting cells. These results demonstrate a role of the Hippo/Warts pathway in a non- autonomous regulation of a cell death program.

D1066C Survival of proliferative, radio-resistant polyploid cells in *Drosophila* requires FANCD2. *Heidi Bretscher*, Don Fox. Duke University Medical Center, Durham, NC.

Crucial checkpoints detect DNA damage and arrest the cell cycle, allowing cells to repair DNA or undergo apoptosis. These responses maintain a stable genome. DNA damage checkpoints are also exploited therapeutically to kill proliferative tumor cells. However, many tumors eventually become resistant to death by DNA damage. Resistance is associated with mutations in DNA checkpoint responders, and/or inactivity of canonical checkpoint regulators. How such resistant cells can proliferate and survive DNA damage is poorly understood.

Cells lacking elements of the DNA damage response are not limited to pathological conditions. The endocycle, a conserved cell cycle in which cells alternate between S and G phases, increasing in ploidy without increasing in cell number, is known to silence elements of the DNA damage response. However, many endocycled cells do not divide. In the *Drosophila* rectum, we previously found endocycled papillar cells re-enter mitosis as polyploid cells.

Here, we show that papillar cells also acquire radiation-resistance during the endocycle, allowing us to study the effects of a silenced DNA damage response on subsequent mitosis. We find papillar cells accumulate DNA breaks and acentric DNA fragments during endocycles. Rather than undergo high fidelity DNA repair prior to mitosis, papillar cells divide with broken DNA. Amazingly, papillar cells employ an active mechanism to properly segregate acentric DNA fragments during anaphase, enabling cell survival and proper organogenesis. We find that this response requires the Fanconi Anemia FANCD2/FANCI heterodimer and frequent partner Bloom helicase. Lack of FANCD2 results in failure to incorporate acentric DNA into daughter nuclei, leading to micronuclei. This causes papillar cell death and formation of a non-functional organ. Interestingly, this response is independent of canonical DNA damage response proteins. Our work sheds light on a non-canonical chromosome patching mechanism used to accurately segregate broken acentric DNA during mitosis. We speculate that this response may be shared by other cells lacking an intact DNA damage response, such as radiation-resistant tumor cells.

D1067A Tousled-like kinase regulates G2/M transition through Tak1 to activate p38a MAPK. *G. Liaw*. Natl Yang-Ming Univ, Taipei, TW.

To maintain genome integrity, cell-cycle checkpoints are monitoring DNA damages and abnormal chromosome segregation caused by environmental stresses. Numerous studies have demonstrated that *Tousled-like kinase* (*Tlk*) activity is required for the faithful segregation of chromosomes and the DNA repair. Previous studies show that *tlk* overexpression delays G2/M transition. The delay is relieved by reduced *p38a* activity, consistent with both *Tlk* and *p38a* activities required for stalling G2/M transition under heat shock. Phosphorylation of p38 is significantly increased by overexpressed kinase-dead Tlk (Tlk^{KD}), suggesting that Tlk is a cofactor of a kinase that activates p38a. To find the kinase, proteins in Tlk co-immunoprecipitated complexes are identified by mass spectrometry. Using the esyN program, I find four identified proteins, Rm62, MEP-1, Hsc70-5 and EF1a48D that directly or indirectly link to Tak1. The Tlk^{KD}-facilitated p38 phosphorylation is abolished by removing *Tak1* activity, consistent with both *Tlk* and *Tak1* activities required for stalling G2/M transition under heat shock. Similarly, responding to heat shock, G2/M transition is unstoppable in eye discs with reduced two gene activities among *EF1a48D*, *Hsc70-5*, *Tak1* and *Tlk*.

Taken together, Tlk acts as a positive mediator of Tak1 that activates p38a to delay G2/M transition induced by either *Tlk* overexpression or heat shock. *Tlk* activity likely coordinate G2/M checkpoint and responses to environmental insults to maintain genome integrity.

D1068B Rescue from Ring Chromosome Dominant Lethality by Mutations in *lok* (Chk2) and *p53*. *H. LIN*. UNIVERSITY OF UTAH, SALT LAKE CITY, UT.

Ring chromosomes are known for a number of unique properties, including mitotic and meiotic instability, and dominant lethality. There is substantial cytological support for an underlying mechanism of chromatin bridging as the cause of these phenomena. Sister chromatid exchange and sister chromatid union have been proposed as mechanisms to produce chromatin bridges that may lead either to chromosome loss or lethality.

We constructed new ring *XY* chromosomes that produce strong dominant embryonic lethality. These ring chromosomes produce very few gynandromorphs, suggesting that the mechanism that causes dominant lethality is different than the mechanism that produces gynandromorphs. To determine the nature of the lethal mechanism, we investigated whether the canonical DNA damage response is responsible for embryonic death of zygotes with a ring chromosome. By crossing ring *XY* males to *lok* (encoding Chk2) or *p53* homozygous females we found that both maternal genotypes are able to strongly rescue viability of progeny that inherit the ring chromosome.

Rescue by *lok* or by *p53* differs significantly in that *lok* mutant mothers produce gynandromorph offspring, while *p53* mothers do not. This further suggests that the ring chromosome loss and ring chromosome dominant lethality have distinct causes, and that Chk2 and P53 respond to distinct chromosome abnormalities. Chk2 may normally detect aberrant behavior that would otherwise lead to chromosome loss, while P53 may detect chromosome breakage events. In wildtype embryos, both events would evoke checkpoints that result in lethality, but in mutants, distinct outcomes can be detected. We are continuing to investigate the nature of *lok* and *p53* rescue.

D1069C DNA replication proteins: two mutations better than one? *Chris I. Knuckles*, Wayne A. Rummings, Lucas T. Hopkins, Tim W. Christensen. East Carolina University, Greenville, NC.

Essential to the survival of cellular life is proper replication and maintenance of the genome. Replication proteins Mcm10 and RecQ4 have well-characterized essential roles in assembly, initiation, and proper functioning of the eukaryotic replication machinery. Specifically, Mcm10 aids in the recruitment of Pol-α/primase as well as tethering the ssDNA stabilizing replication protein A (RPA) to the CMG complex, and RecQ4 delivers the GINS complex to the replication fork, which is essential for replication initiation. Past evidence suggests that Mcm10 is not required for RecQ4 chromatin localization or association with the CMG complex, conflicting with previous reports that Mcm10 mediates the interaction between RecQ4 and Mcm2-7 in an S-CDK dependent manner. A recent study demonstrated that truncating residues from the N-terminus (Sld2-like) domain of RecQ4 eliminated the Mcm10 interaction surface. We have found that a homozygous mutation of RecQ4 is lethal in fruit flies that do not also possess a C-terminal domain (CTD) truncation allele of Mcm10, suggesting that Mcm10's RecQ4 interaction surface lies in the CTD of Mcm10. Additionally, we have used the Yeast Two-Hybrid System to demonstrate the interaction between Mcm10's CTD and RecQ4. Specifically, our results indicate that the Mcm10-RecQ4 interaction is essential for high-stress replication states – like embryogenesis and endoreplication in larval salivary glands – but not for normal cell cycles. The Mcm10 CTD truncation rescue of lethal RecQ4 phenotypes can reveal much about how the cooperative roles of these proteins affect DNA replication, but this requires further investigation. Our research aims to explore the nature of the Mcm10-RecQ4 interaction by conducting genetic studies, protein interaction assays, stem cell proliferation analysis in the Drosophila ovary, and the generation of null Mcm10 and RecQ4 alleles using the CRISPR/Cas9 system. We propose that the Sld2-like domain of RecQ4 interacts with the CTD of Mcm10, mediating its associations with the replication fork and other replication factors like RPA and Ctf4.

D1070A A Y chromosome variant mediates sex ratio of surviving *Blm* null embryos. *Abbie Olson*[1], Mallory McDermott[1], Lindsey Riggs[1], Leigh Latta[1], Mia Levine[2,3], Eric Stoffregen[1,3]. 1) Lewis-Clark State College, Lewiston, ID; 2) University of Pennsylvania, Philadelphia, PA; 3) Co-senior authors.

Repetitive DNA poses distinct challenges to the DNA replication machinery, particularly during the rapid syncytial cell cycles of early *Drosophila* embryo development. The maternally deposited Blm DNA helicase mediates such challenges that arise during this unique stage of development. Surviving progeny from *Blm* mutant mothers show a female sex bias, which has been attributed to the absence of the highly repetitive Y chromosome DNA sequences in the developing embryo. If survival to adulthood is dependent on total repetitive DNA content, then Y chromosomes that harbor variable amounts of heterochromatin (a proxy for repetitive DNA content) may show variation in male survivorship. To investigate this possibility, we crossed Y chromosomes isolated from a worldwide sample of *Drosophila melanogaster* populations into a common genetic background and quantified bulk heterochromatin using a classic position effect variegation assay ("PEV"). After classifying these Y chromosomes as strong or weak suppressors of PEV, we crossed them to *Blm* homozygous females. Contrary to our prediction, the strongest suppressor of PEV (i.e., the line presumed to have the most bulk heterochromatin, and thus the most Y-linked repetitive DNA) did not exacerbate the female-biased sex ratio among survivors. Indeed, PEV suppression was a poor predictor of progeny sex ratio. However, we discovered one Y chromosome that consistently altered the ratio of female:male survivors compared to all other Y chromosomes tested. Our data implicate the existence of particular Y chromosome features that pose distinct, Blm-dependent challenges to DNA replication. Currently, we are exploring both the identity of these repetitive features and the molecular mechanism underlying Y-dependent replication challenges that arise during syncytial embryonic cycling.

D1071B Aging impairs double-strand break repair by homologous recombination in Drosophila. *H. A. Ertl*[1], L. Delabaere[2], D. Massey[1], F. Sohail[1], E. J. Beinenstock[1], H. Sebastian[2], I. Chiolo[2], J. R. LaRocque[1]. 1) Georgetown University Medical Center, Washington DC; 2) University of Southern California, Los Angeles, California.

Aging is characterized by genome instability, which contributes to cancer formation and cell lethality leading to organismal decline. The high levels of DNA double-strand breaks (DSBs) observed in old cells and premature aging syndromes are likely a primary source of genome instability, but the underlying cause of their formation is still unclear. DSBs might result from higher levels of damage or repair defects emerging with advancing age, but repair pathways in old organisms are still poorly understood. Here we show that pre-meiotic germline cells of young and old flies have distinct differences in their ability to repair DSBs by the error-free pathway homologous recombination (HR). Repair of DSBs induced by either ionizing radiation (IR) or the endonuclease I-SceI is markedly defective in old flies. This correlates with a remarkable reduction in HR repair measured with the DR-*white* DSB repair reporter assay. Strikingly, most of this repair defect is already present at 7 days of age. Finally, HR defects correlate with increased amount of Rad51 expression and recruitment to damage in old organisms, suggesting that HR in older flies is defective after Rad51 recruitment. These data reveal that DSB repair defects arise early as the organism ages, and, contrary to previous conclusions, suggest HR defects as a leading cause of genome instability in older animals.

D1072C Determining the role of a novel protein, Ankle1, in a resolvase complex of *Drosophila melanogaster*. *M. Hartmann*, J. Sekelsky. University of North Carolina at Chapel Hill, Chapel Hill, NC.

DNA double strand breaks are repaired through homologous recombination in mitotically dividing cells and germ line cells undergoing meiosis. Intermediates formed through homologous recombination are processed by structure-specific resolvases resulting in crossovers and noncrossovers. There are several known eukaryotic resolvases with a number of functions, but there are many aspects of the complexes yet to be uncovered. Specifically, I aim to elucidate the multiple complexes that function as resolvases and in what contexts they operate. Three main resolvases being studied include GEN1/Yen1, and the complexes MUS81-EME1 and SLX1-SLX4. In *Drosophila*, we believe MEI-9- ERCC1 in conjunction with MUS312 (SLX4) is the major meiotic resolvase. However, there are residual crossovers present in a *mus312* mutant and MUS312 may interact with other proteins in a mitotic context. Therefore, I hypothesize SLX4 assembles multiple complexes and that there are other proteins yet to be discovered forming resolvase complexes. MEI-9 and MUS81 are both nucleases that belong to the ERCC4 nuclease family of proteins, and both MEI-9 and MUS81 have binding partners that contain inactive nuclease domains. It is suspected that MUS81-EME1 work in concert with SLX1-SLX4 to resolve junctions, so due to the similarities between MUS81 and MEI-9, I hypothesize that there is another protein similar to SLX1 working in concert with the MUS312-MEI9-ERCC1 resolvase complex. We have identified a candidate gene, *Ankle1* that may act in a complex through interactions with the scaffolding protein MUS312 in concert with MEI-9 – ERCC1. *Ankle1* is a GIY-YIG nuclease domain protein, similar to SLX1. In humans, Ankle1 has been shown to possess endonuclease activities both in vitro and in vivo. I am interested in determining if the *Drosophila* Ankle1 protein has the same endonuclease properties. My goal is to study the *in vitro* activities of Ankle1 and correlate these data with genetics studies. Components of the enzyme complexes will be determined by direct protein-protein interactions and proteomic studies. Preliminary protein-protein interaction studies using the yeast two-hybrid assay have shown an interaction of Ankle1 with MUS312 and MEI-9. I have used the CRISPR/Cas9 gene editing tool to create a mutant of *Ankle1* in *Drosophila*, and I plan to use this mutant for further genetic characterization. Mitotic and meiotic repair assays will be done with single and multiple mutants in *Drosophila melanogaster* to deduce functions and epistatic relationships. Through this research, I will elucidate the interactions of Ankle1, while uncovering the roles of resolvases in recombination.

D1073A Does remodeling of the Myb-MuvB transcriptome promote the switch from mitotic cycles to endocycles? *M. Rotelli*, B. Calvi. Indiana University, Bloomington, IN.

The endocycle is a cell cycle variant that is composed of alternating G and S phases without mitosis. Repeated endocycles result in polyploidy and large cell size. Cells switch from mitotic cycles to endocycles (M-E switch) during the normal development of numerous organisms, including protozoa, insects, plants, and mammals. It is known that the endocycle oscillator is regulated by alternating activity of Cyclin E / CDK2 and APC/C^{cdh1}. Yet, the molecular mechanisms that remodel the mitotic cycle into the endocycle are mostly not understood. We previously analyzed the transcriptome of endocycling cells in the *Drosophila* larval salivary gland and fat body. This study revealed that endocycling cells have dampened expression of genes that are regulated by the E2F1 / Dp and Myb-MuvB (MMB a.k.a. dREAM) transcription factor complexes. It is well established that the MMB can act as either a transcriptional activator or repressor to regulate mitotic cell cycle progression in a variety of organisms, including humans. Many of the MMB-regulated genes that are required for mitotic entry / progression and cytokinesis were expressed at lower levels during fly endocycles. We are currently exploring whether remodeling of the MMB from a transcriptional activator to repressor promotes the M-E switch.

Genetic ablation of mitosis can create induced endocycling cells (iECs). For example, RNAi knockdown of Cyclin A or overexpression of the APC/C activating subunit Fzr (CDH1) both block mitosis and switch cells to an endocycle oscillator. These iECs represent an opportunity to investigate mechanisms of the M-E switch independent of developmental inputs. Our current results suggest that these iECs also have dampened expression of MMB-regulated genes. These results suggest that MMB activity is integrated with the master cell cycle control system, the mechanisms of which we are defining further. It has been shown that polyploid cells in several tissues of the mouse have a transcriptome signature similar to that which we described for fly endocycles. The insights into endocycle regulation revealed by our study, therefore, will likely be conserved across organisms including humans. Moreover, evidence suggests that some cancer cells inappropriately switch to endocycles, which may lead to genome instability and cancer progression. Therefore, this study also has broader impact for understanding the molecular circuitry of these cancer iECs.

D1074B Hippo signaling regulates tissue regeneration from a quiescent cell population. *J. H. Meserve*, R. J. Duronio. UNC-Chapel Hill, Chapel Hill, NC.

Following tissue damage, stem or precursor cells often undergo proliferation to replace lost cells. This proliferation is tightly regulated as inappropriate cell cycle re-entry can lead to tumor growth and cancer. It is unclear how proliferation, often of cells that are normally quiescent, is controlled following tissue damage. I have investigated the mechanism driving regeneration in a quiescent tissue using the *Drosophila melanogaster* eye imaginal disc as a model. The eye disc contains a population of quiescent, undifferentiated cells that re-enter the cell cycle and undergo compensatory proliferation following damage driven by *GMR-hid*. To identify genes involved in this process, I performed an RNAi screen of ~400 transcription factors. Knockdown of the transcription factor *scalloped* completely blocked compensatory proliferation. Knockdown of Scalloped's transcriptional co-activator Yorkie also disrupts compensatory proliferation. Furthermore, in *GMR-hid* discs, both Scalloped and Yorkie are required to drive accumulation of Cyclin E, a previously characterized transcriptional target that is essential for entry into S-phase. Yorkie is a transcriptional effector of the Hippo pathway, which has been implicated in regeneration in *Drosophila* and vertebrates. Our genetic data suggests that activation of Yorkie is likely driven by inhibition of Hippo pathway components by the LIM domain containing-protein Ajuba. Based on previous work in other labs and our own data manipulating cytoskeletal activity, we propose a model where changes in cellular tension induced by massive tissue extrusion drives activation of Ajuba and, ultimately, compensatory proliferation. In addition to the mechanism driving compensatory proliferation in the developing eye, we are also interested in the eventual fate of these compensatory proliferating cells, as it is currently unclear whether these cells can faithfully replace all cells lost following tissue damage. One possibility is cells that re-enter the cell cycle and proliferate are able to adopt all retinal cell type fates, including neuronal photoreceptors which differentiate before compensatory proliferation occurs; alternatively, the fate of these cells may be limited to lineages that have not yet differentiated at the time compensatory proliferation occurs. To investigate this question, I am performing lineage tracing experiments to determine the fate of these compensatory proliferating cells. These results will be important for understanding how cell fate might be affected following tissue damage and whether the mechanisms driving cell cycle changes, from quiescent to proliferating, also drive changes in cell fate that contribute to productive regeneration.

D1075C miRNA regulation of *dacapo* expression in the *Drosophila* embryo. *J. Petley*, C. Swanson. Arcadia University, Glenside, PA.

Cyclin-dependent kinase inhibitors (CKIs) play important roles in embryonic development by directly inhibiting the cell cycle machinery to promote cell cycle exit and limit tissue growth. The *Drosophila* CKI Dacapo (Dap) contributes to normal cell cycle exit in multiple developmental contexts, including the embryonic epidermis, nervous system, and eye. Because of its function as a potent cell cycle inhibitor, altered Dap expression can significantly disrupt normal development. For example, in the embryonic epidermis, premature Dap expression can induce early cell cycle exit, while the absence of Dap expression leads to delayed cell cycle exit and inappropriate cell division. Thus Dap expression must be carefully controlled to ensure normal tissue growth and differentiation. Indeed, multiple mechanisms have been shown to contribute to the regulation of Dap expression, including transcriptional regulation via a complex cis-regulatory region, miRNA-mediated translational inhibition, and regulated proteolysis via interaction with the $CRL4^{Cdt2}$ ubiquitin ligase. We have further examined the role of miRNA-mediated regulation of Dap expression in the embryonic epidermis. We have found that a *dap* transgene expressed under the control of the endogenous cis-regulatory region but lacking the endogenous 3'UTR is expressed prematurely in the embryonic epidermis. In addition, miRNA "sensor" transgenes that include the *dap* 3'UTR are repressed in comparison with controls. Finally, embryos lacking expression of a miRNA known to bind the *dap* 3'UTR, *miR-7*, exhibit premature *dap* expression. Future experiments will continue to explore the functional significance of miRNA-mediated repression of Dap expression in the embryonic epidermis.

D1076A Modulation of $CRL4^{Cdt2}$ activity in the syncytial embryo. *J. Speciale*[1], B. Duronio[2], C. Swanson[1]. 1) Arcadia University, Glenside, PA; 2) University of North Carolina, Chapel Hill,.

$CRL4^{Cdt2}$-mediated protein destruction is a developmentally critical phenomenon required for proper progression of both canonical and non-canonical cell cycles. $CRL4^{Cdt2}$ induces replication-coupled destruction of its substrates, which include the Drosophila proteins Double-parked (Dup), E2f1, and Dacapo (Dap). Despite its critical role in overseeing S phase progression and maintaining genome stability, we have observed modulation of $CRL4^{Cdt2}$ function in several developmental contexts. For example, our preliminary data suggest that $CRL4^{Cdt2}$ activity may be modified in the rapid, non-canonical cell cycles of the syncytial embryo. More specifically, we have observed differential stability of substrates in this context: Dup appears to be targeted for destruction during S phase as normal, while E2f1 and Dap are stable. These data suggest that $CRL4^{Cdt2}$ is active, but that at least two of its substrates are protected during these early cell cycles. We have identified sequences within the E2f1 protein that are required for protection in the early embryo. Continuing experiments seek to elucidate the mechanism, and ultimately the developmental function, of differential $CRL4^{Cdt2}$ substrate stabilization during early embryonic development. Our findings suggest that $CRL4^{Cdt2}$ activity may be modulated to accommodate unique cell cycle programs such as the rapid cycles of the syncytial embryo.

D1077B Identifying regulators of meiotic entry in male *Drosophila melanogaster*. *J. E. Tomkiel*[1], G. K. Yasuda[2], B. T. Wakimoto[3], B. Giri[1]. 1) Univ North Carolina, Greensboro, NC; 2) Seattle University, Seattle, WA; 3) University of Washington, Seattle, WA; 4) Univ. North Carolina, Greensboro, NC.

Many aspects of meiotic entry in male Drosophila are controlled by the activity of the meiotic specific *cdc25* homologue *twine, a* protein tyrosine phosphatase that activates p34cdc2. Mutations in *twine* and its translational regulators *boule* and *off-schedule* prevent meiotic entry. A second G2-M translational regulatory step occurs after *twine* expression, and is controlled by the Cytoplasmic Polyadenylation Element Binding (CPEB) protein orb2. Similarly, mutations in *orb2* result in a prolonged G2-M arrest. To identify other genes responsible for

regulating the G2-M transition, we have identified male sterile mutations that cause a similar meiotic phenotype. Seven chromosome 2 and eight chromosome 3 mutations were identified from a collection of previously identified male sterile mutations (Wakimoto BT, Lindsley DL and Herrera C., 2004). These represent thirteen complementation groups: twelve unique genes and one complementation group consisting of three mutations that fail to complement the amorphic *twineHB5* allele. We have taken a bioinformatics approach to confirm the 3 new twine alleles and identify the mutated genes in the 12 remaining lines. We created strains bearing four mutations each (i. e. transheterozygous for mutations on both chromosome 2 and 3), and performed whole genome sequencing at 11x coverage to identify the causative mutations. We identified new missense mutations in *twine* for each of the three non-complementing mutations. Towards identify the unknown genes, we have filtered variants in the genome sequence data by chromosome and eliminated variants shared between lines. We are presently completing recombination mapping with respect to dominant mutations to further delimit variants to candidate genes.

D1078C Epithelial tricellular junctions act as interphase cell shape sensors to orient mitosis. *F. Bosveld*[1], O. Markova[1], B. Guirao[1], C. Martin[1], Z. Wang[1], A. Pierre[2], M. Balakireva[1], I. Gaugue[1], N. Christophorou[1], D. K. Lubensky[3], N. Minc[2], Y. Bellaïche[1]. 1) Institut Curie, Paris, FR; 2) Institut Jacques Monod, Paris, FR; 3) University of Michigan, Ann Arbor, USA.

The orientation of cell division along the long axis of the interphase cell -the century -old Hertwig's rule- has profound roles in tissue proliferation, morphogenesis, architecture and mechanics. In epithelial tissues, the shape of the interphase cell is influenced by cell adhesion, mechanical stress, neighbour topology, and planar polarity pathways. At mitosis, epithelial cells usually adopt a round shape to ensure faithful chromosome segregation and to promote morphogenesis. The mechanisms underlying interphase cell shape sensing in tissues are therefore unknown. Here we show that in ***Drosophila*** epithelia, tricellular junctions (TCJs) localize microtubule force generators, orienting cell division via the Dynein-associated protein Mud independently of the classical Pins/Gα_i pathway. Moreover, as cells round up during mitosis, TCJs serve as spatial landmarks, encoding information about interphase cell shape anisotropy to orient division in the rounded mitotic cell. Finally, experimental and simulation data show that shape and mechanical strain sensing by the TCJs emerge from a general geometric property of TCJ distributions in epithelial tissues. Thus, in addition to their function as epithelial barrier structures, TCJs serve as polarity cues promoting geometry and mechanical sensing in epithelial tissues.

D1079A A new toolbox for the fly *Sciara* - a new/old model system that disobeys the rules for chromosome movement on spindles. *S. A. Gerbi*, Y. Yamamoto, J. Urban, J. Bliss. Brown Univ BioMed Division, Providence, RI, USA.

The fly *Sciara* has long been recognized as an outstanding model system to elucidate questions of chromosome mechanics. *Sciara* offers many unique biological features, several of which impact chromosome movement on spindles:

(a) chromosome imprinting; **(b)** a monopolar spindle in male meiosis I; **(c)** non-disjunction of the X chromosome in male meiosis II; **(d)** chromosome elimination in early embryogenesis; **(e)** sex determination; **(f)** evolution towards parthenogenesis; **(g)** germ line limited (L) chromosomes; **(h)** DNA amplification in salivary gland polytene chromosomes; **(i)** high resistance to radiation.

We have now developed a toolbox to enable *Sciara* research, and we welcome new investigators [http://brown.edu/go/sciara-stocks]. We are completing the *Sciara* genome with cutting edge approaches for assembly using long reads from the PacBio RSII and Oxford Nanopore MinION sequencing platforms, using Illumina reads for polishing, and using BioNano Irys optical maps for scaffolding. Genome annotation used RNA-seq data from the *Sciara* transcriptome interrogating both sexes at multiple stages. We have used the genomic data to identify sequences of "DNA puffs" that represent sites of DNA amplification in salivary gland polytene chromosomes regulated by ecdysone.

We have developed methods for transformation of *Sciara* to manipulate its genome. DNA has been introduced into ectopic sites in the *Sciara* genome using piggyBac. We present here a new method for site-specific integration of large DNA into the *Sciara* genome. Previously, others have accomplished this by homologous recombination (HR). However, HR is inefficient; instead, the preferred pathway in most cells is non-homologous end-joining (NHEJ), but its primary previous application has been to create small indels. We have coupled NHEJ with obligate ligation-gated recombination (ObLiGaRe) for high efficiency precise insertion of large DNA into a unique double-strand break. This approach is easily applicable to a broad range of organisms, including those where a transformation system has not been available.

With the new toolbox of the genome assembly and transformation methodology, the time is now ripe to elucidate many canonical processes using the unique biological features of *Sciara*.

D1080B Cell size regulation in Drosophila sensory organ precursor asymmetric cell divisions. *Nitya Ramkumar*[1], Nelio Rodrigues[2], Buzz Baum[1]. 1) University College London, London, GB; 2) The Francis Crick Institute Lincoln's Inn Fields Laboratory Room 605 44 Lincoln's Inn Fields London WC2A 3LY.

Asymmetric cell division is the unequal segregation of cell fate determinants into daughter cells following mitosis. In addition, some asymmetric divisions lead to daughter cells of unequal sizes. The regulation and functional significance of the unequal daughter cells size are unknown. To investigate this, we are using the bristle pattern in the adult fruit fly, which arises in the notum (dorsal thorax) during pupariation. The sensory organ precursor (SOP) cells undergo a series of asymmetric divisions to give rise to the cells of the mecahnosensory organ. During the first division, cell fate determinants, Numb and Partner of Numb localize to the anterior cortex, while Bazooka localizes to the posterior and each segregates into the anterior and posterior daughter cells respectively. In addition, the resulting daughter cells are of unequal sizes, a larger posterior cell and a smaller anterior cell. Chromosome passenger complex (CPC) are a complex of proteins, that localize early in mitosis to the kinetochores. During anaphase, they move from the kinectochores to the spindle midzone, and specify the position of the future cleavage furrow. Owing to their dynamic localization pattern during mitosis, we are investigating their role in the asymmetric SOP divisions.

Additionally, using genetic tools, we aim to perturb the daughter cell size and the relative size of the cells to determine the significance of cells size during lineage specification.

D1081C Characterizing the Role of Rough Deal (Rod) Protein in *Drosophila* Male Meiosis. *Qiutao He*, Bruce McKee. Department of Biochemistry and Cellular and Molecular Biology, The University of Tennessee, Knoxville, TN.

Meiosis is a specialized cell division in sexual reproduction which produces the haploid gametes from diploid precursor cells. Two separated stages are involved in this process – meiosis I and meiosis II. Meiosis I is considered as a reductional division since the homologous chromosomes segregate to opposite spindle poles while equational sister chromatids separation takes place in meiosis II. Accurate chromosome segregation enable the genetic fidelity and prevent the generation of aneuploidies. Spindle assembly checkpoint (SAC) is a surveillance mechanism to ensure completion of bipolar alignment before chromosome segregation and the role of SAC in meiosis has not been fully characterized. Rough Deal is a protein involved in SAC and responsible for the recruitment of other SAC-related proteins onto kinetochores during cell division. In mitosis, mutations in *rod* lead to stretched chromatid arms, anaphase lagging chromatids and chromosome mis-segregation while null mutations are lethal. In present study, we took advantage of a fully viable meiosis-specific *rod* allele to assess the role of *rod* in male meiosis. Genetic cross experiments exhibited high rates of sex chromosome nondisjunction in both stages of meiosis. Although chromosome morphology, conjunction and cohesion were normal throughout prophase I, the loss of centromere cohesion on autosomes appeared at metaphase I and later stages based on anti-CID immunostaining and FISH analysis. Cytological data in anaphase I revealed additional abnormalities, such as uneven chromosome segregation and lagging chromosome, and equational sex chromosome segregation was also observed at this stage. Future studies will concentrate on how *rod* mutation affect centromere cohesion and chromosome segregation in *Drosophila* meiosis. .

D1082A Cohesion without cohesin in *Drosophila* meiosis. *A. Mukherjee*, B. McKee. University of Tennessee, Knoxville, TN.

Meiosis is essential for sexual reproduction and therefore occurs in all eukaryotes, including single celled organisms that reproduce sexually. The proper segregation of homologous chromosomes and sister chromatids in meiosis requires multiple functions of a multi-subunit protein complex known as cohesin. Cohesin forms a ring around duplicated sister chromatids and prevents them from separating prematurely. In Drosophila, mitotic cohesin is composed of four subunits: the long coiled-coil proteins SMC1 and SMC3 and two non-SMC subunits SCC1/RAD21 and SCC3/SA and all four subunits are required for mitotic cohesion. However, RAD21 is dispensable for meiotic cohesion and although SMC1 and SMC3 are present on meiotic centromeres and on synapsed chromosome arms, as expected, their functions in meiosis remain poorly characterized. Moreover, unlike in other eukaryotes in which screens for meiotic cohesion genes have revealed meiosis-specific cohesin paralogs such as the conserved RAD21 paralog REC8, similar screens in Drosophila identified three novel genes that encode meiosis-specific centromere proteins (SOLO, ORD and SUNN (SOS)) with no apparent similarity to cohesins. Loss-of-function mutations in all three genes disrupt centromere cohesion and SMC1/3 centromere localization, and cause random chromatid segregation. We are investigating the role of cohesins in *Drosophila* male and female meiosis by using germ-line specific RNAi (RNA interference) to deplete one of the core cohesins - SMC3. When we knockdown SMC3 in the male germline we observe premature loss of cohesion between the sister centromeres of some (but not all) chromosomes during prophase I, and numerical mis-segregation of major autosomes (2^{nd} and 3^{rd}). Surprisingly, however, cohesion and segregation of the sex chromosomes is nearly unaffected by depletion of SMC3. Even more remarkably, SMC3 knockdown in the female germline has no apparent effect on centromere cohesion. This result is probably not due to incomplete depletion of SMC3 as synaptonemal complexes (which require SMC1 and SMC3 as components of the lateral elements) are completely eliminated in the SMC3-depleted oocytes. We show that despite the absence of cohesin proteins from centromeres, SOLO localizes normally to the centromeres in SMC3 knockdown in both male and female germlines. These results suggest the hypothesis that SOLO along with SUNN and ORD can provide cohesion to centromeres in the absence of the cohesins. This is the first clear evidence for a non-cohesin-based cohesion mechanism in any eukaryote.

D1083B The role of the endoplasmic reticulum during asymmetric cell division in *Drosophila melanogaster*. *S. A. Beyeler*. San Francisco State University, San Francisco , CA.

A crucial step for multicellular organism success is proper asymmetric cell division. Little is known about how organelles like the endoplasmic reticulum (ER) are partitioned during asymmetric cell division. While we know the ER will divide asymmetrically during asymmetric cell divisions, we don't know what is regulation this movement. Drosophila melanogaster offers a great model for asymmetric cell division, in their larval neural stem cell divisions. Our lab has shown the intermembrane ER protein, jaugunal, will partition to only one daughter cell during asymmetric cell division in embryonic neural stem cells. As jagunal null mutant flies die in 2^{nd} instar we want to investigate if this death is due in part to improper asymmetric cell division in larval neural stem cells. This lead to the hypothesis that jagunal is part of a regulation pathway partitioning the ER during asymmetric cell division in larval neural stem cells. To test this, we used fluorescent immunostaining with confocal microscopy to visualize known cell polarity markers, aPKC (localizes apically) and prospero (localizes basally), to see if they were affected during cell division by a lack of jagunal. We found that both aPKC and prospero localize properly in the absence of a functional jagunal. This could mean that jagunal and the ER is acting upstream of cell polarity to affect daughter cell fate. Future studies will investigate jagunal's role in notch/delta signaling in the larval brain to find a regulation mechanism for ER asymmetry.

D1084C To get more for less: Thermodynamic versus active mechanisms of the nucleolus assembly. *H. Falahati*, E. Wieschaus. Princeton University, Princeton, NJ.

Cells are unique in their ability to avert thermodynamic equilibrium by spending energy, and yet to exploit the laws of thermodynamic to minimize their energetic costs. Although biochemical assays can determine whether a single reaction is active or thermodynamically driven, addressing this question becomes particularly challenging for complex in vivo phenomena such as the formation of multicomponent membrane-less organelles. There is growing evidence supporting the possibility that such organelles form as a new phase, separating from the cytoplasm or nucleoplasm. Any in vivo assessment of this hypothesis, however, requires a robust and unambiguous manipulation of the factors affecting phase transition processes. To tackle this problem we have developed an in vivo approach for unambiguous disentanglement of a thermodynamically driven phase separation from an active process. This approach is based on the different predictions of each of the two models in response to changes in temperature. To employ this approach we used a microfluidics device for accurate temperature control during confocal imaging, and quantitative image analysis to study the mechanism by which six different nucleolar proteins localize to the nucleolus in *D. melanogaster* embryos. Our results indicate that the nucleolus assembly in vivo is an interplay between thermodynamically driven phase separations and active assembly processes, with the localization of the individual proteins following one of these two distinct mechanisms.

D1085A SIRS is a spindle-independent mechanism of chromosome separation in mitotic polytene tissues. *B. Stormo*, D. Fox. Duke University, Durham, NC.

The canonical cell cycle alternates rounds of chromosome replication with chromosome segregation. This process ensures that at metaphase each chromosome is composed of a single pair of chromatids that can be bi-oriented by the spindle and segregated evenly. However, many cells undergo an alternate cell cycle termed the endocycle in which multiple rounds of DNA replication take place without intervening segregation. Endocycled tissues are traditionally thought of as being terminally differentiated. However, as we previously showed in the Drosophila rectum, as well as following treatment with many common mitosis blocking drugs, endocycled cells return to the mitotic cell cycle. How cells respond to the challenge of segregating reduplicated chromosomes is not well understood.

The Drosophila rectum provides a developmentally accessible model to answer this question., During rectal development, cells undergo two rounds of the endocycle and then undergo two rounds of mitosis. We find that despite re-entering mitosis with re-duplicated chromosomes, papillar cell division is relatively error free. Using a combination of live-imaging, pharmacology, genetics, and classical cytology we have defined a process that we call Separation Into Recent Sisters (SIRS). SIRS is a spindle- independent process that separates the developmentally programmed polytene chromosomes in the Drosophila rectum into individual pairs of sister chromatids prior to anaphase.

In contrast to the perfectly executed polytene separation during SIRS, mitosis after aberrant (non-programmed) endocycles is highly error prone and results in aneuploidy and cell death. SIRS is lacking in such cells, and as a result polytenes form conjoined chromosomes at anaphase. Further, we have found that while conjoined chromosomes following induced endocycles activate the mad1/mad2 dependent spindle checkpoint, SIRS instead relies on a checkpoint-independent function of mad2 to regulate the length of mitosis. Together, these data give important insights into how endocycled cells respond when they return to mitosis. These findings have relevance to both developing polyploid tissues as well as the many pathological conditions in which polyploidy plays a role.

D1086B ER-associated membrane contribution to cellularization furrows in *D. melanogaster* embryo morphogenesis. *E. Holt*, B. Riggs. San Francisco State University, San Francisco, CA.

Cellular blastoderm formation in the early *Drosophila melanogaster* embryo occurs as a synchronous compartmentalization of thousands of cortical nuclei during a specialized cytokinetic event known as Cellularization. This process is characterized by the formation of cellularization furrows invaginating between adjacent nuclei. This invagination has been shown to require the reorganization of intracellular membrane stores through endocytic pathways and most recently has been associated with morphological changes to microvillar membrane reservoirs. Currently it is not known if there are additional intracellular components involved in proper furrow formation and as such we are interested in the role of the endoplasmic reticulum and its capacity to act as an intracellular source of membrane. In preliminary investigations we find the ER-associated small GTPase Rab10, known to mark areas of ER-tubule growth, localizes to the ingressing cellularization furrow. We hypothesize that ER-tubule growth constitutes a novel source of membrane contribution during cellularization furrow formation. Here we investigate the role of Rab10 during morphogenesis of the early *D. melanogaster* embryo. Using time-lapse confocal microscopy we find that cellularization furrow formation kinetics are negatively affected in Rab10-deficient embryos using Rab10.RNAi under GAL4/UAS control. This data suggest a possible role for ER-associated membrane addition during growth phases of furrow ingression. A more detailed functional analysis is necessary to identify the role of ER growth during cellularization.

D1087C A cell cycle-regulated ArfGAP-Arf1 pathway for Golgi organization and cleavage furrow biosynthesis. *F. Rodrigues*, W. Shao, T. Harris. University of Toronto, Toronto, Ontario, CA.

Biosynthetic traffic drives plasma membrane growth. For early cleavage of the *Drosophila* embryo, this growth is rapid, but regulated, for repeated cycles of furrow ingression and regression. We report that furrow ingression requires Asap, a conserved Arf GTPase activating protein (GAP). Although Asap has several known effects, our genetic analyses argued that furrow loss with *asap* RNAi was linked predominantly to reduced Arf1 function at the Golgi. Moreover, Arf1 levels at the Golgi displayed a direct relationship with total cellular levels of Asap. Although Asap lacks Golgi enrichment, a conserved residue for Arf1 regulation by Asap was required for Golgi organization and output. We propose that

Asap maintains Arf1 activity at the Golgi by recycling Arf1 from post-Golgi compartments. This role appears essential for cell division, and is thus unlike the roles of characterized Golgi Arf GAPs which act redundantly for cell viability. Strikingly, Asap re-localized to the nuclear region at metaphase, a shift that coincided with transient Golgi re-organization preceding cleavage furrow regression. Thus, we further propose that Asap sequestration underlies periods of Golgi attenuation and furrow regression during the cell cycle.

D1088A Anillin and Citron Kinase, Sticky, collaborate during the contractile ring-to-midbody ring transition. *D. Wernike*, N. El Amine, A. Kechad, G. Hickson. Sainte-Justine Hospital Research Center, Montreal, Quebec, CA.

Cytokinesis, the final phase of cell division, physically separates one cell into two daughter cells. Its spatiotemporal control is crucial for metazoan development, and failures in the cytokinetic machinery often are associated with the formation of cancer. During cytokinesis, an actomyosin contractile ring (CR) constricts and transitions into the midbody ring (MR), a structure that forms around the midbody as it matures from the midzone microtubules (MTs) that become compacted during CR closure. In *Drosophila* S2 cells, we recently showed that the conserved scaffold protein Anillin promotes the transition from CR to MR. During this maturation process, the Citron kinase Sticky retains Anillin's N-terminus for incorporation into the MR, while the septin Peanut acts on the C-terminus of Anillin to remove excess Anillin and membrane via shedding. We found that Anillin-depleted S2 cells not only fail to form a proper MR, but also are deficient in forming a constricted zone of MTs at the midbody. Interestingly, Sticky-depleted cells, which also fail MR formation and cytokinesis, exhibit no earlier MT defects at the midbody. Hence, MR formation (Sticky-dependent) and midbody maturation (Sticky-independent) are two separable Anillin-dependent events, which we aim to further dissect in this study. Using a diverse panel of Sticky and Anillin truncations combined with live imaging and RNAi of endogenous proteins, we are currently defining the contributions of specific domains of Sticky and Anillin required for their normal CR localization and retention at the MR. Because shedding depends on Anillin, and not on Sticky, removal of Sticky likely occurs via its association with Anillin. Surprisingly, we found that Sticky-ΔRBD-FP is shed, but fails to retain Anillin at the MR, suggesting that Sticky's association with Anillin and its ability to form a stable MR may be separate events. Also, our preliminary data from immunoblotting suggest that the very N-terminus of Anillin is sufficient to pull-down Sticky from S2 cell lysate. Taken together, this work will strengthen our understanding about how the cell cortex is organized and re-organized in a mechanistic way to ensure the robustness of cytokinesis.

D1089B Mechanotransduction mechanisms in compensatory cellular hypertrophy. *K. Morimoto*[1,2], E. Suzuki[2], W. Deng[3], Y. Tamori[2]. 1) Keio University, Fujisawa, JP; 2) National Institute of Genetics, Mishima, JP; 3) Florida State University, Tallahassee, FL.

In multicellular organisms, tissue integrity and organ size are maintained through removal of aberrant or damaged cells and compensatory proliferation. The sensing and removal of aberrant cells by their neighbors involve cell competition, a remarkable homeostatic process at the cellular level. In proliferating tissues, cell division is the primary strategy winner cells use to compensate for the loss of loser cells during cell competition. In post-mitotic *Drosophila* follicular epithelia, however, we have shown that the loss of local tissue volume resulting from loser-cells elimination triggers sporadic cellular hypertrophy to repair the tissue. This ''compensatory cellular hypertrophy'' (CCH) is implemented by polyploidization through the endocycle, a variant cell cycle composed of DNA synthesis and gap phases without mitosis, dependent on activation of the insulin/IGF-like signaling (IIS) pathway. Furthermore, several lines of evidence in our study suggest that the IIS-dependent CCH is triggered by tensile forces resulting from the elimination of loser cells. Using stretched follicle cells as a model system, we found that a transient receptor potential (TRP) channel is involved in the process of mechanotransduction. Given the fact that similar hypertrophic cellular growth can be observed in different contexts such as *Drosophila* epidermis, mammalian hepatocytes or corneal endothelial cells, the tension-induced compensatory cellular hypertrophy is likely a conserved strategy for postmitotic tissue homeostasis.

D1090C A screen using FijiWings reveals the E3 Ubiquitin Ligase Neuralized as a novel Tribbles target. *A. Shipman*, L. Dobens. Sch Biological Sci, UMKC, Kansas City, MO.

The Drosophila wing is an ideal tissue to examine how cell proliferation is coordinated with cell growth to pattern proper tissue size. We have developed FijiWings, a set of macros that automate plug-ins in the ImageJ/Fiji software, to accomplish morphophometric analysis of wing features. FijiWings uses pattern recognition to outline wing veins and calculate tissue size and image processing to identify individual trichomes and calculate cell size. Using a misexpression approach, we have shown previously that FijiWings can effectively detect changes in both tissue and cell size resulting from manipulations of the insulin signaling pathway. We have used FijiWings to reveal that misexpression of the Tribbles pseudokinase, which binds and blocks the activity of key regulatory molecules that mediate insulin-regulated cell growth and cdc25-triggered cell proliferation, decreases overall wing tissue size but increases the size of individual cells. Using Fijiwings, we screened the Basler collection of growth regulatory genes for modifiers of the Tribbles wing misexpression phenotype and identified several potential Trbl interactors, including multiple E3 ubiquitin ligases. One of these E3 ubiquitin ligases is Neuralized, a component of the Notch signaling pathway, and here we present data from several approaches designed to validate the interaction between Tribbles and Neuralized to better understand a general mechanism for Tribbles regulation of RING-finger E3 ubiquitin ligases.

D1091A Crosstalk between mitochondrial fusion and the Hippo pathway in controlling cell proliferation during *Drosophila* development. *Q. Deng*[1,2], T. Guo[1,2], X. Zhou[2,3], Y. Xi[1,2], X. Yang[1,2], W. Ge[1,2]. 1) Institute of Genetics, Zhejiang University School of Medicine, Hangzhou,China 310058; 2) College of Life Sciences, Zhejiang University, Hangzhou, China 310058; 3) Skate Key Laboratory of Biotherapy and Cancer Center, West China Hospital,Sichuan University, Chengdu, China.

Cell proliferation and tissue growth depend on the coordinated regulation of multiple signaling molecules and pathways during animal development. Previous studies have linked mitochondrial function and the Hippo signaling pathway in growth control. However, the underlying

molecular mechanisms are not fully understood. Here we identify a Drosophila mitochondrial inner membrane protein ChChd3 as a novel regulator for tissue growth. Loss of ChChd3 leads to tissue undergrowth and cell proliferation defects. ChChd3 is required for mitochondrial fusion and removal of ChChd3 increases mitochondrial fragmentation. ChChd3 is another mitochondrial target of the Hippo pathway, although it is only partially required for Hippo pathway mediated overgrowth. Interestingly, lacking of ChChd3 leads to inactivation of Hippo activity under normal development, which is also dependent on the transcriptional co-activator Yorkie (Yki). In addition, depletion of two other mitochondrial fusion compoents, Opa1 and Marf, inactivates the Hippo pathway as well. Taken together, we propose that there is a crosstalk between mitochondrial fusion and the Hippo pathway which is essential in controlling cell proliferation and tissue homeostasis in Drosophila.

D1092B Yorkie activates transcription by recruiting the histone deacetylase HDAC1 and promoting histone deacetylation. *W. Ge*[1,2,4], F. Wang[1,2], Z. Nan[1,2], Z. Wang[1,2], J. Zhang[3], P. Guo[1,2], J. Lv[1,2], X. Xu[1,2], L. Wu[1,2], T. Guo[1,2], Q. Deng[1,2], Q. Zhou[3], X. Yang[1,2,4], Y. Xi[1,2,4]. 1) Institute of Genetics, Zhejiang University School of Medicine, Zhejiang University, Hangzhou, China 310058; 2) College of Life Sciences, Zhejiang University, Hangzhou, China 310058; 3) Life Sciences Institute, Zhejiang University, Hangzhou, China 310058; 4) Department of Genetics, Zhejiang University School of Medicine, Zhejiang University, Hangzhou, China 310058.

Control of organ size is a fundamental aspect in biology and plays important roles in development. The Hippo pathway is a conserved signaling cascade that controls tissue and organ size through the regulation of cell proliferation and apoptosis. Here, we report on the roles of *Drosophila* histone deacetylase HDAC1 in regulating the Hippo signaling pathway. Loss of HDAC1 function causes tissue undergrowth and the downregulation of Hippo target gene expression. Furthermore, Hippo pathway-mediated overgrowth and target gene expression are dependent on HDAC1. Mechanistically, we show that HDAC1 associates with the transcriptional coactivator Yorkie (Yki), and they co-occupy sites on chromatin at Hippo target gene loci. Finally, we provide evidence that Yki recruits HDAC1 to promote histone H3 lysine 27 (H3K27) deacetylation at Hippo target genes. Taken together, our findings suggest that recruiting of the histone deacetylase HDAC1 to Hippo target genes by Yki is required to activate gene expression, and highlight a positive role for HDAC1 in controlling specific gene expression program.

D1093C Role of Tctp interaction with a septate junction protein Coracle in imaginal disc development. *S. Lee*. KAIST, Daejeon, KR.

Translationally controlled tumour protein (TCTP) is a highly conserved protein in eukaryotes and has been implicated in multiple events, including growth control. To find new roles of TCTP *in vivo*, we performed biochemical and genetic screens for TCTP-interacting genes. From this screen, we identified Coracle (Cora) as a new binding partner of TCTP. Cora is a septate junction protein of the protein 4.1 family required for maintaining the transepithelial barrier and the apical basal epithelial polarity together with Yurt, Neurexin Ⅳ and Na^+/K^+ ATPase. We show that *cora* mutations enhance the phenotypes of TCTP knockdown by RNAi expression, resulting in synthetic lethality or headless flies depending on the affected tissues. Biochemical analyses indicate that Cora forms a complex with TCTP by direct binding. Loss of Cora causes decreased levels of TCTP protein and affects Arm expression. Loss of TCTP also shows reduction of Cora and Arm expression. Further, TCTP and Cora show overlapping localization in epithelial tissues. We also identified additional biochemical and genetic interaction of TCTP with other septate junction genes. Taken together, this study suggests a novel linkage between TCTP and the septate junction proteins implicated in growth regulation.

D1094A A matter of growth and death: An unexpected output of the Hippo Network in the *D. melanogaster* trachea. *S. S. McSharry*, C. Feng, G. J. Beitel. Northwestern University, Evanston, IL.

The infamous and highly conserved Hippo Network, well known for controlling cell and tissue size, also regulates the size of the tube-shaped tracheal system of the developing fruit fly, but in an unexpected way. Mutations in HN genes cause tracheal size phenotypes that are the opposite of the same mutations in other tissues. For example, a loss-of-function mutation in Yorkie (Yki), the transcription factor regulated by the Hippo Network, yields reduced eye or wing size, due in part to increased apoptosis. However, as we have previously reported, the same Yki mutation causes tracheal overgrowth without affecting cell number. This phenotype is ultimately due to loss of Yki-dependent over-activation of Ice, the fly homologue of the mammalian executioner caspase-3, a protein that is both necessary and sufficient to execute cell death in most cells. Here, we report that activated (cleaved) caspase is enriched at the apical surface of tracheal cells in both wild-type and Yki mutant embryos. This result suggests that the non-apoptotic target(s) of Ice that regulate tube size may also be localized to the apical surface. Moreover, compartmentalization of Ice at the apical surface may allow activation of non-apoptotic targets without cleavage of apoptotic targets. Increased staining of activated Ice is observed in some tracheal tube-size control mutants, and we are presently using this assay to investigate which tracheal genes may regulate tracheal tube size by modulating Ice activity. We also present results from epistasis experiments that explore the complex relationships between and among members of the HN such as Yki and Ice, and known regulators of tracheal size, including components of the septate junction.

D1095B Kibra and Merlin orchestrates activation of Hippo pathway core kinases cassette independent of Expanded *in vivo*. *T. Su*, M. Ludwig, R. Fehon. The University of Chicago, Chicago, IL.

Kibra, Merlin and Expanded function as upstream components of the Hippo pathway to regulate the activity of the core kinase complex, consisting of Hippo, Salvador and Warts. Previous biochemical studies indicated that Kibra, Merlin and Expanded form a complex and function together to activate the downstream kinases, but genetic experiments have suggested that Merlin and Kibra function in parallel to Expanded. To better understand the functional relationships between upstream components in relation to the core kinases, we have examined their functions in living tissues using fluorescently-tagged, endogenously expressed pathway components. Interestingly, while a recent study has shown that Expanded organizes pathway components in the apical junctional region of imaginal epithelial cells, we find that Merlin and Kibra

organize a distinct signaling complex at the apical cortex that is medially rather than junctionally localized. Gain- and loss-of-function experiments indicate that Kibra plays a key role in organizing this apical medial signaling center by recruiting both Salvador and Hippo. Merlin functions to promote Kibra-mediated recruitment of Salvador. Furthermore, we have found that Crumbs, which is localized junctionally, functions to negatively regulate the activity of this signaling complex by tethering Kibra at the junctions, despite the fact that loss of function Crumbs mutants have a modest overgrowth phenotype. Together, our studies reveal the complexity with which Hippo pathway activity is regulated, and suggest new mechanisms for pathway control in developing epithelia.

D1096C Characterization of *rio* (*CG11340*) as a regulator of tissue-specific growth in the larval trachea of *Drosophila*. *Robert Ward*, Kayla Wilson, Erin Suderman. Univ Kansas, Lawrence, KS.

During post-embryonic development in animals, organs and tissues grow at different rates relative to each other, which is likely tied to the unique requirements of each organ's function during development. Tissue-specific growth mechanisms can generate allometric differences that vary between individuals within a species and play critical roles in interspecific divergence. The fact that organs grow at different rates suggests that there must be tissue-specific mechanisms to control their differential post-embryonic growth trajectories, although surprisingly we know little about them. To gain a better understanding of these tissue specific growth mechanisms, we have been characterizing mutations that specifically alter the growth of the larval trachea in *Drosophila melanogaster*. Larval trachea growth is well suited for these studies since the trachea shows allometric growth during the larval stages, the trachea can be imaged and measured in living animals, gene expression can be specifically altered in the trachea using *breathless-GAL4*, and genes have been identified that appear to regulate tissue-specific growth in the larval trachea. Specifically, animals with mutations in *uninflatable (uif)* and *Matrix metalloproteinase 1 (Mmp1)* have larval tracheae that are roughly half the relative size of those in wild type animals. Through a screen of EMS-induced larval lethal mutations, we identified three allelic mutations that show substantially increased trachea to body length proportions in late third instar larvae. We named this gene *rio* based upon its long and winding tracheal phenotype. Whole genome sequencing followed by deficiency and specific mutation complementation tests revealed that *rio* mutations map to *CG11340*, which encodes a glycine gated chloride channel. Characterizing of *rio* mutants indicated that the enhanced tracheal growth occurs primarily in late 3rd instar, and in an extended late larval period (beyond when their heterozygous siblings pupariate). Enhanced tracheal size is not associated with increased cell number per metamere. In addition, the taenidia in *rio* mutant trachea appear normal. Interestingly, Uif expression appears to be generally higher in *rio* mutant trachea, with small numbers of individual cells showing a stochastic strong upregulation of Uif expression. *RNAi* of *CG11340* in trachea using *breathless-GAL4* recapitulates these tracheal phenotypes, whereas *RNAi* of *CG11340* in various imaginal discs have no obvious effect on growth or differentiation of these tissues.

D1097A Genetic manipulations of EGF ligands in motor neurons affect myoblast proliferation and muscle patterning in Drosophila. *Darren Wong*, Kole Sedlack, Erin Enright, Katie Lincoln, Arya Chalke, Kumar Vishal, Fernandes Joyce. Miami University, Oxford, OH.

Two phases of myogenesis occur in *Drosophila*- the embryonic period during which larval muscles develop, and the pupal period during which adult muscles develop. Adult myogenesis is dependent on innervation and embryonic is not (Fernandes and Keshishian, 1999). This allows us to explore how the nerve may signal to myoblasts or organizer cells. This is important, because vertebrate skeletal muscles also depend on innervation for their development. We use the indirect flight muscles of the fly as a model system- these ar esome of the largest muscles in the fruifly. Based on previous studies [Fernandes and Keshishian, 2005], the influence of motor neurons on myoblast proliferation was proposed to be brought about through the founder cell. This has been confirmed by 2 sets of preliminary data (1) Ablation of founder cells causes a 30% reduction in BrDU positive myoblasts, which is similar to what is seen during denervation (2) Blocking EGF signal reception in myoblasts causes 20% reductions in proliferation and causes patterning defects in 95% of animals. To test if the motor neuron was the source of signals, we have used two approaches- Blocking the synthesis of EGF ligands and overexpressing the signals. The number of adult nuclei in sections of adult muscles can be used as an indicator of the extent of myoblast proliferation. Our results thus far show that spitz and vein are predominant EGF ligands that mediate the neuronal influence on organizer cells to influence myoblast proliferation. Blocking synthesis with RNAi caused decreased number of nuclei in adult muscles, and conversely overexpression caused an increase in the number of nuclei. Our results indicate that the EGF signaling pathway operates during the motor neuron mediated proliferation of myoblasts during myogenesis.

D1098B Investigating the role of epithelial curvature in growth control. *Lina ZHANG*[1,2], Tiantian JI[1,2], Xianwei CHEN[1,2], Hongkai LIU[1,2], Yan YAN[1,2]. 1) HKUST, Division of Life Science, Hong Kong, China; 2) HKUST, Center for Systems Biology and Human Health, Hong Kong, China.

Epithelial cells are highly polarized cells with distinct apical and basolateral domains defined by specific protein complexes. The conserved Scribble module localizes at the basolateral region. It consists of three scaffold proteins, namely Scribble (Scrib), Discs large (Dlg) and Lethal giant larvae (Lgl). The Scribble module was originally identified in *Drosophila* as a group of "neoplastic tumor suppressor genes" (nTSGs). *Drosophila* imaginal disc cells homozygous mutant for the Scribble module genes grow into tumors that are disorganized, undifferentiated, lethal and transplantable. In mammals, loss of the Scribble module function is also linked with epithelial tumor progression.

We noticed that the cells deficient for the Scribble module show location-dependent growth phenotypes. Specifically, the Scrib and Dlg deficient cells can only grow into tumor-like masses in the highly curved epithelial regions including the hinge region of wing imaginal discs and the polar region of the follicular epithelia. We found that the ectopic activation of myosin II activity induces folding in the flat wing pouch region and rescues the Scrib and Dlg deficient cells from cell competition-induced elimination. The folded wing hinge region exhibits higher Yki activity. Moreover, MDCK and MCF10A cells cultured on curved surfaces display differential YAP/TAZ activity correlating with the substrate

curvature. These data indicate that the curvature might play a role in modulating the epithelial baseline Yki activity and thus influence the growth outcome of the nTSG mutant cells.

D1099C Elucidating the role of various signaling pathways in *lgl* derived tumors in *Drosophila* egg chambers. *Deeptiman Chatterjee*, Wu-min Deng. Florida State University, Tallahassee, FL.

Major signaling pathways have been conserved between *Drosophila* and humans, making the former a good model organism for cancer research. Loss of polarity and tissue organization being one of the prominent characteristics of epithelial-derived cancers, it has been widely studied in *Drosophila*. Some of the first apical-basal polarity genes that regulate tissue organization have been studied in *Drosophila* egg chambers, the spatiotemporal development of which is quite well characterized. Many of these genes act as tumor suppressors in the epithelial follicle cells as they maintain the polarity and prevent defective overgrowth in the epithelia. One such tumor suppressor is the *lethal Giant Larvae* or *lgl*, a polarity-determining gene that works in consort with others such as *dlg* and *scrib* to characterize the basolateral region of the epithelial cell. Loss of *lgl* function results in rampant loss in polarity and an overgrowth phenotype. The spatiotemporal dynamism of follicular epithelia provides a unique opportunity to elucidate how various signaling pathways, namely the *JAK-STAT, JNK, Hippo* and *Notch*, act with each other to promote overgrowth and metastasis in *lgl*-derived tumors. Using various cell signaling reporters, antibody staining and tissue-specific Gal4 drivers to knockdown gene expression, we have found growth-promoting roles of JAK-STAT and JNK pathways in *lgl* tumors. We have also found that while the Hippo pathway is important in promoting Notch signaling in the regulation of cell differentiation and growth, its disruption causes a loss of polarity in the epithelia. We have also investigated the role of the *Hippo* and *Notch* pathways in causing overgrowth phenotype in *Drosophila*..

D1100A Pointed/ETS acts as a novel tumor suppressor that regulates Ras-mediated cellular senescence. *T. Ito*[1], M. Enomoto[1], T. Igaki[1,2]. 1) Laboratory of Genetics, Graduate School of Biostudies, Kyoto University, Kyoto, Japan; 2) PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan.

Oncogenic activation of Ras is often not sufficient for driving malignant progression, because it can cause premature senescence. This suggests that mutations in the regulators of cellular senescence could cooperate with oncogenic Ras to drive tumor progression. Here, we performed a genetic screen in *Drosophila* eye imaginal discs to identify dominant mutations that cause tumor progression in conjunction with oncogenic Ras (Ras^{V12}). Using the MARCM technique, we induced mosaic expression of Ras^{V12} in the eye discs and simultaneously introduced a series of heterozygous chromosomal deficiencies. As a result, we identified a mutation in the ETS transcriptional activator Pointed (Pnt) as a strong dominant-enhancer of Ras^{V12}-induced tumorigenesis. In addition, overexpression of Pnt dramatically inhibited Ras^{V12}-induced tumor overgrowth. Interestingly, we found that loss of Pnt abrogated Ras^{V12}-induced cellular senescence. Our data show that Pnt acts as a novel tumor-suppressor that regulates Ras-mediated cellular senescence in *Drosophila*.

D1101B Myc regulates cytoophidium formation. *J. L. Liu*, G. Aughey, S. Grice. Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3PT, United Kingdom.

The coordination of metabolism with cell growth is critical for regulation of organismal development. Therefore there is significant interplay between metabolic enzymes and key developmental regulators such as transcription factors. The enzyme CTP synthase (CTPsyn) is essential for metabolic homeostasis as well as growth and development, due to its role in synthesising precursors for many fundamental cellular macromolecules such as RNA and lipids. However, the mechanisms by which CTPsyn is regulated during development are little understood. Here we have shown that Myc, an oncogene and a key development regulator, is necessary and sufficient for the assembly of CTPsyn-containing macrostructures termed cytoophidia. We show that the presence of CTPsyn is required for Myc to mediate its effect on cell growth during *Drosophila* development. Roles for CTPsyn and Myc in tumourigenesis have been well established and both proteins have been considered promising therapeutic targets. By better understanding the relationship between these two proteins, we can gain important insights, not only into tumour pathology and aetiology, but also metazoan developmental processes.

D1102C A distinct tumor suppressor role of Snr1 from the SWI/SNF complex in *Drosophila* imaginal tissues. *Gengqiang Xie*, Wu-Min Deng. Florida State University, Tallahassee, FL.

Components of the SWI/SNF chromatin-remodeling complex are among the most frequently mutated genes in various human cancers, yet only SNF5, a core member of the SWI/SNF chromatin-remodeling complex, is found mutated in malignant rhabdoid tumors (MRTs), which are primarily found in young children. How SNF5 functions differently from other members of the SWI/SNF complex remains unclear. Here we use *Drosophila* imaginal epithelial tissues to demonstrate that Snr1, the conserved homolog of human SNF5 (hSNF5), prevents tumorigenesis by ensuring normal endosomal-trafficking-mediated signaling cascades. We show that disruption of *snr1* causes massive apoptosis in the wing pouch area and obvious overgrowth in the notum region of imaginal discs. Blocking apoptosis by coexpression of the anti-apoptotic protein p35 in *snr1*-knockdown cells results in neoplastic tumorigenic overgrowth in imaginal epithelial tissues, whereas depletion of any other members of the SWI/SNF complex does not induce similar phenotypes. The *snr1*-knockdown-induced tumors show multiple hallmarks of cancer, including sustained proliferation, altered metabolism, and dedifferentiation. Unlike other components of the SWI/SNF complex which are only detected in the nucleus, Snr1 is observed in both the nucleus and the cytoplasm. The cytoplasmic function of Snr1 is probably necessary for endosomal trafficking, because components of the trafficking signaling pathway are enriched in *snr1*-depleted cells and transmembrane proteins are accumulated in the cell surface. Further experiments demonstrate that aberrant regulation of multiple signaling pathways, including Notch, JNK, and JAK/STAT is responsible for *snr1*-loss-of-function-mediated tumor progression. RNA profiling of *snr1*-

depleted tumors by RNA-Seq analysis confirms that, indeed, several signaling targets are transcriptionally degregulated. Our data provide an alternative way to understand the mechanism of how hSNF5 suppresses MRTs during early childhood. The detailed results will be presented in the 57th Annual *Drosophila* Research Conference @TAGC.

D1103A Oncogenic properties of Troponin-I. *S. Casas-Tinto*, A. Ferrús. Cajal Institute, Madrid, Madrid, ES.

Human tumors of various tissue origins show an intriguing overexpression of genes not considered oncogenes, such as that encoding Troponin-I (TnI), a well-known muscle protein. Out of the three TnI genes known in humans, the slow form, *TNNI1*, is affected the most. *Drosophila* has only one gene that encodes all forms of TnI, *wupA*. Here, we carried out a study on excess- and loss-of function conditions for *wupA* in *Drosophila*, and assayed *TNNI1* down regulation in human tumors growing in mice. *Drosophila* TnI excess-of-function causes tissue overgrowth and potentiates the phenotypes due to oncogenic mutations in Ras, Notch and Lgl genes. By contrast, TnI loss-of-function reduces proliferation and antagonizes the overgrowth due to these oncogenic mutations. The conserved Hippo signaling pathway requires the up-regulation of TnI for tumor overgrowth. Troponin-I defective cells exhibit aberrant number of centrosomes, multiple double strand DNA breaks and undergo Flower-dependent cell competition in the wing disc. Human tumor cell lines treated with a human Troponin-I peptide arrest in $G_{0.}$ In addition, the proliferation of non-small-cell lung carcinoma xenografts in mice is restrained by down-regulation of the *TNNI1* gene. Thus, Troponin-I reveals as a novel oncogene that links abnormal cell signaling, proliferation, genome integrity and tumor growth, and may open the possibility for a novel therapeutic target/marker.

D1104B A shared loser molecular signature identifies stress signaling pathways common to loser cells, which play distinct roles in cell competition. *M. P. Dinan*, I. Kucinski, E. Piddini. Gurdon Institute, University of Cambridge, Cambridge, UK.

Cell competition is a process by which a population of fitter cells (winners) outcompetes neighbouring weaker cells (losers), inducing their elimination by cell death or differentiation. This mechanism has been proposed to a play role in tissue health and turnover and in disease states such as cancer. There are many known mutations, which can induce a loser status, however despite its discovery four decades ago, the molecular properties of cells that earmark them as losers have not been identified.

To identify molecular differences between winner and loser cells we generated and analysed RNAseq data from control (wild-type) cells and from a subset of loser mutations, from *Drosophila* wing imaginal discs in the absence of cell competition. We focused on two mutations, which are seemingly functionally unrelated, yet share the loser phenotype: *Minute* (ribosomal) heterozygous mutations and a mutation in *mahj* (involved in cell polarity and protein degradation). To enrich for genes involved in the loser cell status we focused on the intersection of genes that were differentially expressed in both of these mutants compared to the wild type. This intersection includes genes indicative of a constitutively activated stress response, encompassing JNK, JAK/STAT, p53/DNA damage and oxidative stress response pathways. We then assessed the functions of these pathways in loser cells. While some pathways have an autonomous pro-survival role, we found that others can specifically modulate cell competition. In particular the JAK/STAT signaling pathway, activated by constitutive production of Unpaired ligands from loser cells, is important not only for loser cell proliferation, but also for cell competition, as it is responsible for the overgrowth of winner cells. Thus, winner cell overgrowth does not require winner-loser recognition, but stems directly from excessive availability of proliferative signals.

In summary, loser cells carrying *Minute* and *mahj* mutations exhibit a common molecular signature, which involves activation of stress and pro-survival pathways. JAK/STAT signaling autonomously promotes proliferation while, at the same time non-autonomously boosts growth of the competing winner population.

D1105C Dissecting a common principle underlying cell competition and wound healing. *C. Iida*, S. Ohsawa, M. Yamamoto, T. Igaki. Kyoto university graduate school of biostudies, Kyoto, Kyoto, JP.

Cell competition is a form of cell-cell interaction that can eliminate oncogenic mutant cells from the epithelia tissue. For instance, clones of oncogenic cells mutant for apico-basal polarity genes such as *scrib* or *dlg* are actively eliminated from the tissue when surrounded by wild-type cells. We have recently identified the Sas-PTP10D system as a ligand-receptor system required for this tumor-suppressive cell competition. To investigate the physiological role of the Sas-PTP10D system, we focused on the process of wound healing, as it is crucial for epithelial homeostasis and has been considered to share a common principle with cancer, namely cancer as an overhealing wound. After cutting the *Drosophila* wing imaginal disc, the wound is usually healed during development and it generates a normal wing in the adult. We found that defects in the Sas-PTP10D system significantly inhibited the wound healing. Our data also indicated that the Sas-PTP10D system regulates wound healing by modulating the Hippo pathway through EGFR and JNK signaling. The mechanism by which the Sas-PTP10D system regulates wound healing will be discussed.

D1106A Functional analysis of the Ribosome protein mutant in cell competition. *Zhejun Ji*, Abhijit Kale, Marianthi Kiparaki, Nicholas Baker. Albert Einstein College of Medicine, Bronx, NY.

Cell competition can occur between distinct genotypes within multicellular organs. We mapped a recessive mutation that prevents competition of RpL36+/- cells to the gene encoding Ribosomal Protein S12. Null alleles of RpS12 are recessive lethal, and we have generated several transgenic fly lines and showed that null alleles can be rescued by genomic constructs lacking the major introns and their encoded snoRNAs, confirming that it is the protein coding region that is essential. We have also produced RpS12 specific antibodies and demonstrated their specificity by immunoprecipitation and immunostaining assays. We have described the localization of the RpS12 protein in wild type and Minute genotypes, and in the suppressor allele for cell competition. Immunostaining of Drosophila salivary glands and wing imaginal discs

showed that RpS12 is mainly distributed in the cytoplasm. A substantial amount of RpS12 is also detected around the nucleolus. There is no clear difference for the cellular localization among these three genotypes. Future work will be focused on mapping the region of RpS12 protein that is important in cell competition and its mechanism of action.

D1107B RpS12 role in cell competition. *Marianthi Kiparaki*, Zhejun Ji, Abhijit Kale, Nicholas Baker. Albert Einstein College of Medicine, Bronx, NY, NY.

Cell competition is a homeostatic mechanism, which non-autonomously determines the fate of the cells- to die (lose) or to thrive (win). This phenomenon was originally described 40 years ago to occur between wild type cells (winners) and cells that are haploinsufficient for ribosomal proteins (which are called Minute cells-losers). Since then, cellular differences in a handful of genes have been described to trigger cell competition. Previous members in our lab conducted an EMS unbiased genetic screen to identify mutations that permit the survival of Minute cells during cell competition. Interestingly, one of these mutations was mapped in the ribosomal protein gene RpS12. RpS12 homozygous mutant flies are viable, fertile, with no obviously identifiable phenotype. We are currently in the process of investigating the role of RpS12 in cell competition. By sucrose density gradient analysis, we have shown that the mutant form of RpS12 is able to assemble into the ribosomes in vitro (S2 cells) and in vivo (fly tissues). Our polysome analysis also showed that RpS12 exists in extra-ribosomal fractions. Our efforts are now being directed towards the characterization of the potential extra-ribosomal role of RpS12 by purification of RpS12 complexes. It is tempting to think RpS12 as an internal ribosome-quality control factor that helps to identify the less ribosome-efficient cells in the tissue and eliminate/restrict them for the flourishing of the organism.

D1108C Epithelial Slit-Robo signaling regulates tumor-suppressive cell competition. *J. Vaughen*[1], T. Igaki[1,2]. 1) Laboratory of Genetics, Graduate School of Biostudies, Kyoto University; 2) PRESTO, Japan Science and Technology Agency (JST).

Dynamic cellular interactions occur between heterogeneous cell populations during both development and tumorigenesis. Intricate cellular communication also underpins 'cell competition', a conserved tumor-suppression mechanism whereby aberrant "loser" cells are actively eliminated by neighboring "winner" cells. For example, while *Drosophila* tissue mutant for conserved apicobasal-polarity gene *scribble* (*scrib*) forms lethal neoplasms, mosaic clones of *scrib* cells are eliminated by neighboring wild-type cells. While cell competition is conserved in vertebrates and can similarly function as a tumor-suppressor, the mechanisms underlying how cell competition enforces homeostasis remain incompletely understood.

To gain insight into cell competition, we conducted a genetic deficiency screen in *Drosophila* eye epithelia mosaic for *scrib* and wild-type cells. We identified the axon-guidance signaling system, Slit-Roundabout (Robo), as essential for proper *scrib* cell elimination. Heterozygosity for the secreted ligand *slit* or the transmembrane receptors *robo1* or *robo2,* but not *robo3*, caused aberrant *scrib* clone overgrowth. Moreover, Slit-Robo antibodies accumulated around specific populations of apically extruding *scrib* cells. Through genetic perturbation of Slit-Robo within or around *scrib* clones, we demonstrate that Slit acts locally on Robos inside *scrib* cells to extrude the aberrant loser cells away from the main epithelium. One source of the Slit ligand required for *scrib* cell competition is *scrib* loser cells, suggesting that Slit-Robo signaling is autocrine in the context of *scrib* competition.

Surprisingly, while Robo-overexpression in *scrib* cells drastically enhanced apical extrusion of *scrib* clones, it also unexpectedly blocked *scrib* cell elimination. Instead, excessive extrusion merged *scrib* clones in the apical lumen and prevented proper cell competition. Thus, balanced Slit-Robo signaling and extrusion are essential for *scrib* cell competition and homeostasis. Notably, either loss or gain of Slit-Robo signaling is associated with various human epithelial cancers. Because cell competition also eliminates human cell lines mutant for *scrib*, epithelial Slit-Robo signaling could broadly enforce tumor-suppression and epithelial homeostasis through the removal of aberrant, precancerous loser cells.

D1109A The ligand Sas and its receptor PTP10D drive tumor-suppressive cell competition. *M. Yamamoto*, S. Ohsawa, K. Kunimasa, T. Igaki. Kyoto University, Kyoto, Japan.

Normal epithelial cells often exert anti-tumor effects against neighboring oncogenic cells. In *Drosophila* imaginal epithelium, clones of oncogenic cells mutant for apico-basal polarity genes such as *scrib* or *dlg* are actively eliminated from the tissue by cell competition when surrounded by wild-type cells. We and other groups have previously shown that JNK signaling is required for this cell elimination. However, the initial event occurring at the interface between normal cells and polarity-deficient cells remained unknown. To address this, we performed an EMS-based genetic screen in *Drosophila* eye imaginal disc and identified the ligand Sas and the receptor-type tyrosine phosphatase PTP10D as the cell-surface ligand-receptor system that drives tumor-suppressive cell competition. At the interface between wild-type "winner" and polarity-deficient "loser" cell clones, winner cells relocalize Sas to the lateral cell surface while loser cells relocalize PTP10D to the lateral cell surface. This leads to trans-activation of Sas-PTP10D signaling in loser cells that restrains EGFR signaling, thereby enabling elevated JNK signaling in loser cells to cause cell elimination. In the absence of Sas-PTP10D, elevated EGFR signaling in loser cells switches JNK's role from pro-apoptotic to pro-growth by impairing Hippo signaling, thereby causing overproliferation of polarity-deficient cells. These findings uncover the mechanism by which normal epithelial cells recognize oncogenic neighbors to trigger cell competition.

D1110B The subcellular distribution of Tribbles is linked to the regulation of growth and patterning by insulin and BMP signaling pathways. *L. L. Dobens*, Zachary Fischer, Jin-Yuan Fan, Alexander Nail. Univ Missouri, Kansas City, Kansas City, MO.

The pseudokinase Tribbles is a conserved adaptor protein that binds and mediates the proteasomal degradation of key signaling mediators to balance tissue growth and proliferation. Tribbles family members are found in all animals and share conserved targets, notably Akt kinase, which Tribbles binds and degrades to block insulin responses. Recently, mammalian Tribbles 3, has been shown to bind SMURF to block Smad

4 degradation and promote BMP signaling in tissue culture cells. We used a structure-function analysis of the highly conserved central pocket domain of Tribbles to identify a mutation Trbl[SLE/G] that changes the protein's subcellular distribution from cortical/nuclear to strongly cortical. This change in subcellular distribution of Trbl[SLE/G] results in a strong block of Akt phosphorylation and insulin-mediated growth. Misexpression of Trbl[SLE/G] in the wing unexpectedly disrupts cross vein formation suggesting that Tribbles may regulate BMP signaling and in support of this, we observe increased levels of phosphoMad levels in cell misexpressing Trbl[SLE/G]. We will present on-going work in several model tissues to test the notion that the conserved pocket domain of Tribbles regulates its subcellular distribution with consequent effects on BMP- and insulin-mediated growth.

D1111C Prominin-like, a homology of mammalian CD133, regulates body size by affecting dilps and inhibiting TOR pathway in *Drosophila*. *H. Zheng*[1], X. Wang[1], P. Guo[1], Y. Zhang[2], W. Ge[1], Y. Xi[1], X. Yang[1]. 1) ZheJiang University, HangZhou, CN; 2) Beijing Genomics Institute,ShangHai,CN.

CD133, a pentaspan membrane glycoprotein, is extensively used as a surface marker to identify and isolate stem-like cells. However, the molecular and biological functions of CD133 are not well understood. Here, we demonstrate that the *Drosophila* Prominin-like, a homology of mammalian CD133, regulates body size. We find that Prominin-like expression levels are regulated by ecdysone signaling and increase dramatically in the fat body during metarmorphsis. Interestingly, the *prominin-like* mutants exhibit bigger body size and excess body weight accompanied with higher fat deposits. Varied dilps transcriptional levels and up-regulated TOR are observed in the mutants. Whilst, overexpressing Prominin-like in insulin-producing cells (IPCs) induces a smaller body size. Taken together, these findings reveal that Prominin-like controls fly body size through affecting dilps and inhibiting TOR pathway, which may suggesting a novel function of CD133 on metabolism in cancer stem cells (CSCs) and cancers.

D1112A Acinus Links Autophagy and Hippo Signaling. *L. K. Tyra*, N. Nandi, H. Kramer. UT Southwestern Medical Center, Dallas, TX.

In a genome wide screen for interactors of Drosophila Acinus, a regulator of autophagy and endocytic trafficking, we discovered a strong genetic interaction between Acinus and members of the Hippo signaling pathway. For example, loss of one copy of Hippo suppresses the rough eye phenotype produced by GMR-Gal4 over-expression of Acinus. Additionally, over-expression of Acinus suppresses overgrowth caused by over-expression of the gain of function Yorkie-S168A. In order to better understand these genetic interactions, we screened Acinus gain and loss of function for developmental growth phenotypes. Over-expression of Acinus in the wing causes small wings with Engrailed-Gal4, Ms1906-Gal4, or Nubbin-Gal4. Similar to known negative regulators of Yorkie, knock-down of Acinus by two independent RNAi lines using the Engrailed-Gal4, Nubbin-Gal4, or Ms1096-Gal4 drivers causes wing over-growth. Finally, RNAi knock-down of Acinus in the wing causes increased expression of the *expanded, four-jointed,* and *thread (diap1) LacZ* reporters, which are used to measure Yorkie transcriptional activity. Taken together, these data suggest that Acinus is a negative regulator of the transcriptional output of the Hippo signaling cascade. A complex, yet poorly understood connection between autophagy and Hippo signaling has been shown in *Drosophila*; however a clear mechanism to connect these two critical processes remains unknown. Understanding if the connection between Acinus and Hippo signaling is autophagy dependent is a current focus of our research. Moreover, elucidating the mechanism by which Acinus is repressing transcriptional targets of Hippo signaling will provide valuable insight into its function in cellular processes critical for developmental cell growth, as well as cancer.

D1113B The interaction of social environment with genetic background, expression of Extra-Cellular Superoxide Dismutase and oxidative stress in *Drosophila melanogaster*. *J. D. Parker*[1,2], T. Segre[1], J. Tromblee[1], J. Binns[2]. 1) SUNY Plattsburgh, Plattsburgh, NY; 2) University of Southampton, Southampton UK.

Ruan and Wu (2008) discovered that social environment (specifically the presence of young individuals) can rescue the phenotype of a cytoplasmic superoxide dismutase mutant. We extended these findings by testing the presence of young individuals on an under expressing extra-cellular superoxide dismutase (EC SOD3) mutant fly line (Blackney et al. 2014), with and without an acute oxidative stress. We observed that young helpers extended the lifespan of the EC SOD3 mutants relative to the control line after both were exposed to paraquat. We also found that the presence of young helpers extended lifespan on one of two wild caught lines, but only after they were cleared of Wolbachia. We conclude that the observed positive social effects on aging seen in Ruan's study are dependent on other aspects of the enviornment including oxidative stress, endosymbiont infection and genetic background.

D1114C Hypoxia inhibits insulin signaling and regulates lipid metabolism in *Drosophila melanogaster* larvae through CDK8-dependent inhibition of the Gbb pathway. *z. Shen*, J. Martinez-Agosto. UCLA, Los angeles, CA.

How an organism responds to oxygen availability in order to modulate its own metabolism balance remains an unresolved question in the field of developmental biology. In our study, we use Drosophila larvae as a model to study how hypoxia disrupts metabolism homeostasis. We have previously shown that hypoxic conditions cause growth restriction and lipid aggregation in larger droplets in the larval fat body, the main site of fat metabolism. However, the mechanism of lipid aggregation remains unknown. Here we demonstrate that Gbb signaling decreases in larval fat body tissue under hypoxia conditions. Overexpression of the ligand, Gbb does not rescue these lipid metabolism defects under hypoxia conditions. However, overexpressing an activated form of Mad protein, the transcription factor downstream of GBB signaling, rescues hypoxia-induced lipid metabolism defects in larval fat body. Therefore, a decrease in Gbb signaling is responsible for the larval lipid metabolism defects observed under hypoxia conditions. Interestingly, down-regulation of CDK8 in the larval fat body restores both lipid metabolism defects and Mad activity under hypoxic conditions. Therefore, an increase in CDK8 activity decreases GBB signaling and plays an important role in

hypoxia-induced lipid metabolism defects.

We have previously demonstrated that the larval growth defects observed under hypoxic conditions are due to a block in insulin signaling. Drosophila insulin-like peptides (DILPs) are produced by insulin-producing cells (IPC) located in the larval brain. After production, they are secreted to promote protein synthesis and cell growth. Our laboratory has previously shown that hypoxia disrupts the insulin signaling pathway by causing retention of Dilp2 in the IPCs. Fat body specific over-expression of InR under hypoxia conditions does not rescue lipid metabolism defects under hypoxic conditions. However, knockdown of the downstream negative regulator of InR signaling, PTEN rescues hypoxia-induced fat body lipid loss. Interestingly, fat body specific knockdown of PTEN under hypoxic condition also increases Mad activity under hypoxia conditions. Therefore, inhibition of GBB and insulin signaling pathways regulates fat body lipid storage under hypoxic conditions.

In conclusion, a reduction in insulin signaling and enhanced activity of CDK8 in the fat body causes a decline in Gbb/BMP signaling that is required for the maintenance of proper lipid metabolism during times of limited oxygen availability.

D1115A Assessing the Role of *Drosophila melanogaster* HP1B Protein in Aging. *A. Thomas*, B. Mills, N. Riddle. UAB, Birmingham, AL.

Aging is marked by a gradual change in macromolecules that, over time, negatively affect biological processes such as gene expression and the maintenance of chromatin structure. Age-related aberrant gene expression can lead to a number of health complications, making age the single greatest risk factor for the development of many human diseases. Heterochromatin proteins play important roles in chromatin structure and gene regulation, suggesting a link to aging. To further investigate, we have chosen the *Drosophila melanogaster* protein HP1B, a member of the Heterochromatin Protein 1 (HP1) family of proteins. HP1 proteins are highly conserved across eukaryotes, playing a vital role in the formation and maintenance of heterochromatin. To probe for a connection between HP1B and aging, we assayed two *HP1b* null mutant strains for phenotypes related to aging. We measured longevity, as well as various forms of stress resistance. Our results show that fly strains lacking HP1B have both increased starvation and oxidative stress resistance. In addition, although they have shorter maximum lifespans compared to the wildtype, they have a longer average lifespan. These results support a potential connection between chromatin proteins and aging and suggest that chromatin proteins should be evaluated as targets for future aging therapies.

D1116B *Fs(1)h* is required in fat body for normal lifespan, metabolism, and immune function in *Drosophila*. *Jessica Sharrock*[1,2], Marc S. Dionne[2]. 1) Department of Immunobiology and Centre for the Molecular and Cellular Biology of Inflammation, King's College London, London SE1 1UL, UK; 2) Department of Life Sciences and MRC Centre for Molecular Bacteriology and Infection, Imperial College London, South Kensington Campus, London SW7 2AZ, UK.

Bromodomain containing proteins (BCPs) play important roles in transcriptional regulation and chromatin remodelling. The inhibition of these proteins affects the production of pro-inflammatory cytokines by macrophages, suggesting they play an important role in the regulation of immunity and inflammation. The main aims of this project were to identify how tissue specific knockdown of BCPs in *D. melanogaster* affects survival, the production of antimicrobial peptides and metabolic function. RNAi was used to specifically knock down each of the BCPs in the *Drosophila* genome in the fat body, an organ which plays important roles in the humoral immune response and metabolism. Following the RNAi screen one gene of interest was identified, *Female sterile (1) homeotic* (*Fs(1)h*). *Fs(1)h* has been shown to be critical for proper patterning of the early embryo, but its functions in the adult fly are essentially unknown. Flies with *Fs(1)h* knocked down in the fat body alone show reduced lifespan, increased expression of immune-response genes known as antimicrobial peptides, and strong metabolic changes. These metabolic changes include increased triglyceride levels and reduced free sugar and glycogen, and are accompanied by a severe reduction in phosphorylated AKT (pAKT), an important downstream effector of PI3 kinase. Remarkably, reduction of *foxo* in *Fs(1)h* knockdowns completely abolishes their short-lived phenotype. Furthermore, reduced *foxo* dosage also eliminated overexpression of antimicrobial peptides in uninfected *Fs(1)h* knockdowns; the effect on antimicrobial peptide expression after infection was heterogeneous. In conclusion, *Fs(1)h* is required in fat body for normal lifespan, metabolism and immune function; its effects on lifespan and uninfected immune activity are mediated by a requirement for *Fs(1)h* in regulation of Akt activity.

D1117C The microbiota affects ADH protein level and influences alcohol sensitivity in Drosophila. *M. A. Blundon*, A. M. Pyzel, T. W. Lau, S. L. Oliver, R. Eutsey, J. A. Cohen, J. H. Huang, N. L. Hiller, J. S. Minden, B. M. McCartney. Carnegie Mellon University, Pittsburgh, PA.

Symbiotic relationships between microbes and animals are ubiquitous in nature. The animal microbiota is a vast and diverse population of bacteria and other microbes living symbiotically with their hosts that has a profound influence on many aspects of host physiology, including metabolism, immunity, development, and behavior. However, little is known about the molecular crosstalk between microbes and host that mediate these effects, particularly in the area of brain function and behavior. To address this problem, we employed a proteomic technique call Two-dimensional Difference Gel Electrophoresis (2D-DIGE) to examine the protein differences in heads between conventionally-reared (CV) flies and "germ free" (GF) flies, those raised in a sterile environment. We found that the level of Alcohol Dehydrogenase (ADH), a key enzyme in alcohol metabolism, is elevated in the heads of GF males and females, suggesting that GF flies would be less sensitive to the effects of ethanol (EtOH) exposure. Indeed, we found that GF males are significantly less sensitive (T_{50}=16.4 mins) to the immobilizing effects of EtOH vapor than their CV counterparts (T_{50}= 11.8 mins). This increased EtOH sensitivity is partially reverted when restoring the normal Drosophila microbiota to GF flies 0-24 hours after eclosion. This suggests that the microbiota plays an important role in the response of Drosophila to EtOH vapor. We are currently testing whether the microbiota also affects EtOH recovery, tolerance, and preference in Drosophila. These data provide a new insight on the effect of the microbiota on alcohol metabolism that may lead to a deeper understanding of the microbiota's role in alcoholism. .

D1118A A temperature-dependent shift in dietary preference alters the viable temperature range of *Drosophila*. *M. Brankatschk*[1], T. Gutmann[2], B. Brankatschk[2], M. Grzybek[2], U. Coskun[2], S. Eaton[1]. 1) MPI-CBG, Dresden, Saxony, DE; 2) PLID, Dresden, Saxony, DE.

How cold-blooded animals adapt their behaviour and physiology to survive seasonal changes in temperature is not completely understood - even for well-studied model organisms like *Drosophila melanogaster*. Here, we show that *Drosophila* respond to high and low temperature extremes by modifying their feeding behaviour. Above 15˚C, *Drosophila* feed and lay eggs on yeast. In contrast, below 15˚C, *Drosophila* prefer to feed and lay eggs on plant material. The different lipids present in yeast and plants improve survival at high and low temperatures, respectively. Yeast lipids promote high temperature survival by increasing systemic insulin signalling. This expands the range over which developmental rate increases with temperature, suggesting that faster nutrient utilization is required to fuel biochemical reactions driven faster by kinetic energy. In addition to speeding development, yeast lipids increase fertility. Thus, yeast provide cues that could help Drosophila to exploit a transient summer food resource. Plant lipids, on the other hand, maintain membrane lipid fluidity at low temperature, and increase cold-resistance of larvae and adults. The cold-resistance and lowered insulin signalling conferred by feeding on plants allows adults to survive for many months at temperatures consistent with overwintering in temperate climates. Thus, temperature-dependent changes in feeding behaviour produce physiological changes that could promote seasonal adaption.

D1119B A genetic screen for novel neuronal genes regulating lifespan extension in *Drosophila melanogaster*. *T. Chen*, W. Lien, C. Chan. National Taiwan University, Taipei, Taiwan.

Rab27 is a neuron-specific GTPase expressing in a small subset of brain neurons in *Drosophila melanogaster*. Recently we found that loss of *rab27* leads to lifespan extension and this effect is dosage-dependent. To discover the genetic network regulating lifespan extension within *rab27*-expressing neurons, we perform an F1 modifier screen. The *rab27Gal4-KO* allele is not only a null, but also possesses Gal4 activity that allows for expression within *rab27*- expressing neurons including the Mushroom bodies and the Insulin-producing cells. In heterozygous *rab27KO* background, approximately 2000 GS lines would be expressed by *rab27Gal4-KO* to identify modifiers of *rab27*-independent longevity phenotype. Similarly, a screen in hemizygous *rab27Gal4-KO* background was performed to identify novel *rab27*-independent regulators of lifespan. Known lifespan regulators such as *hsp27* and *S6K* were isolated from the screen, indicating the validity of the screen. For secondary screen, we set a statistical cutoff to determine top candidates. To distinguish the neuron-specific lifespan extensors, genes that extend lifespan via expression from fat bodies were excluded. To examine whether these genes exerts neuronal or organismic protection, both paraquat- and starvation-induced oxidative stress tests were performed. To reveal the underlying mechanisms, further examinations are ongoing, including metabolic analyses of hemolymph glucose and stored energy of TAG. *In vivo* analyses of several neuron-specific stress-resistant genes will be presented.

D1120C Functional characterization of multiple promoter alleles of the *bellwether* gene in *Drosophila melanogaster*. *J. Frankenberg Garcia*[1,2], M. A. Carbonne[2], T. F. C. Mackay[2], R. R. H. Anholt[2]. 1) School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK; 2) Program in Genetics, W. M. Keck Center for Behavioral Biology, and Department of Biological Sciences North Carolina State University, Raleigh, NC, USA.

Lifespan varies among individuals, but the genetic factors that contribute to variation in lifespan are not completely understood. *Drosophila melanogaster* presents a powerful genetic model system to explore the genetic underpinnings of longevity, since its lifespan is limited and both the genetic background and environment can be controlled precisely. A previous study identified sequence variants associated with differences in lifespan between five long-lived lines originally selected for delayed reproduction (O lines) and their five unselected controls (B lines). Two single nucleotide polymorphisms in the promoter of the *bellwether* (*blw*) gene (A>G and G>T) were associated with differences in lifespan between the O and B lines. To assess whether associated polymorphisms in the *blw* promoter may contribute to differences in lifespan by modulating gene expression, we amplified 500 bp upstream promoter sequences containing all four possible haplotypes (AG, AT, GG, GT) and assessed promoter activity in an *in vitro* luciferase reporter system. Our results show that the AG haplotype characteristic of the B lines showed ~18% greater expression of luciferase than the GT haplotype, which is associated with the long-lived O lines. Current experiments are designed to assess to what extent alternative *blw* alleles modulate lifespan in a common genetic background. The *blw* gene codes for the alpha subunit of the mitochondrial ATP synthase, and thus may represent a possible molecular link between metabolic rate and lifespan.

Supported by NIH grant R01-AG043490..

D1121A Endogenous L-2-hydroxyglutarate synthesis coordinates aerobic glycolysis with epigenetic modifications in *Drosophila.* *Alexander Hurlburt*, Hongde Li, Maria Sterrett, Geetanjali Chawla, Jason Tennessen. Indiana University, Bloomington, IN.

2-hydroxyglutarate (2HG) has emerged as a potent oncometabolite that inhibits enzymes involved in metabolism, chromatin modification, and cell proliferation; however, neither the D-2HG nor the L-2HG enantiomers are tumor specific. In fact, both compounds are synthesized in a diversity of cells, but the presence of 2HG in healthy tissues is commonly disregarded as a byproduct of metabolic error. This presumption is further supported by observations that the only known causes of 2HG accumulation are hypoxia and mutations in either *Isocitrate Dehydrogenase* or in the enzymes that degrade these compounds. We have discovered that L-2HG is more than an aberrant metabolite, as *Drosophila* larvae generate millimolar concentrations of L-2HG during normal development. Larval L-2HG, however, does not accumulate simply to due defects in metabolic repair pathways. Rather, we have found that this compound is the direct product of aerobic glycolysis, as most L-2HG is generated from glucose and dependent on activation of the *Drosophila* Estrogen-Related Receptor, which promotes L-2HG synthesis by up-regulating *Lactate Dehydrogenase* (*Ldh*) gene expression. As a result, high levels of larval LDH activity directly synthesize L-2HG for 2-oxoglutarate. Furthermore, we have found that the resulting L-2HG pool is stabilized, in part, by lactate, which inhibits L-2-

hydroxyglutarate dehydrogenase (L-2HGDH) activity and controls L-2HG abundance. Finally, we demonstrate that L-2HG influences DNA methylation and position effect variegation, indicating that this compound coordinates metabolism with epigenetic modifications during fly development. In conclusion, our findings suggest that L-2HG is more than an aberrant compound produced by enzymatic infidelity, but rather acts as part of an ancient mechanism to coordinate glycolytic flux with chromatin formation.

D1122B Hemocytes regulate responses to nutrition via the Jak/STAT pathway. *K. Kierdorf*, J. Dou, M. S. Dionne. Imperial College, Department of Life Sciences and MRC Centre for Molecular Bacteriology and Infection, London, UK.

Hemocytes are the myeloid cells of the fly. In adult flies, 95% of hemocytes are plasmatocytes, which are similar to tissue resident macrophages in mammals. Upon infection or inflammation, hemocytes phagocytose invaders and release different cytokines. However, less is known about the physiological function of hemocytes and how they affect tissue homeostasis and metabolism. Recently, it was shown that resident tissue macrophages in the fly are major regulators of glucose metabolism and lifespan upon metabolic stress. Adult flies on a lipid-enriched diet showed enhanced storage of triglycerides, hyperglycemia, and decreased insulin sensitivity followed by a severe reduction in lifespan. In this context, hemocytes release the Jak/STAT activating cytokine *unpaired-3* (*upd3)* and over-activate the Jak/STAT pathway in various organs including gut and muscles; this is a major driver of shortened lifespan due to high-fat diet. *upd3* activation by lipid is dependent on the scavenger receptor *croquemort* and the Jun-kinase *basket*. Here, we further investigate the physiological consequences of macrophage cytokine release and dysregulation. We suggest that hemocytes might be a major nutritional sensor regulating tissue homeostasis via release of Jak/STAT-activating cytokines. We and others have observed that several tissues including adult muscle exhibit physiological Jak/STAT signaling in the absence of any overt inflammatory stimuli. As skeletal muscle is the major glucose consuming organ in most multicellular organisms, and lipid-driven *upd3* causes changes in glucose homeostasis, we are now exploring the impacts of hemocyte-derived cytokine signals on muscle metabolism and systemic health. Importantly, we suggest that cytokine signaling in this system is important in the response of muscles to nutritional changes, even in the absence of overt inflammatory stimuli.

D1123C The regulation of lipid storage by sex determination genes in *Drosophila*. *Cezary Mikoluk*[1], Alexis Nagengast[2], Justin DiAngelo[1]. 1) Penn State Berks, Reading, PA; 2) Widener University, Chester, PA.

Excess nutrients are stored as triglycerides mainly in the adipose tissue of an animal. These triglycerides are located in structures called lipid droplets within adipose cells. Previous genome-wide RNAi screens in *Drosophila* cells identified splicing factors that play a role in lipid droplet formation. Our lab identified the SR protein, 9G8, as an important factor in fat storage as decreasing its levels results in augmented triglyceride storage in the fat body. However, whether 9G8 interacts with other proteins to affect lipid metabolism is unclear. Previous *in vitro* studies have implicated 9G8 in the control of doublesex (DSX) splicing by binding to transformer (TRA) and transformer2 (TRA2) to regulate sex determination; any function of these proteins in regulating metabolism is unknown. The goal of this study is to determine whether 9G8 acts with TRA, TRA2, or DSX to regulate fat storage *in vivo*. To test this hypothesis, we measured triglyceride and glycogen levels in flies with TRA^{dsRNA}, $TRA2^{dsRNA}$, and DSX^{dsRNA} induced in the fat body. Decreasing TRA, TRA2 and DSX in the larval fat body has no effect on nutrient storage. However, decreasing the expression of these sex determination genes in the adult fat body resulted in an increase in triglyceride levels, a phenotype similar to the 9G8 knockdown flies. Interestingly, glycogen also accumulated when the sex determination genes were decreased in the adult fat body. To determine whether this increase in energy stores was the result of excess food consumption, we performed CAFÉ assays to measure feeding over a 24 hour period. Feeding was not increased in the TRA^{dsRNA}, $TRA2^{dsRNA}$, and DSX^{dsRNA} flies, suggesting the triglyceride and glycogen accumulation phenotype was not the result of increased food intake. Together, these results suggest a link between mRNA splicing, sex determination and lipid metabolism and may provide insight into the mechanisms underlying tissue-specific splicing and nutrient storage in the fat body.

D1124A Lipolytic effects on insulin signaling in the *Drosophila* larval fat body. *Emily Scott*, Stephen Roth, Nigel Muhammad, Michelle Bland. University of Virginia, Charlottesville, VA.

When nutrients are abundant, insulin-like peptides are secreted and promote the storage of glucose as triglycerides in lipid droplets in the fat body. During starvation, lipolysis is activated to provide energy for the body in the form of free fatty acids and glycerol. In mammals, fatty acids released during lipolysis also act intracellularly to dissociate the TORC2 complex, which is an upstream kinase in the insulin signaling pathway. Inhibiting lipolysis by adipocyte-specific deletion of *adipocyte triglyceride lipase* (*ATGL*) improves insulin sensitivity. Here we investigated whether lipolysis affects *Drosophila* insulin-like peptide (DILP) sensitivity in fruit fly larvae. We find that fat body-specific knockdown of the *Drosophila ATGL* homolog *brummer* (*bmm*) leads to triglyceride accumulation not only in the fasted but also in the fed state, indicating that *Drosophila* larvae exhibit basal lipolysis even during periods of nutrient intake. We examined whether loss of *bmm* affected DILP signaling in a model of inflammation-induced insulin resistance. Fat body-specific expression of the constitutively-active $Toll^{10b}$ receptor activates immune signaling and leads to reduced DILP signaling and, consequently, decreases in both triglyceride storage and cell size. We find that larvae expressing $Toll^{10b}$ in fat body are refractory to the triglyceride-elevating effects of *bmm* knockdown, suggesting that triglyceride storage defects in this background are due to reduced de novo lipogenesis and not elevated lipolysis. Accordingly, loss of *bmm* expression did not rescue cell size in clones of fat body cells expressing $Toll^{10b}$, indicating that elevated lipolysis does not play a role in DILP resistance on this background. DILP signaling can be stimulated acutely by incubating larvae at 37˚C for two hours, a stimulus that activates TORC2 through an unknown mechanism. Wild type type but not TORC2-deficient *rictor* null larvae exhibit a robust increase in phosphorylation at the TORC2 site on Akt (Ser505) following heat exposure. However, Ser505 phosphorylation is reduced when fasted, lipolytic larvae are exposed to heat, providing a new model in which to study lipolysis and insulin signaling. Together, our results suggest that the physiological states such as fasting immune

system activation, dictate the nature of the interactions that occur between the insulin signaling pathway and lipid anabolic and catabolic processes.

D1125B Studies of the *Drosophila Lactate Dehydrogenase* gene reveal compensatory metabolic networks that support larval development. *Jason M. Tennessen*, Geetanjali Chawla, Hongde Li, Maria C. Sterrett. Indiana University, Bloomington, IN 47405.

The metabolism of proliferating cancer cells not only generates energy, but also synthesizes the biomolecules required for growth. In response to these metabolic demands, cancer cells rely on a metabolic program known as aerobic glycolysis, which synthesizes biomass from carbohydrates. *Drosophila* also uses aerobic glycolysis to support the nearly 200-fold increase in body size that occurs during larval development and we are now using the fly to understand how the inhibition of aerobic glycolysis affects growth and physiology. Our initial studies have focused on the *Drosophila Lactate Dehydrogenase* gene (*Ldh*; also known as *ImpL3*), which encodes the hallmark enzyme of aerobic glycolysis. We have confirmed that LDH is widely expressed in larval tissues, with notably high levels occurring in the muscle and brain. Furthermore, this expression occurs independent of oxygen availability; however, LDH expression in the salivary glands and imaginal discs is enhanced under hypoxic conditions. Furthermore, we have generated *Ldh* loss-of-function mutations with the goal of understanding how this enzyme influences growth and biosynthesis. Our preliminary metabolic characterization reveals that *Ldh* mutants accumulate excessive glycogen stores and exhibit a 95% decrease in lactate production. However, despite these metabolic defects, *Ldh* mutants grow at a normal rate and experience an impenetrant lethal phase during the mid-L3. These relatively mild phenotypes suggest that larval metabolism can compensate for loss of LDH activity. To test this possibility, we analyzed the *Ldh* mutants using a combination of GC-MS-based metabolomics and RNAseq. These analyses revealed several putative compensatory metabolic networks that are activated in *Ldh* mutants. For example, loss of LDH activity induces a significant increase in glycerol-3-phosphate (G3P) metabolism, as mutant larvae exhibit a 300% increase in glycerol-3-phosphate levels and 4-fold increase in *glycerol-3-phosphate dehydrogenase* gene expression. Overall, these studies represent a unique opportunity to both understand how the disruption of aerobic glycolysis affects biosynthesis and to explore the metabolic plasticity that underlies larval growth.

D1126C The GATOR1 function in Drosophila development. *Youheng Wei*, Weili Cai, Mary Lilly. NICHD/NIH, Bethesda, MD.

Target of Rapamycin Complex 1 (TORC1) is a master regulator of metabolism in eukaryotes that integrates information from multiple upstream signaling pathways. The evolutionary conserved complex Gap activity towards Rags (GATOR) inhibits TORC1 activity in response to amino acid limitation. In yeast, GATOR1 mutants show proliferation defects during nitrogen source scarcity, but not when cultured in nutrient rich conditions. In humans, the GATOR1 complex has been implicated in a wide array of human pathologies including cancer and hereditary forms of epilepsy. These observations strongly suggest that in metazoans the GATOR1 complex has acquired functions beyond coordinating a response to amino acid limitation. However, the precise requirement of GATOR1 in animal physiology remains largely undefined. Here we generate null mutants of the GATOR1 components *nprl2*, *nprl3* and *iml1* in *Drosophila.* We find that all three mutants have inappropriately high TORC1 activity. Moreover the GATOR1 mutants are semi-lethal with a percentage of homozygous animals dying as pharate adults. Notably, the *nprl2* and *nprl3* mutant adults exhibit profound ataxia. This ataxia is metabolic in origin in that it is rescued by the expression of Nprl3 in the fat body, but not muscles or neurons, of *nprl3* mutants. Consistent with the theory that inhibiting TORC1 activity extends lifespan, the *nprl2* and *nprl3* mutants have shortened lifespan. Finally, our data confirm that in addition to its role in the development and physiology of Drosophila raised under standard culture conditions, the GATOR1 complex has retained a critical role in the response to nutrient stress. Notably, *nprl2* and *nprl3* mutants fail to activate autophagy in response to amino acid stress and are extremely sensitive to both amino acid and complete starvation. In summary we find that the TORC1 inhibitor GATOR1 contributes to multiple aspects of the development and physiology of Drosophila.

D1127A The contribution of host genetic variants to microbiota-mediated juvenile growth. *D. Ma*[1], Claire-Emmanuelle Indelicato[1], Maroun Bou-Sleiman[2], François Leulier[1], Bart Deplancke[2]. 1) IGFL, ENS de Lyon - CNRS UMR 5242 - INRA USC 1370, Lyon, Rhone-Alps, FR; 2) Laboratory of Systems Biology and Genetics (LSBG) EPFL-SV-IBI-LBSG,Station 19 CH-1015 Lausanne.

The metazoan gut microbiota comprises a rich and diverse community of commensal bacteria species. It plays a vital role to maintain systemic homeostasis in multiple host organs and thus sustains optimal host health. Consequently, various aspects of host development and physiology, ranging from juvenile growth to adult lifespan, are altered in germ-free (GF) animals. We have previous shown that on a low yeast diet, GF *Drosophila* experience retarded larval growth and delay in development. Interestingly, mono-associating axenic fly embryos with a single strain of the gut commensal species, *Lactobacillus plantarum* (**Lp^{WJL}**), can re-accelerate growth and partially restore developmental timing, and this growth-promotion effect is mediated through the host ToR signaling pathway.

To identify additional host genetic variants and networks that respond to the growth promotion effect via Lp^{WJL}, we conducted a genome-wide association study (GWAS) on the Drosophila Genetic Reference Panel (DGRP) based on the variation in larval growth difference between the GF and their Lp^{WJL} –monoxenic siblings among the strains. We obtained a set of polymorphisms associated to genes with novel and unknown functions, as well as genes involved in host metabolism, hormonal signaling and immune response. Here we present the validation of selected candidates implicated in host response to Lp^{WJL} –mediated juvenile growth. Specifically, we focus on the role of Dawdle, the activin-like TGF-β ligand that has been shown to regulate host metabolic adaptation to the nutritional environment. In summary, our finding suggests that the genetic basis of the juvenile host response to the presence of the intestinal microbiota is complex and poly-genic.

D1128B Genetic mapping and molecular characterization of the genes *lysine* and *red cell*. *S. L. St. Clair*[1], L. K. Golden[1,2], U. Ashraf[1], K. R. Cook[1,2], J. M. Tennessen[1]. 1) Indiana University, Bloomington, IN 47405; 2) Bloomington Drosophila Stock Center, Bloomington, IN 47405.

We are investigating a fifty-year-old observation that *lysine*^{1} *red cell*^{1} (*lys*^{1} *rc*^{1}) double mutants accumulate pigmented fat cells in the head and thorax of adult flies (Grell, 1961). Toward this goal, we have confirmed that *lys*^{1} *rc*^{1} mutants exhibit pigmented cells and have used GC-MS to determine that lysine levels are increased 50-fold in *lys*^{1} single mutants. These results demonstrate that the *lys*^{1} *rc*^{1} mutant phenotypes stem from a metabolic defect and implicate lysine catabolism in the development of adult adipose tissue. As a first step towards studying the molecular mechanisms that underlie these phenotypes, we used deficiency mapping to identify the genes that are disrupted by the *lys*^{1} and *rc*^{1} mutations. We have successfully used a GC-MS-based approach to map the *lys*^{1} mutation to an ~10,000 bp region of chromosome 2 that contains the gene *lysine ketoglutarate reductase/saccharopine dehydrogenase* (*LKRSDH*), which encodes an essential enzyme in lysine catabolism. Furthermore, we have determined that *lys*^{1} fails to complement *LKRSDH*^{MB10942} and have identified the causal mutation as a large insertion in the second exon of *LKRSDH*. We have also mapped *rc* based on a previous observation that *rc*^{1} single mutants accumulate pigmented fat cells if starved during the post-critical weight period of L3 development. We used this phenotype to demonstrate that *rc*^{1} is located within an ~60,000 bp region of chromosome 2 and we are now employing a whole-genome sequencing approach to identify the causal lesion. Overall, our studies suggest that degradation of the essential amino acid lysine influences the metabolism and development of adult adipose tissue.

Grell E.H. (1961). The genetics and biochemistry of red fat cells in *Drosophila melanogaster*. *Genetics* 46: 925-933.

D1129C Investigating the role of dietary iron on reactive oxygen species levels in *Drosophila*. *Rebecca A. Vaders*, Alexis A. Nagengast. Widener University, Chester, PA.

Aging is a complex process that has a variety of underlying causes. Reactive oxygen species (ROS) cause oxidative damage to proteins, lipids and carbohydrates, and contribute to aging. Although calorie restriction has been linked to an increased lifespan, our previous results showed that *Drosophila melanogaster* raised on a higher nutrient corn syrup-based diet lived longer than those on a lower nutrient molasses-based diet. We hypothesize that increased iron levels in molasses are contributing to increased ROS and the observed decreased lifespan. An indirect measurement of ROS levels was detected through superoxide dismutase (SOD) expression in males and females using quantitative polymerase chain reactions (qPCR), only to find SOD levels did not vary significantly between the two diets but varied between sexes. Through the use of a UAS-*MitoTimer* labeled reporter gene, ROS is measured more directly by the irreversible change from green to red fluorescence in the presence of mitochondrial oxidative stress. An evident trend between the expression of SOD and *MitoTimer* fluorescence has been detected and differences between *MitoTimer* fluorescence on the molasses, corn syrup and iron supplemented-corn syrup foods are being investigated. Using iron supplementation to further study the changes in lifespan and ROS opens windows to better understand how SOD reacts with other biochemical pathways in order to affect oxidative stress.

D1130A The *Drosophila* Estrogen-Related Receptor acts as a nutrient sensor to coordinate larval growth with nutrient availability. *M. C. Sterrett*, S. L. St. Clair, J. M. Tennessen. Indiana University, Bloomington, IN.

All growth during the *Drosophila* life cycle is restricted to larval development, when animals increase their body size ~200-fold over the course of four days. To support this exponential growth, larvae rely aerobic glycolysis, a unique metabolic program ideally suited to synthesize biomolecules from carbohydrates. Our previous work demonstrated that aerobic glycolysis is transcriptionally-activated during embryogenesis, when the *Drosophila* ortholog of the Estrogen-Related Receptor (ERR) class of nuclear receptors coordinately up-regulates genes involved in glycolysis, the pentose phosphate pathway, and lactate production. We have discovered that dERR activity is not restricted to embryogenesis; rather, dERR also promotes aerobic glycolysis during larval development. Our preliminary analyses demonstrate that dERR protein is expressed in key metabolic tissues, including the fat body, intestine, and muscle. This expression pattern suggests that dERR coordinates glucose-derived biosynthesis with growth conditions. Consistent with this model, we have discovered that the dERR ligand-binding domain (LBD) is activated when larvae are fed a yeast-based diet but not sugar-only or starvation media. Furthermore, the addition of insulin to larval organ cultures fails to activate the dERR LBD, suggesting that dERR represents a novel mechanism for linking dietary conditions with sugar metabolism. We have also determined that dERR is covalently modified under starvation conditions, suggesting that a nutrient-sensitive enzyme controls dERR activity. Finally, we have demonstrated that many dERR target genes are transcriptionally down-regulated upon starvation, indicating that diet-induced changes in dERR activation are functionally significant. Overall, our studies indicate that dERR promotes aerobic glycolysis in response to dietary compounds and suggest that mammalian ERRs also act as nutrient sensors that coordinate biosynthesis with rapid growth.

D1131B *reaper* expression in the wing imaginal disc causes non-autonomous larval growth inhibition. *C. D'Ancona*, A. Reyes, A. Kesick, A. Halme. University of Virginia, Charlottesville, VA.

In response to localized imaginal disc damage, *Drosophila* larvae can alter systemic developmental progression and growth in order to coordinate the regeneration of damaged tissues with the growth and development of undamaged tissues. Previously, we have demonstrated that targeted expression of the TNF homologue *eiger,* in the wing imaginal discs produces localized apoptosis and tissue damage, and limits the growth of undamaged imaginal discs. However, larval tissue growth is not affected by *eiger*-induced damage in the wing. In contrast, expression of the pro-apoptotic gene *reaper* in wing imaginal discs strongly restricts larval growth. *reaper*-induced damage in the wing is observed in early L1 stage larvae and persists throughout larval development, producing pupae that are significantly smaller than *eiger*-expressing or control pupae. Therefore, these two distinct apoptotic stimuli produce different non-autonomous effects on larval growth.

To better characterize which apoptotic pathways can produce this non-autonomous larval growth inhibition, we have begun to examine larval

growth after the expression of several different apoptotic stimuli. In *Drosophila*, *reaper*-induced programmed cell death is critical during development for sculpting final adult tissue shape, deleting pre-adult structures, and regulating cell number. Unlike *eiger*, which induces apoptosis through JNK signaling, *reaper* induces apoptosis through direct inhibition of Diap1. We observe that expression of other Diap1 inhibitors *hid* and *grim* in the wing discs also causes a strong non-autonomous larval growth inhibition. In contrast, disruption of the wing imaginal disc epithelia by inhibition of the neoplastic tumor suppressor *avalanche/Syntaxin-7* does not alter larval growth. Finally, preliminary tests with varied doses of X-irradiation suggest that a subset of irradiated larva may undergo similar growth inhibition. Therefore, the larval growth inhibition resulting from *reaper* expression in the wing disc is a specific damage response not seen with all apoptotic stimuli. This distinct systemic response may reflect a different mode of tissue repair in response to *reaper*-induced damage.

D1132C *Drosophila* Adipokinetic hormone regulates food intake, metabolic rate, and expression of neuropeptide genes with metabolic functions. *Martina Gáliková*, Peter Klepsatel, Yanjun Xu, Ronald P. Kühnlein. Max Planck Institute for Biophysical Chemistry, Research Group Molecular Physiology, Am Faßberg 11, D-37077 Göttingen, Germany.

Adipokinetic hormones (AKHs) induce mobilization of energy reserves in insects, acting thus analogously to the human hormone glucagon. Experiments in the model insect *Drosophila melanogaster* have recently shown that loss of AKH results in adulthood-specific onset of obesity coupled with hypoglycemia. Nevertheless, not much is known about the exact mechanism whereby AKH affects fly physiology. Therefore, we investigated the physiological roles of this hormone, focusing on the metabolic rate and food intake control, and on the interactions between AKH and other fly neuropeptides known to regulate metabolism. Our gain- and loss-of-function experiments show that despite its anti-obesity effect, AKH is an orexigenic peptide. This hormone also affects expression of several other neuropeptide genes involved in feeding behavior, including orexigenic neuropeptides *CCHamide-2* and *neuropeptide F.* In addition, AKH affects metabolic regulators like *corazonin, limostatin,* and *insulin-like peptides 2, 3, 5* and *6.* Altogether, our work shows that next to the regulation of the feeding behavior and metabolic rate, AKH signaling controls *Drosophila* metabolism also by affecting metabolic regulators from the family of insect neuropeptides. AKH should be thus viewed as a central regulator of energy homeostasis and neuroendocrine signaling in *Drosophila* adults.

D1133A The Membrane Attack Complex / Perforin-like protein Torso-like regulates *Drosophila* growth and developmental timing. *Michelle Henstridge*[1], Travis Johnson[1,2], Christen Mirth[1], James Whisstock[2,3], Coral Warr[1]. 1) School of Biological Sciences, Monash University, Clayton VIC 3800 Australia; 2) Department of Biochemistry and Molecular Biology, Monash University, Clayton VIC 3800 Australia; 3) ARC Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton VIC 3800, Australia.

Membrane Attack Complex / Perforin-like (MACPF) proteins are best known for their roles in mammalian immunity, where they function to disrupt cell membranes by forming oligomeric pores. However, several MACPF proteins perform essential, but poorly understood, roles in development. The sole *Drosophila* MACPF protein, Torso-like (Tsl), is well known for its role in early embryonic patterning where our studies suggest it enables secretion of a specific growth factor, Trunk, the ligand for the Torso receptor tyrosine kinase[1]. We and others have recently discovered that *tsl* is also expressed in the major fly endocrine gland, the prothoracic gland, where it has an additional role in the regulation of growth and developmental transitions[2,3]. We have further shown that this role of Tsl is independent of Trunk and Torso[3], raising the possibility that Tsl also influences the secretion of other growth factors. *tsl* null mutants exhibit a delay in time to pupariation and reduced adult body size, phenotypes that closely resemble those observed when insulin signalling is reduced. To investigate a role for Tsl in insulin signalling we performed genetic interaction experiments between *tsl* and components of the insulin signalling pathway. Over-expression of PI3K in the prothoracic gland results in a shorter time to pupariation. This phenotype is suppressed in a *tsl* mutant background, providing evidence that Tsl interacts with the insulin signalling pathway. We are currently performing additional genetic interaction experiments to determine where in the pathway Tsl functions, and in addition, whether secretion of insulin-like peptides is impaired in *tsl* mutants. Finally, we are also screening other candidate growth factors, neuropeptides and their receptors for roles in the prothoracic gland, and testing for interactions with Tsl. Our studies of Tsl will provide fundamental insight into the regulation of important insect growth pathways, many of which play highly conserved roles in humans.

1. Johnson, Henstridge *et al.* (2015) *Nat. Commun.* **6**, 8759; 2. Grillo *et al.* (2012) *Sci. Rep.* **2**, 762; 3. Johnson *et al.* (2013) *PNAS* **110**, 14688-14692.

D1134B Relationship between Heme Biosynthesis and Ecdysone Production during *Drosophila* Larval Development. *Nhan Huynh*[1], Qiuxiang Ou[1], Kirst King-Jones[1], Kim F. Rewitz[2], Michael B. O'Connor[3]. 1) Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada; 2) Department of Biology, University of Copenhagen, Copenhagen, Denmark; 3) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, USA.

The steroid hormone ecdysone plays a key role in insect development. In *Drosophila*, ecdysone pulses trigger all major developmental transitions including hatching, molting and puparium formation. While the downstream actions of ecdysone have been well studied, we only have a limited understanding of how ecdysone production is regulated so that distinct pulses are formed during development. We recently started to examine the relationship between heme biosynthesis and ecdysone production, because the majority of steroidogenic enzymes, the cytochrome P450s, require heme as a cofactor. From a genome-wide RNAi screen that we had conducted in collaboration with Mike O'Connor's and Kim Rewitz's labs, we carried out a secondary screen aimed at identifying genes involved in the regulation of heme production. This screen was limited to the prothoracic gland (PG), the principal source of larval ecdysteroids, and led to the identification of the 34 candidate genes. PG-specific RNAi against these genes caused larval or pupal arrest and/or significant delays in development. Importantly, all RNAi lines displayed enlarged ring glands, 20 of which exhibited strong red autofluorescence in the PG, consistent with fluorescent heme

precursor accumulation in this tissue. Here, we present preliminary findings for two candidate genes, Su(Var)2-10 and AGBE, and how they relate to heme and ecdysone regulation.

D1135C Identifying new growth regulatory receptors in *Drosophila melanogaster*. *Melissa J. Saligari*[1], Patrick Farrell[1], Michelle A. Henstridge[1], Karyn A. Moore[1,2], Travis K. Johnson[1,2], James C. Whisstock[2,3], Coral G. Warr[1]. 1) School of Biological Sciences, Monash University, Clayton, Victoria, Australia; 2) Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia; 3) Australian Research Council Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Victoria, Australia.

In animals, control of the rate and duration of growth is coordinated by endocrine systems which produce secreted peptide hormones and steroid hormones. Many of the key growth pathways that are crucial for human development, such as the insulin signaling pathway, were first identified and characterised in *Drosophila*. In insects, developmental transitions are regulated by pulses of production of the steroid hormone ecdysone in the larval prothoracic gland (PG). Multiple environmental factors and signaling pathways regulate ecdysone production. To find new regulators of growth we used RNAi to knockdown a set of candidate neuropeptide and growth factor receptors in the PG using *phantom-Gal4*. We found that knocking down Adipokinetic hormone receptor (AkhR) causes larval lethality. Knocking down Pigment-dispersing factor receptor (Pdfr) causes a severe developmental delay and increases adult weight. Neither receptor has previously been shown to have a role in the PG. We tested amorphic alleles of *AkhR* and *PdfR* (*AkhR*^{1}, Gronke *et al.* 2007; *PdfR*^{5304}, Hyun *et al.* 2005) and found that homozygous mutants were developmentally delayed and larger than their heterozygous controls. We are currently investigating how these specific receptors perturb growth and interact with the PTTH/Tor and insulin pathways, which are known to operate in the PG to control ecdysone production. Identifying novel growth factor receptors and signaling pathways that regulate developmental timing and body size in *Drosophila* is not only interesting in its own right, but may prove vital in deepening our understanding of human growth disorders, obesity and cancer.

Gronke et al. 2007, PLoS Biol. **5**(6): e137; Hyun et al. 2005, Neuron **48**(2): 267-278.

D1136A The influence of the RU486 steroid on *Drosophila* feeding and lifespan. *Erin S. Keebaugh*[1], Ryuichi Yamada[1], Sonali A. Deshpande[1], Margaux R. Ehrlich[1], Alina Soto Obando[1], Frédéric Marion-Poll[2], William W. Ja[1]. 1) The Scripps Research Institute, Jupiter, FL 33458, USA; 2) Evolution, Génomes, Comportement & Ecologie, CNRS, IRD, Université Paris-Sud, Université Paris-Saclay Gif-sur-Yvette, France; AgroParisTech Paris, France.

Insects that develop and feed on plants are exposed to a wide range of plant-derived steroidal compounds. The perception of plant steroids can influence insect food choice and feeding behaviors; furthermore, some plant steroids serve as insecticides and are toxic to plant-feeding insects. RU486 is a synthetic steroid that is added to *Drosophila* food to activate transgenes using the powerful GeneSwitch system, which facilitates the spatial and temporal control of transgene expression. Because of the structural similarity between RU486 and plant-derived steroids, and since GeneSwitch assays may be influenced by altered food intake, we tested if RU486 influences fly feeding behaviors. We found that flies show a reduced proboscis extension response (PER) towards RU486 mixed into a variety of diets. Furthermore, flies show reduced feeding on food containing RU486. Since changes in food consumption can have an impact on aging and health, we next tested if RU486 influences the lifespan of multiple *Drosophila* lines exposed to RU486 across a variety of diets. We found that RU486 can modulate lifespan in a diet-dependent manner, especially on diets where dietary restriction-mediated longevity is observed. Our findings demonstrate that RU486 is taste-aversive to flies and can influence feeding behavior and lifespan. Thus, future studies using the GeneSwitch system should consider the impact of potential RU486-specific effects.

D1137B Analysis of the effects of acetyl-para-aminophenol on *Drosophila melanogaster* development. *Terry Blaszczak*, Raluca Ciupuliga, Stephanie Fonseca, Fareha Nazneen, Mary Kimble, Elyse Bolterstein. Northeastern Illinois University, Chicago, IL.

Acetyl-para-aminophenol (acetaminophen) is a known toxin in humans, but the extent of its toxicity is not fully understood. The difference between an effective dose and an overdose of acetaminophen is very minimal, and acetaminophen overdoses resulting in hospitalizations are relatively common. This study investigates the effects of acetaminophen on the development of *Drosophila melanogaster* exposed to different light/dark conditions to better understand the mechanisms and antioxidant pathways that are affected by acetaminophen. Our objectives include determining the dose-response relationship of acetaminophen on *Drosophila* development under different light conditions and measuring the antioxidant activity in adults raised on acetaminophen food to determine oxidative stress levels. This research will increase our understanding of the toxic effects of acetaminophen on developing organisms.

To determine the acetaminophen dose-response relationship, instant food was added to fly vials and rehydrated with 30mM to 70mM acetaminophen solutions. Oregon R wild type flies were added to each vial and placed in different light cycle conditions. Once the eggs hatched, the rate of larval development was monitored and the number of adults emerging was recorded every day until all flies had eclosed (emerged). A separate trial placed collected eggs in vials containing acetaminophen food of different concentrations. Vials were placed either in constant dark or twelve-12 hour light/dark cycles. The number of flies eclosed was recorded every 12 hours until all flies had emerged. Our results show that acetaminophen doses less than 30 mM had minimal effect on development. Doses of 30 mM to 60 mM affected survival percentages and time to eclosion, and doses greater than 70 mM showed very low survival rates. Additionally, light cycles affect survival and development rates, with cultures exposed to a twelve-hour light/dark cycle experiencing the least effect of acetaminophen. Currently, assays testing oxidative stress of adult flies raised on acetaminophen food are underway. Flies will be separated according to day eclosed, sex, light conditions, and acetaminophen dosage. Fly homogenates from the most recent dosage study will be used to extract proteins and determine the relative antioxidant activity in flies exposed to different light conditions and acetaminophen concentrations. Because flies develop more

slowly and show greater response to acetaminophen when reared in constant darkness, it is predicted that exposure to higher concentrations of acetaminophen and constant darkness will result in greater antioxidant activity.

D1138C BLM and WRNexo protect against aging and tumorigenesis in Drosophila. *Elyse Bolterstein*[1], Rob Salomon[2], Mitch McVey[3], Deirdre Cassidy[1], Matthew Kuo[1], Joshua Kruithof[1], Imad Rebiai[1], Luhan Zhou[1]. 1) Northeastern Illinois University, Chicago, IL; 2) Tufts Medical Center, Boston, MA; 3) Tufts University, Medford, MA.

Members of the RecQ family of helicases are known as the "guardians of the genome" due to their essential roles in DNA repair, replication, and recombination. Mutations in the RecQ helicases, WRN and BLM, cause Werner and Bloom syndrome respectively. These autosomal recessive diseases are characterized by patients' increased risk of cancer and early onset of aging-related pathologies. Similar to Bloom syndrome patients, *Blm* mutant flies have shortened lifespans and a higher incidence of tumors, particularly in the gut and ovaries of aged females. We have found that flies mutant in the Drosophila homolog of WRN, *WRNexo*, also exhibit decreased lifespans and higher tumor incidence compared to age-matched wild type controls. In contrast to *Blm* mutants, *WRNexo* flies accumulate tumors in the testes, suggesting tissue-specific mechanisms of these two proteins. *WRNexo* mutants also exhibit other physiological signs of aging such as degeneration of the flight muscles and decreased locomotor activity. Additionally, we observed that mutant flies have increased antioxidant activity that declines with age, suggesting that the aging phenotypes may be due to increased oxidative damage. We propose that WRNexo and BLM physically interact with one another to protect against physiological signs of aging, which is a hypothesis supported by our recent finding that *WRNexo* and *Blm* mutants share similar phenotypes in response to replication stress. To this effect, we are investigating these interactions by performing a co-immunoprecipitation assay using *WRNexo* transgenic mutants.

D1139A The Interaction of Wolbachia and Oxidative Stress with Genetic Background in *Drosophila Melanogaster*. *F. Capobianco*, S. Nandkumar, J. Parker. SUNY Plattsburgh, Plattsburgh, NY.

It is recognized that the rate of aging varies among individuals according to genetic background. The intracellular bacterium, Wolbachia, is found in most arthropods and nematodes, including *D. melanogaster*. Here we explore how Wolbachia infection and oxidative stress interact on aging with infected and uninfected *D. melanogaster* in two different genetic backgrounds.

Two wild caught lines, naturally infected with Wolbachia, were cleared of the bacterium. Infected and uninfected flies from the wild caught lines were acutely treated with oxidative stressors (Paraquat, Hydrogen Peroxide) and a nitric oxide stressor (L-Arginine). When treated with paraquat, levels of superoxide anions increase within the cells. Treatment with hydrogen peroxide causes accumulation of itself outside of the cells. L-Arginine increases the amount of nitric oxide within the cells.

It was discovered that Wolbachia increases the sensitivity of oxidative stress in a manner dependent on the genetic background and that the effect Wolbachia itself has on lifespan varies according to genetic background. It was also seen that Wolbachia increases the sensitivity to hydrogen peroxide based on genetic variation and length of the fly line's lifespan. We propose that Wolbachia may be working like Superoxide Dismutase, only in long lived fly lines.

The system factors that can govern aging (infection and oxidative stress) are not universal, but are specific to the individual's genetic makeup. This raises the possibility that selection on Wolbachia infection is related to the lack of selection predicted by the mutation accumulation hypothesis for aging.

D1140B Oxidative insult induces clock-dependent, rhythmic expression of stress-related genes in *Drosophila*. *E. Chow*, R. Kuintzle, T. Bonar, D. Hendrix, J. Giebultowicz. Oregon State University, Corvallis, OR.

Circadian rhythms provide temporal coordination between organisms and their environment, and contribute to healthy aging in animals from flies to humans. Clock genes regulate many biological processes, and a disrupted circadian system increases risk for oxidative damage, neurodegenerative diseases, and metabolic disorders. Expression of many genes appears to be clock-dependent in young flies. However, as aging weakens circadian rhythms and synchrony, it has been unclear how expression of clock-controlled genes may change with age. Using RNA-Seq to obtain a large-scale overview of transcriptome differences around the clock in heads of young and old flies, we found a group of genes that had constitutively low expression in young, but were greatly upregulated and rhythmic in heads of old flies. Genes showing this expression pattern were called "Late Life Cyclers," or LLCs. Further investigation revealed that many LLCs were involved in stress response, specifically oxidative stress. These included, among others: small heat shock protein *Hsp22*, cytokine *bnl*, dehydrogenase *ImpL3*, and Hsp40-like *CG7130*. Since the most robustly rhythmic LLCs' expression peaked at lights-off, or Zeitgeber Time (ZT)12, we tested whether this would be affected by longer or shorter periods of light during a 24h cycle. In flies collected in light:dark (LD) 16:8 or LD 8:16, the peak expression of most LLCs tested by qRT-PCR shifted to follow the time of lights off. In constant darkness (DD), expression was low and arrhythmic, suggesting that light is a factor in regulation of these genes. To mimic oxidative stress known to occur in old flies, we exposed young flies to 100% hyperoxia (HO). We found that LLCs were indeed upregulated and rhythmically expressed upon HO exposure in LD12:12, similar to what was seen in old flies. When flies were exposed to HO in DD, these genes were barely upregulated compared to normoxia controls, again resembling expression in old flies in DD. The inability of these genes to be overexpressed and rhythmic in the absence of light despite strong oxidative insult led us to investigate the role of the photoreceptive protein encoded by the gene *cryptochrome* (*cry*). CRY, a flavoprotein, has a key role in circadian regulation by causing degradation of the core clock protein TIM in the presence of blue light. However, other possible roles for *cry* remain unknown. We tested whether CRY was necessary for LLC upregulation, by measuring their expression profiles in young *cry*-null mutants, cry^{01} and cry^{02} held in LD under HO. We observed a loss of typical LLC rhythms and their reduced overall expression compared to wild type controls. Our data suggest that CRY has a novel role in stimulating expression of neuroprotective genes in response to light and oxidative stress.

D1141C The *fluted*^{1} (*fl*^{1}) mutation is a deletion in the CG5873/cysu gene and is required for wing integrity. *E. P. Spana*, Bio414 Team fluted (2012-2016). Duke University, Durham, NC.

The Drosophila wing makes an excellent model system for many developmental processes. From growth control and proliferation through patterning and signal transduction to eventually cell fate establishment and differentiation, the wing magically presents something for everyone. We have used this system in an undergraduate course in Genetics and Development to identify genes responsible for proper wing development.

The *fluted* (*fl*^{1}) mutation was discovered by Helen Redfield in 1921 and over 90 years later we have molecularly mapped that mutation to the genome and further characterized its phenotype. After reviewing the historical mapping data, students in the Bio414L course designed deficiency and transposon insertion complementation tests and identified insertions in CG5873 that failed to complement *fl*^{1}. Whole genome sequencing revealed that the lesion in *fl*^{1} is an approximately 1 kb deletion that removes most of exon 1 and the start of translation—not surprising since *fl*^{1} behaves as a genetic null. What is surprising is that *fl*^{1} is temperature sensitive. At 25° the wings have longitudinal creases between L3 and L4 and between L4 and L5. At 18°, these creases are much more severe, and at 29° the wings are almost wild-type. We used time-lapse imaging to identify when the creases present and found that after inflation, the wings were perfectly flat, but during the drying stage the wings creased and withered. These wings then slowly disintegrated over time where after a week, much of the intervein tissue had broken away.

The CG5873/cysu gene encodes for a Heme Peroxidase and has been linked to the Duox gene in wing development (Hurd et al, 2015 PLoS Genetics 11(11): e1005625) where it functions to remove the reactive oxygen species (ROS) created by Duox. It is possible that in *fluted* wings, an excess of ROSs are created that causes the deterioration of the wing. Increasing the temperature could alleviate this by causing the ROSs to volatilize at a higher rate.

D1142A Alternative splicing and isoform expression in the honeybee flight muscles. *A. J. Ayme-Southgate*, L. Galloway, E. Risner, J. Vance. Col Charleston, Charleston, SC.

Phenotypic plasticity allows individuals to, for example, adapt their physiological responses to their environment, as well as to the stage of their life cycle. Within a honeybee colony, worker bees start their adult life as nurses involved with building the honeycombs, feeding the larvae, and cleaning the hive. As such, nurse bees only need to fly limited distances and for short amounts of time. Some of the nurse bees transition later in life (around 21 days) to foragers whose job is to collect water and nectar, and as such foragers need to be excellent flyers. The nurse-forager transition event is a model system for understanding the molecular implication of phenotypic plasticity. This event necessitates major changes in the bee's behavior, but also in the efficiency and power generated by the flight muscle system. RNA sequencing analysis of flight muscle tissues isolated from nurse and foragers at different ages reveal differential gene expression of genes involved in several biological processes, including stress response, immunity, and protein synthesis. Proteins involved in muscle sarcomere structure, in particular proteins of the myofilaments undergo shift between isoforms during the transition. Quantitative RT-PCR analysis for proteins of the troponin complex, as well as the elastic C-filament will be presented. The generation of these alternative splice variants depends on the activity of regulatory splicing factors, such as muscleblind, lark, and others. The initial analysis of *Apis mellifera* splicing machinery and in particular regulatory splicing factors will be discussed in the context of the nurse-forager transition.

D1143B *Cellular senescence and oxidative stress are aging mechanisms that depend on genetic background*. *P. Bejo*, F. Capobianco, J. Parker. SUNY Plattsburgh, Plattsburgh, NY.

Cellular senescence (CS) is a state of metabolically active cells that experience an irreversible growth arrest upon some type of stress. Recently CS has been tied to age-related diseases (Zhu et al, 2015). Physiological aging is also associated with increased levels of ROS, but it is not understood how senescence and oxidative stress react to induce aging. We assessed the effect of senolytic drugs (that selectively kill senescent cells) on the longevity of wild-type *Drosophila melanogaster*. In our study, we show that a combination of senolytics (Dasatinib and Quercetin) increase the lifespan of one wild-type line Drosophila melanogaster, with no effect on the other. Previous experiments of our lab show a difference in oxidative stress response between these two genetic backgrounds. We are now using this combination of senolytic drugs and paraquat treatment to understand the relationship between oxidative stress and CS on physiological aging.

D1144C Epigenetic Regulation of Aging in *Drosophila melanogaster* . *Q. Brent Chen*[1,2], Trudy F. C. Mackay[1,2]. 1) Department of Biological Sciences and Program in Genetics, North Carolina State University, Raleigh, NC 27695, USA; 2) W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC 27695, USA.

As the average lifespan of the world population continues to increase, deciphering the biological underpinnings of natural variation in aging and lifespan are becoming critical to managing aging-related diseases. While many genes regulating lifespan have been identified, the epigenetic factors regulating lifespan and aging are largely unexplored. Here, we use five *Drosophila melanogaster* lines selected for postponed reproductive senescence for over 170 generations (O lines) and five lines from the same base population without selection maintained (B lines) to assess differential epigenetic modifications. The O lines live approximately twice as long as the B lines. We are using ATAC-seq to determine changes in open chromatin from a variety of tissues in both sexes of the O and B lines at several developmental and adult time points; and ChIP-seq to target histone modifications at different ages in these divergent lines. In conjunction with previous genomic, transcriptomic, metabolomic, and phenotypic data, we will derive putative causal relationships between epigenetic modifications and natural variation in lifespan.

D1145A Histone deacetylases affect aging-related muscle fiber loss.. *E. Gerberich*, A. Bryantsev. Kennesaw State University, Kennesaw, GA.

Progressive loss of individual muscle fibers in the elderly is a characteristic hallmark of aging-related muscle wasting condition – sarcopenia. We recently developed a model to address aging-related fiber loss (ARFL) on the basis of *Drosophila* jump muscle. In this study, the ARFL rates were analyzed in the muscles with depleted chromatin-remodeling factors, histone deacetylases (HDACs). *Drosophila* and human HDAC3 and HDAC4 share significant homology and are expressed in various muscle tissues. We used RNAi to down-regulate these HDACs in adult flies by means of a jump-muscle specific Gal4 driver. Our results show that the decreased expression of either HDAC3 or HDAC4 strongly promotes muscle fiber loss in aging flies. We hypothesize that this effect might be mediated by changes in HDAC-controlled expression of apoptotic factors. Our study identifies HDACs as important regulators of muscle longevity and survivorship, with potential implications in the development of human sarcopenia.

D1146B The Role of Activin Signaling in Drosophila Cardiac Aging. *Ping Kang*[1], Rolf Bodmer[2], Karen Ocorr[2], Hua Bai[1]. 1) Iowa State University, Ames, IA; 2) Sanford Burnham Medical Research Institute, La Jolla, CA.

Our previous study suggests that activin regulates muscle aging via autophagy. In this studywe investigated whether genetic manipulation of activin signaling within fly hearts affect cardiac functions during aging. Cardiac-specific expressed activin type I receptor Babo led to pre-matured cardiac aging phenotypes and altered cardiac functions at young age. In contrast, cardiac-specific knockdown the expression of key activin genes, daw and babo results in preserved cardiac function with age (e.g. prevents the age-dependent increases of heart period and diastolic intervals, as well as the incidence of arrhythmia). Interestingly, reduction in cardiac activin prolongs lifespan. These data suggest that activin signaling plays a key role in cardiac aging.

D1147C Regulation of dense-core granule replenishment by autocrine BMP signalling in *Drosophila* secondary cells. *Siamak Redhai*[1], Josephine Hellberg[1], Mark Wainwright[1], Sumeth Perera[1], Felix Castellanos[1], Benjamin Kroeger[1], Carina Gandy[1], Aaron Leiblich[1], Laura Corrigan[1], Thomas Hilton[1], Benjamin Patel[1], Shih-Jung Fan[1], Freddie Hamdy[2], Deborah Goberdhan[1], Clive Wilson[1]. 1) University of Oxford, Oxford, GB, Department of physiology, anatomy and genetics; 2) University of Oxford, Oxford, GB, Nuffield Department of Surgical Sciences, John Radcliffe Hospital.

Regulated secretion typically involves the trafficking of specific secretory products into dense-core granule (DCG) compartments, which mature and are then released by exocytosis after stimulation. These compartments must subsequently be rapidly replenished to retain secretory capacity. However, the mechanisms involved are largely unexplored. We have studied this problem in fixed and live secondary cells (SCs) of the *Drosophila* accessory gland (AG), which contain huge secretory compartments (> 5 µm diameter), thus providing a unique system to study DCG biogenesis and secretion. Here we demonstrate that each SC contains approximately ten large DCGs loaded with the Bone Morphogenetic Protein (BMP) Decapentaplegic (Dpp). When flies mate, roughly four DCG compartments are rapidly released from SCs, and autocrine BMP signalling, as measured by nuclear pMad staining, is immediately increased. Two of the secreted compartments are restored within 6 hours and by 24 hours, complete replenishment is achieved. The release of secretory compartments is dependent on their maturity, limiting the secretion of additional DCGs during rapid multiple matings. Interestingly, the replenishment of DCGs is regulated by BMP signalling, which is also required for secretion of these compartments, potentially through its effects on DCG maturation. Our study therefore highlights a central function for Dpp in matching biogenesis of new DCGs to elevated release of these compartments from SCs after mating. Notably, BMP signalling is crucial for secretion in other cell types, including insulin-secreting pancreatic beta cells and *Drosophila* motor neurons. Our new model potentially reflects a more general role for BMPs in providing a read-out for DCG release that ensures DCG numbers are maintained following regulated secretion.

D1148A Male proteins mediate the binding of sex peptide binding to sperm to prolong post-mating responses in *D. melanogaster* females. *Akanksha Singh*[1], Geoffrey D. Findlay[2], Mariana F. Wolfner[1]. 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA; 2) Department of Biology, College of the Holy Cross, Worcester, Massachusetts, USA.

Drosophila melanogaster seminal proteins induce several post-mating responses in mated females, some short-term and others lasting for days. The long term post-mating responses, which include elevation of egg-laying, regulation of sperm release from storage, and changes in mating receptivity and behaviour, are mediated by the seminal protein Sex Peptide (SP), which is retained in the female by its binding to sperm. To test whether male-derived proteins are sufficient for SP binding to sperm, we examined the extent of this binding in ejaculates (produced by fru-GAL4;UAS-dTRP-A1 males in the absence of females), compared to that seen in the bursa and seminal receptacle of mated female. By immunofluorescence and Western blotting we see that SP can bind to sperm in ejaculates, indicating that male contributions are sufficient for this step. However, the level and distribution of this SP-sperm binding differs a bit from that seen on sperm isolated from the bursa or seminal receptacle, suggesting possible assistance by female components. Some male-derived seminal proteins needed to bind SP to sperm are known. To identify the full suite, and any sperm proteins that are required for SP binding, we used a comparative genomic method to pinpoint proteins whose evolutionary rates across the *Drosophila* phylogeny co-vary with the SP network proteins. Twenty-six such candidates are being tested for effects on the persistence of post-mating response and for their effects on SP binding to sperm.

D1149B Exploring chronic drug delivery regimes for aging studies in *Drosophila*. *H. Stratton*, A. Bryantsev. Kennesaw State University, Kennesaw, GA.

Drosophila has been instrumental in advancing studies on aging. Combination of the genetic methods available for *Drosophila* together with pharmacologic approaches can provide even deeper insight into the mechanisms of aging. Here, we studied the effects of chronic administration of the proteasomal inhibitor MG132 on muscle morphology. Besides testing for drug effects, we also specifically probed the feasibility of different oral drug administration regimes. Several conditions were employed:

1. MG132 present constantly in the fly food,
2. MG132 supplied on sucrose-soaked discs as an alternative supplement along with standard fly food,
3. MG132 obligatory supplied during overnight sessions, twice a week

The trials went for 4 weeks at 29^{o}C and then the effects of MG132 were scored by evaluating the abundance of polyubiquitylated proteins on cryosections and in muscle tissue lysates. According to preliminary results, chronic administration of MG132 improves muscle morphology in aged flies. The only successful feeding regime was when the drug was constantly present in the food. Additionally, we found that dimethyl sulfoxyde, a common organic solvent for many drugs, acts as a strong feeding repellent to flies, thereby reducing drug consumption rates. The results of our study emphasize the importance of proper drug administration planning in pharmacologic studies.

D1150C Sestrin, a novel target in the mTOR pathway that mediates benefits of exercise. *A. Sujkowski*[1], M. Kim[2], B. Kim[2], J. Lee[2], Robert Wessells[1]. 1) Wayne State University, Detroit, MI; 2) University of Michigan, Ann Arbor, MI.

Modern lifestyle, often characterized by over-nutrition and lack of exercise, causes prolonged activation of mTOR complex 1 (mTORC1)/S6K and chronic suppression of mTORC2/AKT, which together promote age-related muscle pathologies including insulin resistance, fat accumulation, mitochondrial dysfunction and functional decline. Using *Drosophila* as a model organism, we recently found that Sestrin acts as an important regulator of both mTOR complexes, and that the absence of Sestrin brings about several age-associated pathologies including mitochondrial dysfunction and skeletal/cardiac muscle degeneration. Sestrin dependent regulation of mTOR complexes is important for maintaining muscle health throughout life. We hypothesize that Sestrin can be a novel molecular target in the mTOR signaling network that can limit chronic mTORC1 activation and preserve mTORC2 activity in muscle, thereby promoting life- and healthspan. Transgenic induction of Sestrin in skeletal and cardiac muscle can prevent age-dependent loss of endurance and mobility, while increasing speed, endurance and cardiac performance. Importantly, endurance exercise induces Sestrin expression, while Sestrin deficiency nullifies the effects of long-term exercise in improving mobility and metabolism. These data suggest that Sestrins may mediate the beneficial effects of exercise in preserving muscle health, making the Sestrin protein family a potential therapeutic target for preserving mobility and improving quality of life in later ages.

D1151A Lateral Abdominal Muscles as a model for studying muscle atrophy in *Drosophila* . *N. Tamba*, A. Bryantsev. Kennesaw State University, Kennesaw, GA.

Drosophila has been notoriously instrumental in the uncovering of genetic mechanisms of muscle development, however it is generally considered less attractive for studying post-developmental plasticity of muscles. This is because the most studied muscles, flight and jump muscles, remain unchanged in size and morphology throughout the life of adult flies. We recently found that Lateral Abdominal Muscles (LAMs), small muscles underlying the abdominal wall, can undergo atrophic changes in response to starvation, aging, and experimentally-induced cancer cachexia. The relative ease of abdominal preparations, as well as the multitude of LAMs per fly, makes this model suitable for quantitative analysis. Using genetic approach, we demonstrate that experimental up-regulation of muscle structural gene expression can ameliorate LAM atrophy in aging flies. Our study indicates that LAMs can be a useful model for studying genetic components of muscle atrophy.

D1152B *Mondo/dChREBP* functions in the *Drosophila* intestine to regulate nutrient storage. *Niahz Wince*, Justin DiAngelo. Penn State Berks, Reading, PA.

After a meal, digestion occurs and nutrients are absorbed through the walls of the intestine and transported throughout the body to provide energy for cellular functions. Excess energy is stored as triglycerides and glycogen. Several enzymes act to break down complex macromolecules so they can be absorbed through the intestinal epithelia. However, the full complement of genes that function in the intestine to regulate nutrient absorption and storage are not fully understood. Carbohydrate response element binding protein (ChREBP) is a transcription factor known to function in the liver as a nutrient sensor that stimulates triglyceride storage in response to elevated nutrient levels. In addition to being expressed in the liver, ChREBP expression is enriched throughout the small intestine; however, whether ChREBP functions in the intestine to regulate nutrient absorption or storage is unknown. The *Drosophila* genome contains a single ChREBP homolog known as *Mondo*, and our lab has shown that this gene functions in the fly brain, muscle, and fat body to regulate feeding, nutrient storage, and muscle function. To test whether Mondo functions in the intestine, we measured levels of triglycerides and glycogen in flies where *Mondo* has been decreased specifically in the intestine using RNAi. We have found that lowering *Mondo* levels in the intestine results in a decrease in triglyceride and glycogen levels. This altered nutrient storage phenotype does not seem to be due to less nutrient intake as food consumption is not altered in *Mondo*-knockdown flies. Together, these data suggest that Mondo functions in the fly intestine to regulate nutrient absorption or transport in response to nutrient availability. .

D1153C Kruppel homolog 1 represses dFOXO transcriptional activity and lipolysis. *Hua Bai*[1], Kang Ping[1], Kai Chang[1], Marc Tatar[2]. 1) Iowa State University, Ames, IA; 2) Brown University, Providence, RI.

Insulin/insulin-like growth factor signaling (IIS) plays important roles in animal growth, metabolism, stress resistance and aging. It functions through the downstream forkhead transcription factor FOXO and interlays with many other key signaling pathways. The interaction between FOXO and its co-factors enhances the transcriptional specificity of insulin/FOXO signaling. Here, we identified transcriptional co-regulation of insulin signaling and lipolysis through the interaction between kruppel-like factor Kr-h1 and dFOXO in Drosophila. In fasting larvae, Kr-h1 co-localize with dFOXO in nuclei of fat body cells. Kr-h1 physically binds to dFOXO *in vitro* and acts as a repressor of dFOXO to regulate the transcriptional activation of insulin receptor (*InR*) and adipose TAG lipase *brummer*. The binding of Kr-h1 to the promoters of *InR* and *brummer* requires dFOXO. Juvenile hormone (JH) signaling, a major insect developmental regulator and an upstream regulator of Kr-h1, interacts with dFOXO to control lipolysis and the transcription of *brummer* lipase, and thus, lipolysis. Transcriptome analysis further reveals that Kr-h1 targets many metabolic genes that overlap with dFOXO targets. Thus, the interaction between Kr-h1 and dFOXO may represent a broad mechanism by which metabolic signaling integrates with JH-regulated developmental programs to coordinate organism growth. .

D1154A Transcriptional co-regulation of lipid metabolism by Drosophila dFOXO and Kruppel homolog 1. *K. Chang*, H. Bai. Iowa State University, Ames, IA.

Multiple transcription factors often interact at their genomic binding sites and these interactions enhance transcription specificity and pleiotropic functions in the regulation of diverse cellular processes. Insulin/insulin-like growth factor signaling (IIS) plays important roles in animal growth, metabolism, stress resistance and aging. It functions through the downstream forkhead transcription factor FOXO and interlays with many other key signaling pathways. The interaction between FOXO and its co-factors enhances the transcriptional specificity of insulin/FOXO signaling. We recently identified an intriguing interaction between dFOXO and zinc finger transcription factor Kruppel homolog 1 (Kr-h1). In the study, we further characterized this interaction at the genomic level to identify their direct target genes using Next-Gen sequencing approaches (ChIP-Seq and RNA-Seq). Pathway analysis of identified target genes revealed that dFOXO and Kr-h1 co-regulate a large set of metabolic genes, especially those involved in sphingolipid andglycerolipid metabolism. Our results provide addition evidence supporting the notion that cellular metabolic processes are coordinately regulated by diverse transcriptional programs.

D1155B Loss of *rab27* in the αβ pioneer neurons of the mushroom body extends lifespan by deactivating TOR signaling in *Drosophila*. *W. Lien*[1], Y. Chen[1], C. Wu[2,3], C. Chan[1]. 1) Graduate Institute of Physiology, National Taiwan University, Taipei, Taiwan; 2) Department of Biochemistry and Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan; 3) Department of Neurology, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan.

The mushroom body (MB) is the regulatory center of several brain functions including learning, memory, and sleep in *Drosophila*. Whether MB regulates lifespan has not been investigated. MB is composed of Kenyon cells, calyces and five distinct lobes including the vertical α, α', medial β, β', and γ lobes. Further functional analysis subdivides the αβ neurons into at least 3 subsets: αβ pioneer (αβp), αβ surface (αβs), and αβ core (αβc). Here, we show that Rab27 controls lifespan in the αβp neurons of MB. Rab27 is an evolutionarily conserved Rab GTPase mediating the exocytic machinery in neuroendocrine cells. We characterized the *rab27* knockout (KO) flies, and no obvious developmental defect was observed, except that loss of *rab27* significantly extended lifespan. Specifically, *rab27* knockdown in the αβp neurons promoted longevity. To reveal the signal transduction, we first examined the insulin/IGF signaling (IIS) pathway. *rab27* knockdown in the median neurosecretory cells (mNSCs) by *dilp2-Gal4* did not extend lifespan and suppression of IIS in *rab27KO* background further extended lifespan. Moreover, the level of phosphorylation of AKT (pAKT), a downstream component of IIS, was not decreased in *rab27KO* brains. Thus the longevity phenotype was not due to reduced IIS. Rather, reduction of *rab27* leads to decreased phosphorylation of S6 (pS6), a downstream component of target of rapamycin (TOR) signaling. Consistently, suppression of TOR signaling by feeding rapamycin or expressing either TSC2 or $S6K^{DN}$ does not further increase the longevity of *rab27KO*, indicating that Rab27 functions through TOR signaling but not IIS pathway. We propose that reduction of *rab27* attenuates TOR signaling at the level of pS6. Further molecular evidence will be presented. Taken together, the results pinpointed αβp neurons of MB as an important region to control longevity. We discovered a novel site of TOR function in a specific neuronal circuitry for lifespan extension.

D1156C Activin-Beta/TGF-Beta signaling in skeletal muscle controls insulin signaling, metabolism and final body size. *Lindsay Moss-Taylor*, Michael O'Connor. University of Minnesota, Minneapolis, MN.

Inter-organ communication is essential for regulating development and homeostasis. Mutations in Drosophila *Activin-Beta* (*Act-Beta*) cause accelerated pupariation and reduced final body and organ size. To determine how Act-Beta affects size and timing, we first looked at which cells express Act-Beta and found expression in the Insulin Producing Cells (IPCs), neuroendocrine cells and motor neurons. Overexpression of Act-Beta in either neuroendocrine cells or motor neurons increases body size. Muscle-specific knockdown of the TGF-Beta signaling transducer/transcription factor dSmad2 reduces body size, indicating muscle is a target tissue of the Act-Beta signal. Additionally, levels of phospho-dSmad2 are reduced in skeletal muscle samples of *Act-Beta* mutants and increased in animals overexpressing *Act-Beta* from motor neurons. Levels of phospho-S6K in *Act-Beta* mutants are correlated with phospho-dSmad2 levels, suggesting TGF-Beta signaling regulates insulin signaling. Because insulin signaling controls metabolism, we used GC/MS analysis to identify and quantify levels of metabolites in whole-larval samples of *Act-Beta* mutants. We found intermediates of the energy-producing steps of glycolysis and lactic acid are reduced, indicating reduced flux through glycolysis. Overall, this indicates neuronally-derived Act-Beta signals to the skeletal muscle to regulate levels of insulin signaling and subsequent glycolysis. We have identified over 300 downstream targets of dSmad2 using RNA-seq of *Act-Beta*

mutant skeletal muscle. We are testing *impL2*, an insulin binding protein, as a potential dSmad2 target gene regulating systemic insulin signaling.

D1157A Toll signaling acts through the transcription factor Dif to block DILP-dependent growth in the *Drosophila* fat body. *Nigel Muhammad*, Stephen Roth, Michelle Bland. University of Virginia, Charlottesville, VA.

In *Drosophila* and mammals, activation of the innate immune system leads to insulin resistance. Infiltration of macrophages with active Toll-like receptor 4 signaling into obese adipose tissue leads to reduced insulin signaling and metabolic defects in mammals. In *Drosophila*, expression of constitutively-active Toll^{10b} receptors in the larval fat body activates the innate immune system and leads to reduced Akt activity, and decreases in fat body cell size, triglyceride storage, and whole-animal growth compared with flies expressing control transgenes. Toll signaling disrupts DILP signaling by interfering with phosphorylation of the kinase Akt. However, it is not known whether transcription factors in the Toll signaling pathway are required for the development of insulin resistance. Here we examine the role of the Toll pathway transcription factor and NF-KB homolog Dif in growth regulation in the *Drosophila* fat body. We find that expression of Toll^{10b} or elevated expression of Dif in the larval fat body is sufficient to induce expression of Toll target genes such as *Drosomycin*, *IM2*, *Dif*, and *cactus*, as measured by quantitative RT-PCR. Knockdown of Dif in fat bodies expressing Toll^{10b} restores low, wild-type expression of these genes, with the exception of *cactus*. When Toll^{10b} is expressed in clones of fat body cells, nuclear area and cell size are reduced, phenotype that are reversed by co-expression of constitutively-active myristoylated-Akt. Cell growth phenotypes are also rescued when Dif is knocked down in clones that express Toll^{10b}. Clones of fat body cells expressing constitutively-active PI3K (Dp110^{CAAX}) exhibit massively increased nuclear area, which is completely blocked by co-expression of Toll^{10b} but only partially blocked by elevated expression of Dif. Taken together, these data indicate that the transcription factor Dif is necessary but not sufficient for the Toll signaling pathway to reduce Akt-dependent cell growth. This indicates that an additional factor or factors contribute to growth inhibition even when Dif is over-expressed. This may indicate a requirement for post-translational modification of Dif that is not met unless the Toll pathway is activated at the receptor level or that another transcription factor is activated by Toll signaling for the full response. Further, our data suggest that transcription of unknown genes is likely required for Toll signaling to inhibit DILP signaling. Identification of such genes should shed light on the pathology of inflammation-induced insulin resistance.

D1158B Neprilysins control insulin signaling via cleavage of regulatory peptides. *R. Schiemann*[1], B. Hallier[1], L. Dehnen[1], E. Cordes[1], J. Vitos-Faleato[2], S. Walter[3], A. Malmendal[4], A. Paululat[1], H. Harten[1]. 1) University of Osnabrueck, Zoology & Developmental Biology, Osnabrueck, Germany; 2) IRB Barcelona, Department of Biomedical Research, Barcelona, Spain; 3) University of Osnabrueck, Mass spectrometry, Osnabrueck, Germany; 4) University of Copenhagen, Biomedical Sciences, Copenhagen, Denmark.

Insulin and IGF signaling play important roles during development and growth, but also in the daily life of mature organisms where they regulate metabolism, reproduction, stress responses, aging or lifespan. Despite these critical physiological functions, mechanisms that control insulin production and release are still only partially understood. By showing that modulating expression of a *Drosophila* neprilysin considerably interferes with proper expression of major insulin-like peptides, we provide novel mechanistic insight into this issue and relate neprilysin activity to the regulation of insulin signaling for the first time. Concomitant phenotypes of impaired Neprilysin 4 expression include reduced body size, premature lethality and characteristic changes in the metabolite composition of respective transgenic animals. Significantly, ectopic expression of a catalytically inactive protein variant does not elicit any of the phenotypes, which proves aberrant enzymatic activity and thus impaired peptide hydrolysis as critical parameter. In a screen for novel substrates of Neprilysin 4 we identified numerous peptides known to be involved in regulating insulin-like peptide expression, feeding behavior, or both, and thereby provide a conclusive explanation for the depicted phenotypes. The high functional conservation of significant factors renders the characterized principles applicable to numerous species, including higher eukaryotes and humans.

D1159C Localized epigenetic silencing of a damage-activated WNT enhancer limits regeneration in maturing *Drosophila* imaginal discs. *Robin Harris*, Iswar Hariharan. University of California, Berkeley, Berkeley, CA.

For many organisms, the ability to regenerate damaged tissues diminishes with increasing maturity. The mechanisms responsible are unknown, but their identification is essential to promote tissue regeneration in adult organisms. *Drosophila* imaginal discs, when damaged *in situ*, are able to regenerate, but this ability diminishes dramatically during the third larval instar. This decline correlates with a reduction in the ability to upregulate *wingless* (*wg*, *WNT1*) and *WNT6* expression following damage. Since WNT proteins are upregulated following injury in diverse taxa, the mechanisms that regulate WNT expression following tissue damage are likely conserved.

To understand how damage-induced *wg* expression is activated, we have characterized an enhancer that regulates the expression of two flanking genes, *wg* and *WNT6,* during regeneration. Deletion of this enhancer permits normal development but compromises regeneration. Dissection of the genomic region reveals a bipartite structure that includes a damage-responsive module and a silencing element. The damage-responsive module is robustly activated following various damaging stimuli, requiring the JNK pathway. Notably, this activity is undiminished with increasing maturity. In contrast, an adjacent element, which has no enhancer activity on its own, can progressively attenuate expression mediated by the damage-responsive module by nucleating highly localized epigenetic silencing at the enhancer. Cas9-mediated deletion of this silencing element abolishes silencing and restores damage-induced WNT expression in mature discs, demonstrating that the silencing element is indeed responsible for the age-related loss of damage-induced WNT expression *in vivo*. Importantly, this epigenetic silencing remains restricted to the enhancer, thus allowing adjacent enhancers that are regulated by developmental signals to remain accessible.

As the majority of genes implicated in regeneration also function during development, until now it has been difficult to explain how a maturing organism can limit the capacity to regenerate without compromising normal growth and differentiation. The localized silencing of

damage-responsive enhancers is a mechanism that can prevent the activation of genes following injury, while still allowing those same genes to be expressed by developmental signals. In addition to *wg* and *Wnt6*, this mechanism likely regulates many other regeneration genes. Using sequence motif analysis we have identified a similar bipartite enhancer within the *MMP1* gene. Alongside genome-wide chromatin structure analysis, we will use these approaches to identify other such regulatory regions, and thus comprehensively define the genetic basis for declining regenerative capacity of maturing tissue.

D1160A Establishing a model of BM damage and analyzing its repair. *Angela Howard*, Gautam Bhave, Andrea Page-McCaw. Vanderbilt University , Nashville, TN.

The basement membrane is a sheet-like extracellular matrix that wraps around muscle fibers and underlies epithelia. Because it has been difficult to study in vivo, the basement membrane is often considered to be static, analogous to cement. However, there are indications that the BM is a dynamic system. Basement membrane is able to grow, shrink, repair, and move to assist in tissue attachment as needed. We want to develop a system to analyze basement membrane repair in adult animals. In both mammals and flies, the gut appears to be a relatively dynamic basement membrane suggesting it could be a good system for analyzing repair.

We will analyze basement membrane repair using an adult gut injury model. The gut has a well-defined architecture of epithelial cells (enterocytes) residing on top of a basement membrane sheet, wrapped in visceral muscles also surrounded by basement membrane. To injure the gut, flies are fed Dextran Sodium Sulfate (DSS); DSS administration has been previously used as a model for ulcerative colitis in mice although the etiology is unknown. In Drosophila, DSS induces morphological changes in the architecture of the gut consistent with basement membrane damage. Using both electron and super-resolution microscopy, we observe an increase in BM thickness. Furthermore, preliminary data suggests that DSS becomes lodged in the gut BM. Interestingly, inhibiting or knocking down a collagen-IV crosslinking enzyme, peroxidasin, mimics the tissue changes seen in response to DSS. Currently, we are analyzing how this damage is repaired and the requirements for its repair. We plan to knock down multiple basement membrane components after flies reach adulthood to test their role in repair.

D1161B Systemic influences of methionine metabolism in fat body for *Drosophila* imaginal disc repair. *S. Kashio*[1], F. Obata[1,2], L. Zhang[1,3], T. Katsuyama[1,4], T. Chihara[1,4], M. Miura[1,4]. 1) The University of Tokyo, Tokyo, JP; 2) The Fancis Crick Institute, London, UK; 3) University of Toronto, Ontario, CA; 4) AMED-CREST, Tokyo, JP.

Regeneration and tissue repair is a fascinating process that allows organisms to maintain homeostasis after tissue injury. Molecular mechanisms for tissue repair and regeneration within damaged tissue have been extensively studied. However, the systemic regulation of tissue repair remains poorly understood. To elucidate tissue nonautonomous control of repair process, it is essential to manipulate genes in uninjured parts of the body, independent of local tissue damage.

In this study, we develop a system in *Drosophila* for spatiotemporal tissue injury using a temperature-sensitive form of Diphtheria toxin A domain (DtA^{ts}) driven by the Q system to study factors contributing to imaginal disc repair. This system enables us to induce reproducible tissue damage and to perform genetic screening with Gal4 system in uninjured tissues. Using this system, we demonstrate that methionine metabolism in the fat body, a counterpart of mammalian liver and adipose tissue, supports the repair processes of wing discs. Local injury to wing discs decreases methionine (Met) and S-adenosylmethionine (SAM), whereas it increases S-adenosylhomocysteine (SAH) in the fat body, implying that SAM consumption is increased at the early stage of tissue repair. Fat body-specific genetic manipulation of methionine metabolism results in defective disc repair, but does not affect normal wing development. These results indicate the contribution of tissue interactions to tissue repair in *Drosophila*, as local damage to wing discs influences fat body metabolism, and proper regulation of methionine metabolism in the fat body, in turn, affects disc repair.

D1162C The Loss of Regenerative Potential in the Aging Drosophila Germline Stem Cell. *R. E. Kreipke*, H. Ruohola-Baker. University of Washington, Seattle, WA.

Aging is a complex biological process, comprised of a large number of both cellular and organismal changes that, together, lead to the progressive decline in tissue and organ function. In addition to declining function in somatic cells, aging tissues are also marked by a decrease in the proliferative capacity of pluripotent cells, hampering their regenerative capacity and ability to respond properly to injury and insult. However, the mechanisms that govern the regenerative competence of aging stem cells still remain unclear. The *Drosophila melanogaster* germline stem cell (GSC) niche offers a powerful model with which to study developmentally regulated changes in stem cell regeneration and their response to insult. In the young fly, GSCs are resistant to the apoptotic effects of ionizing radiation. Dying daughter cells release a protective signal that inhibits the apoptotic machinery in germline stem cells, prompting them to become quiescent rather than apoptotic. The GSCs are able to later re-enter the cell cycle and repopulate the daughter cells. This protective mechanism highlights a key difference between the proliferative capacities of somatic and germline cells. To date, though, the ability of the aged ovary GSC has not been probed. We compared the ability of 2-, 4-, and 6-week old germline stem cells to regenerate daughter cells following exposure to ionizing radiation. We found that, while aged GSCs were able to survive exposure to ionizing radiation, their ability to exit quiescence and re-enter the cell cycle to repopulate the germaria were compromised when compared to young GSCs. This suggests that the mechanisms that preserve the regenerative capacity of GSCs may degrade with age. To identify novel regulators of GSC regeneration, we used newly developed techniques of protein knockdown to disrupt protein function. This will allow us to screen for genes that regulate GSC regeneration following exposure to ionizing radiation and begin to identify genes that contribute to age-related decline in GSC proliferation.

D1163A Spatiotemporal regulation of cell fusion by JNK and JAK/STAT signaling during *Drosophila* wound healing. *J. Lee*, C. Lee, K. Choe. Yonsei University, Seoul, South Korea.

Cell-cell fusion is widely observed during development and disease conditions, including fertilization, myogenesis, and trophoblast formation. Cell fusion brings a dramatic change to participating cells so it should be tightly controlled, but the underlying mechanism is poorly understood. We found that the JAK/STAT pathway suppressed cell fusion during wound healing and delimited the event to the vicinity of the wound in the *Drosophila* larval epidermis. In the absence of JAK/STAT signaling, a large syncytium containing 3-fold the number of nuclei observed in wild-type tissue formed in wounded epidermis. JNK was activated in the wound vicinity and activity peaked at approximately 8 h after injury, whereas JAK/STAT signaling was activated in an adjoining concentric ring and activity peaked at a later stage. Cell fusion occurred primarily in the wound vicinity, where JAK/STAT activation was suppressed by fusion-inducing JNK signaling. We found that the balance of JAK/STAT and JNK signaling determined whether cell fusion was executed or not. JAK/STAT signaling was both necessary and sufficient for the induction of βPS integrin expression, suggesting that the suppression of cell fusion was mediated by integrin protein.

D1164B Effects of exercise and heat shock on lifespan and health span of $A\beta_{1-42}$ *Drosophila melanogaster*. *S. Alsolami*, A. Ospina, I. Silva. California State San Bernardino University, San Bernardino, CA.

Alzheimer's Disease (AD) is an age-dependent neurodegenerative disease pathologically characterized by the formation of amyloid plaques and neurofibrillary tangles. Amyloid Beta 1-42 (Aβ1-42) has been shown to be the most likely peptide to form these plaques that ultimately inhibit neuronal communication and induce neuronal death. Drosophila melanogaster is a model organism that has been used to study numerous neurodegenerative diseases including AD, Parkinson's disease, and ALS. In this study, a Drosophila melanogaster strain expressing human Amyloid Beta 1-42 transgene has been used as an experimental group and a genetically matched line (00C) has served as the control. Previous research has indicated that both exercise and heat shock can increase the lifespan and health span of Drosophila melanogaster. Both Aβ1-42 and 00C strains of Drosophila melanogaster were exercised and heat shocked, each strain was exercised five days a week for three weeks, and heat shocked at 37˚C for twenty minutes twice a week for three weeks. Survival and climbing assays along with heart rate measurements were used to assess lifespan and health span. The survival, climbing, and heart rate assays indicated that exercise and heat shocking the 00C strain improved its lifespan and health span compared to untreated controls while in the Aβ1-42 strain, exercise and heat shock resulted in decreased lifespan, deteriorated health span, and resulted in the impairment of their cognitive capacity compared to the negative control. We've found that exercising and heat shocking the 00C strain improved their cognitive and motor functions and thus increased their lifespan and health span. However, exercising and heat shocking the Aβ 1-42 strain resulted in decreased cognitive ability, and motor function, and as such did not benefit their health span. At this point, it is not clear why the Aβ1-42 flies did not benefit from the treatments, as did their matched controls. Future studies will include heart tissue analysis to identify if other biomarkers associated with heart function were compromised.

D1165C *Characterization of fried/HEATR2 expression and phenotypes*. *Margaret Fisher*, Elina Sigal, Miranda Colman, Jason Morris. Department of Natural Sciences, Fordham University, New York, NY.

fried loss-of-function mutants were first identified in a clonal screen for oogenesis defects. Subsequently, we found that *fried* mutants exhibit behavioral defects during larval stages and eventually die as late larvae or early pupae. Mapping, sequencing and rescue of *fried* mutants showed that *fried* encodes HEATR2, a protein that others have shown to be required for ciliary axoneme formation and to be expressed in mechanosensory neurons in embryos (Diggle, et al., 2014). Here we present the mRNA expression pattern of *fried/HEATR2* in larval and adult tissues based on mRNA *in situ* hybridization using an mRNA null allele as a negative control. We show that *fried/HEATR2* mRNA is strongly expressed in salivary glands and adult egg chambers along with an array of other tissue types. We also discuss loss of function phenotypes in *fried* larvae and pupae.

D1166A The influence of diet and mtDNA genotype on sexual conflict in *Drosophila melanogaster*. *W. C. Aw*[1], M. R. Garvin[2], J. W. O. Ballard[1]. 1) The University of New South Wales, Randwick, NSW, AU; 2) University of Alaska Fairbanks, Juneau, AK, USA.

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The aim of the study is to identify the influence of diet and mtDNA haplotype on sexual conflict in strains of *Drosophila melanogaster* harbouring the same genetic background. Here we focus on sexual conflict conferred by mitochondria as a result of the organelle's maternal mode of inheritance in the majority of metazoans. We hypothesise that changes in diet could be an important factor that affects sexual conflict between males and females. We fed female and male flies one of four diets that differed in their protein: carbohydrate ratios and tested the sexual conflict by examining five organismal (fecundity, longevity, starvation resistant, lipid proportions and paraquat resistance) and four cellular traits (mitochondrial respiration, ROS production, SOD activity and mtDNA copy number). Traits were assayed at 11d and 25d of age. Here, we show that female physiological traits are more susceptible to dietary changes than males suggesting that diet mediates the change in the degree of sexual conflict. We also found that flies harbouring different mtDNA haplotypes have haplotype specific life-history traits. Finally, we reveal sex specific differences in mitochondrial and biochemical functions and suggest these may be associated with sex-specific differences in ageing.

D1167B Intestinal microbes shorten the host lifespan through increased intestinal permeability in *Drosophila melanogaster*. *H. Lee*, S. Lee, W. Seo, K. Min. Inha University, Incheon, INCHEON, KR.

Intestinal microbes that live in a symbiotic relationship with their host are well known to affect host fitness such as metabolism, obesity, and inflammation. However, the effects of intestinal microbes on host lifespan are not well characterized. In this study, we investigated the effects and mechanisms of intestinal microbes on host lifespan using the *Drosophila melanogaster* as a powerful model animal to study host-microbes interaction. We generated axenic flies by dechorionation of eggs with sodium hypochlorite, and measured its lifespan. The lifespan of flies was increased under axenic condition, and it was decreased by the oral ingestion of the extracts of guts from female flies, indicating that the microbes existing in the gut of flies affect host lifespan. To investigate which commensal bacteria affect the host lifespan, adult flies was subjected to oral ingestion with the single species of microbes such as *Lactobacillus brevis*, *Acetobacter persici*, *Lactobacillus plantarum* and *Acetobater malorum* which are dominated in the guts of young or old flies. We hypothesized dysbiosis of the intestinal microbiota leads to systemic influences on aging files with increased intestinal permeability. The incidence of intestinal dysfunction was increased by aging and intestinal dysfunction increased the permeability of microbes in the gut of flies. In addition, we interestingly observed that *L. plantarum, Sphingomonas yunnanensis, L. brevis,* and *Acetobacter indonesiensis* were founded in the hemolymph of flies with intestinal barrier dysfunction. Taken together, our findings suggest the possibility that intestinal microbes decrease the host lifespan with increased intestinal permeability by aging.

D1168C Growing faster or growing too fast? Effects of *Lactobacillus plantarum* on Drosophila fitness. *Mélisandre Téfit*[1,2], François Leulier[1,2,3,4]. 1) IGFL, Lyon, FR; 2) ENS de Lyon, FR; 3) CNRS, FR; 4) UCBL, FR.

In nature, organisms are constantly associated with a variety of microorganisms. The interactions between these microbes and their host can vary along a continuum of different types, with outcomes ranging from obligate symbiosis to lethal infection. In this spectrum, the microbiota occupies the central and balanced part, covering microbial species that establish commensalistic or mutualistic relationships with each other and with the host organism. There is increasing evidence that the microbiota plays a crucial role in different aspects of the host physiology, and disruption in its composition has been linked to several types of pathological situations.

In order to elucidate the fine-tuned dialogue governing the relationships between the microbiota and its host, we use the model *Drosophila melanogaster* associated with one of its natural commensals, *Lactobacillus plantarum*. This rather simple gnotobiotic system allowed us to reveal a growth-promoting effect mediated by *L. plantarum* in nutritionally challenged drosophila larvae. In case of nutrient scarcity, larvae associated with *L. plantarum* develop twice faster than the germ-free ones, giving rise to adults of similar weight and size. However, adjusting development to environmental cues is key to organismal fitness, and yet here animals are growing fast even though the nutritional conditions are poor. What seems like an advantage could then be deleterious at later stages, and the *L. plantarum*-mediated growth acceleration upon nutritional challenge could in turn adversely impact adult fitness.

In this light, we studied different life history traits and showed that adults emerging from the fast-growing, *L. plantarum*-associated larvae perform as well as their slow-growing axenic siblings. They were able to resist starvation equally well and produced equally numerous and fit progeny. In addition, males kept on a low-yeast diet and associated with *L. plantarum* had a markedly increased median lifespan in comparison to the germ-free males. We are now investigating the physiological basis of this differential response, by analysing the metabolic state of these males. In summary, *L. plantarum* acts as a true mutualist of *D. melanogaster*, as it allows the precocious emergence of mature and fertile adults without fitness drawbacks. Furthermore, in certain conditions, this commensal can even increase the lifespan of nutritionally challenged males.

D1169A A Molecular Genetic Analysis of the role of Carbonic Anhydrases in Tracheal Development of *Drosophila melanogaster*. *Grace Jean*[1*], Mark Keroles[1], Ben Russel[1], Sarah McGriff[1], Jyoti Nair[1], Christopher Kowalczyk[1,2], Jaclyn Arvedon[1], James Baker[1]. 1) University of Miami, Coral Gables, FL; 2) Nova Southeastern University, Fort Lauderdale, FL.

The respiratory system of *Drosophila* develops as a fluid-filled epithelium. Late in embryonic development, the fluid is rapidly replaced with gas, an event that occurs within minutes and is independent of contact with outside air. The mechanisms underlying this phase transition remain enigmatic, but carbonic anhydrases may play a crucial role in the process by producing CO_2 gas while simultaneously promoting reabsorption of fluid by the surrounding epithelium.

A bioinformatics study of the carbonic anhydrase gene family in *Drosophila* showed that though humans and flies possess similar numbers of carbonic anhydrase genes (13 and 15 respectively), most of these have evolved after divergence. Based on our analyses, we elected to study two genes that are unique to insect lineages, cah2 and CG6074. Both genes are expressed in the trachea, and have probable signal peptides, suggesting that they are secreted. Also consistent with secretion, Cah2 protein, is glycosylated and has a likely GPI linkage site. These two genes are developmentally and tissue specifically regulated, showing high expression in the tracheal epithelium at the time of tracheal filling.

To functionally characterize these genes, fly lines containing mutagenic MiMIC insertions in both CG6074 and cah2 were obtained from the Bloomington Stock center. Both insertions are in introns and are oriented properly to create early terminations in each of the proteins. The cah2 mutation proved to be lethal in the late embryonic to early larval stages; presenting with a variety of defects in tracheal filling and maintenance of the open airway. Excision lines completely revert the phenotypes indicating that the MiMIC insertion is the cause of the observed defects. We are in the process of generating lines to rescue mutants by tissue specific expression, and generating an endogenously tagged version of the cah2 gene to allow us to directly observe the temporal and spatial expression of the protein. These experiments will enable us to directly test the hypothesis that carbonic anhydrases mediate transitions between fluid-filled and gas-filled states.

D1170B Jak/Stat functions in reproductive aging. *M. Giedt*, D. Harrison. University of Kentucky, Lexington, KY.

Aging is a complex process, subject to genetic and environmental influences. Regulators of aging include cell signaling, nutrient sensing, DNA repair, protein homeostasis, changes in behavior, and stem cell maintenance. Reproductive tissues also display signs of aging, separate from the organism and aging in reproductive tissues can serve as a model for the study of aging overall. Although initially fertile, males mutant for the Jak/Stat ligand Upd3 experience premature loss of fertility, suggesting a minimum level of Jak/Stat activity is required for regulation of reproductive maintenance. However the Jak/Stat pathway is important for a number of different processes that could contribute to reproductive aging. Given the genetic complexity of aging, and its variation between individuals, the genetic architecture of reproductive senescence can be determined by a genome-wide association study (GWAS). Using the DGRP, we performed a GWAS of male reproductive lifespan to identify potential regulators of reproductive aging with links to Jak/Stat signaling. Our results identified candidates that are known targets or regulators of the Jak/Stat pathway. Among these were *pox-neuro (poxn)* a known target of Stat92E in the male posterior lobe. This gene has known functions in chemosensory bristle specification, courtship behavior, and male genitalia development, making it a logical candidate for validation. Ptp61F, a known negative regulator of Jak/Stat signaling was also identified in our analysis. Other loci that are known or predicted modifiers of HSPGs or other extracellular signaling regulators were identified and could have previously undocumented roles in regulation of Jak/Stat activity or other cell signaling processes regulating reproductive maintenance with age. We are currently validating these candidates for their roles in reproductive maintenance through knock down or misexpression of candidates using the Gene Switch system. Data from these analyses will be presented.

D1172A Patterns of Transposable Element Expression in Heads During *Drosophila* Aging. *G. A. Reeves*, C. Highfill, S. Macdonald. University of Kansas, Lawrence, KS.

Transposable elements (TEs) are mobile, selfish genetic factors that are present in large numbers in the genomes of many organisms. TE insertions can lead to deleterious consequences for organism function, and indeed TE proliferation has been linked to a decline in function of the central nervous system and the onset of neurological disease. In addition, age-dependent changes in the expression of certain TE families has been seen in *Drosophila* brain tissue. In order to determine the effects of TE expression and mobilization in the aging brain it is critical to first detail the pattern of TE expression change during the aging process.

Here, we use mRNAseq to evaluate the regulation of TE expression throughout the lifespan of multiple, genetically-distinct, inbred *Drosophila* genotypes, focusing on head-specific expression. In our first experiment we made use of samples of young and old mated females collected from multiple DSPR (*Drosophila* Synthetic Population Resource) strains aged under standard laboratory conditions. Following RNA extraction, library generation and sequencing, we tested for gene expression differences between young and old flies, and for age-related expression changes at 126 TEs. We confirmed previous reports that *Hsp22* expression increases markedly in the heads of old flies, and validated work showing that expression of antimicrobial genes also increases as flies age. We found that 8 TEs show a significant change in expression during aging ($p < 0.05$), all but one of which are Class I retrotransposons. However only 2/8 show the predicted increase in expression with age. For example, while expression of the *Tabor* element (an LTR retrotransposon) increases with age, expression of the *GATE* element (another LTR element) is reduced with age. We found no evidence for the previously reported increased expression of *R1* and *R2* LINE-like retrotransposons or *gypsy* LTR retrotransposons in aged heads of any strain examined.

Concerns with this experiment are the lack of replication within strains, and the use of just two samples of head tissue - young and old - per strain. To both increase our power and more precisely describe the change in TE expression during lifespan, we generated large numbers of mated females from two strains with marked differences in overall lifespan, and sampled flies for mRNAseq at several points throughout the aging process. We anticipate this new experiment will provide a more accurate picture of age-related changes in TE expression in the *Drosophila* head.

D1173B Atypical Paternal Centrioles are Essential for Progeny Embryogenesis. *T. Avidor-Reiss*, A. Khire, E. Fishman, K. Jo. University of Toledo, Toledo, OH.

Centrioles are conserved, self-replicating, microtubule-based 9-fold symmetric subcellular organelles that are essential for proper cell division and functions. Most cells have two centrioles and maintaining this number of centrioles is important for animal development. However, how animals gain their first two centrioles during reproduction is only partially understood. It is well established that in most animals, the centrioles are contributed to the zygote by the sperm. However, in humans, insects, and many other animals, the sperm centrioles are modified in their structure and protein composition, or they appear to be missing altogether. In these animals, the origin of the first centrioles is not clear.

We have discovered that *Drosophila melanogaster* sperm has a novel and atypical second centriolar structure that we named the proximal centriole-like structure (PCL). The PCL contains centriolar proteins but lacks microtubules and has a structure distinct from a typical centriole. Nevertheless, the PCL functions analogously to a centriole in the zygote; after fertilization, it recruits PCM, forms astral microtubules and found in one of the spindle pole, and provides a platform for the formation of a new centriole. Therefore, our data suggests that insect sperm provides two centrioles and suggest a universal mechanism of centriole inheritance among animals that include atypical centrioles.

We have also discovered that both the typical and atypical centrioles of *Drosophila melanogaster* sperm centrioles are remodeled during spermiogenesis. The ultrastructure and protein composition of the two centrioles is modified during spermiogenesis, resulting with two atypical centrioles in the spermatozoa. Paternal protein mutants that regulate this remodeling affect the resulting embryo. Altogether, our findings demonstrate that atypical paternal centrioles play a role in pre- and post-fertilization to ensure embryogenesis.

D1174C The multifunctional transcription factor Suppressor of Hairy-wing is required in spermatogenesis. *T. Duan*. University of Iowa, Iowa City, IA.

Drosophila Suppressor of Hairy-wing [Su(Hw)] is a polydactyl zinc-finger transcription factor that functions as an insulator, activator and repressor protein. Su(Hw) is nearly ubiquitously expressed in all tissues throughout development. One exception is the testis, wherein Su(Hw) is down-regulated mid-way through spermatocyte growth, at a time prior to the onset of the testis-specific transcriptional program. These observations prompted us to test whether Su(Hw) has a role in spermatogenesis. We found that loss of Su(Hw) causes age-dependent male sterility that is accompanied by defects in sperm production. The *su(Hw)* mutant testes are characterized by formation of bulges at the posterior end, which are filled with individualized sperm. Concomitant with pre-mature sperm release within testes, seminal vesicles are small. Based on the timing of Su(Hw) expression and its genome-wide localization within Lamin-associated domains, we hypothesized that Su(Hw) contributes to the developmental program in the testis by conferring transcriptional repression of testis-specific cluster genes in early spermatogenesis. This postulate was tested using microarray analysis to compare gene expression in $su(Hw)^{+/+}$ and $su(Hw)^{-/-}$ testes. Data obtained from these studies revealed that few (2%) of ~300 mis-regulated genes correspond to genes inside the testis clusters. Instead, the majority of these genes correspond to de-repressed neuronal genes, reminiscent of the transcriptional effects associated with Su(Hw) loss in the ovary. Our working model is that expression of one or more Su(Hw) target genes is altered in $su(Hw)^{-/-}$ testes, which interferes with normal spermatogenesis. Current investigations are focused on identifying genes responsible for this block.

D1175A Assessment of age-dependent effects on sperm quality and male fertility in *Drosophila melanogaster*. *H. Elwa*, S. Pitnick, S. Dorus. Syracuse University, Syracuse, NY.

The evolutionary trade-off between investment in somatic maintenance and reproduction predicts that the allocation of energy resources into reproductive traits should increase in an age-dependent manner. As an individual ages, the opportunity for future reproduction decreases; thus, despite resource limitation, individuals should allocate more resources to reproduction to maximize their potential reproductive output. Variation in the amount and timing of resources allocated to reproduction is expected to have significant fitness consequences. However, analyses of age-dependent changes in reproductive fitness often results in conflicting observations. We have examined the effect of male aging on different measures of sperm quality in *Drosophila melanogaster*; including sperm morphology, sperm competitiveness, reproductive output, and larval viability. Young (7-day old) and old (30-day old) RFP sperm-tagged wild type males, who were provided regular opportunities to mate during their lifetime, were used in all experiments. Sperm length measurements revealed an increase in length variation in old males, but no difference in average length between age cohorts. Single mating fertility assays, using standardized females, indicated that the total number of fertilized eggs laid does not differ in relation to male age, but did show a significant deficit in progeny surviving to eclosion for old males. Double mating competitive fertilization experiments were also conducted using a non-virgin wild type male with GFP-tagged sperm as a standard competitor. This revealed that the total number of sperm in the female reproductive tract and the seminal receptacle were reduced in matings with old males. Ongoing experiments are determining whether this is due to reduced sperm production or transfer by old males, or whether more sperm from old males is displaced by competing sperm and ejected by the female. Overall, our results document substantial fertility deficits associated with aging.

D1176B Roles for tissue-specific ATP synthase subunits in mitochondrial shaping and ATP synthase dimerization in *Drosophila*. *K. G. Hales*, E. C. Brunner, M. Bannon, N. Suresh, L. Regruto, B. Jepson, E. M. Sawyer. Davidson College, Davidson, NC.

Mitochondria in post-meiotic early round *Drosophila* spermatids aggregate and fuse into the Nebenkern, which consists of two interwoven large mitochondrial derivatives that together form a spherical structure equal in size to the nucleus. Homozygous *knon* males are sterile, showing defective internal Nebenkern structure and subsequent faulty elongation of mitochondrial derivatives along the growing spermatid axoneme. *knon* encodes a testis-specific isoform of an ATP synthase subunit that, based on its size and structure, may alter ATP synthase dimerization, a phenomenon known to mediate cristae morphology in many systems. We hypothesize that tissue-specific ATP synthase dimerization dynamics may underlie the unusual mitochondrial shaping within the Nebenkern. To test the role of Knon in mitochondrial dynamics and ATP synthase dimerization, we exogenously expressed it in flight muscle and larval brain using the GAL4-UAS system; we assessed mitochondrial structure via fluorescence microscopy, and ATP synthase dimerization via immunoblots of blue native gels. While preliminary data in flight muscle show no large scale alteration of mitochondrial shape, Knon exogenous expression did shift the balance of ATP synthase dimers and monomers, though less than when peripheral stalk components are knocked down via RNAi. Knon expression in flight muscle with simultaneous knockdown of the paralogous broadly-expressed subunit does not restore wild type ATP synthase complex formation as detected by BN PAGE, suggesting that Knon cannot substitute for its paralog in this tissue. Further studies of *knon* and other testis-specific ATP synthase subunit paralogs may help elucidate the basis for unusual mitochondrial dynamics in the Nebenkern.

D1177C Importin α1 is required for maintaining germline stem cells in *Drosophila melanogaster* testes. *G. R. Hime*[1], F. Casagranda[1], K. L. Loveland[2], J. R. Heaney[1]. 1) Dept. of Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria, Australia; 2) Dept. of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia.

Importin-α proteins are required for transporting proteins from the cytoplasm into the nucleus via interaction with Importin-β in all tissues but also have roles in transcriptional regulation and organisation of chromatin and have been suggested to act as developmental switches during germline development. The *Drosophila melanogaster* genome encodes four *Importin-α* genes, also designated *Karyopherin* (*Kap*) genes. We have identified a specific requirement for *Kap-α1* in maintenance of male germline stem cells and spermatogonial differentiation. Loss of function *Kap-α1* mutants are male sterile. They lose germline stem cells (GSCs) and this loss can be rescued by germline specific expression of

Kap-α1 but the rescued animals are still infertile indicating a secondary role of Kap-α1 in spermatogenesis. The GSC loss is accompanied by development of germ cell cysts that contain aberrant numbers of germ cells suggesting that cysts may be differentiating prematurely. The pre-meiotic *Kap-α1* mutant phenotype can be phenocopied by germline expression of a dominant-negative Kap-α1 protein that lacks the Importin-β binding domain. We identified genes known to be expressed in the testis that produce proteins containing a consensus nuclear localisation sequence and screened for the ability of the dominant-negative allele to prevent protein transport into spermatogonial nuclei. From this screen we identified CycH and CG12909 as potential targets of Kap-α1. Germline specific knockdown of CG12909 produces a phenotype similar to that of *Impα1* alleles. The mammalian ortholog of CG12909, LYAR, has been associated with recruitment of the methyltransferase PRMT5 to specific regions of chromatin and symmetric dimethylation of histone H4 Arg3. We are currently investigating if *Kap-α1* mutants genetically interact with CG12909 or have defects in histone dimethylation.

D1178A Functional characterization of a gene family essential for *Drosophila* spermatogenesis. *Benjamin Nicholson*, Maxwell Kearns, Geoffrey Findlay. College of the Holy Cross, Worcester, MA.

Several of the most abundant proteins found in the proteome of mature sperm in *Drosophila melanogaster* are encoded by a single gene family. To examine the functional consequences of the gene duplication events that gave rise to this family and to test whether these genes act redundantly, we used RNA interference to knock down expression of each gene in male testes. For most of the genes, knockdown males exhibited complete or near complete infertility. To investigate the nature of these fertility defects, we produced knockdown males that made sperm labeled with green fluorescent protein (GFP). These males transfer no (or very few) sperm to females during mating, and testis dissections revealed that knockdown males were unable to produce mature sperm. These results suggest that this family of proteins, in addition to being a major component of mature sperm, is also essential for spermatogenesis. Current experiments are focused on determining the stage(s) of spermatogenesis at which each member of this gene family acts, which may allow us to determine the pattern(s) of neofunctionalization or subfunctionalization that occurred after the gene duplication events.

D1179B Analyzing the role of the *aghino* gene in protein and vesicular trafficking during acrosome biogenesis in *Drosophila melanogaster*. *I. Paz*[1], H. Park[2], N. Rigler[2], B. Wakimoto[2]. 1) Heritage University, Toppenish, WA; 2) University of Washington, Seattle, WA.

The *aghino* (*agho*) gene was initially defined by a single male sterile mutation. Previously, we reported that *agho* is one of several genes required for sperm activation during fertilization. Here we show that *agho* is the earliest acting of five known genes in the sperm activation pathway. Analysis of new *agho* alleles recovered by reverse genetics showed that two mutations resulted in defects in biogenesis of the acrosome, a Golgi-derived organelle that is normally localized to the apical tip of mature sperm. We found that in *agho* mutants, sperm lacked acrosomes, but entered the egg efficiently then failed to undergo sperm plasma membrane breakdown, an event required to complete fertilization and initiate embryogenesis. Using a transgene expressing an Agho-Green Fluorescent Protein fusion, we detected the protein at the Trans-Golgi Network, the region of the Golgi that sorts and packages membrane and secreted proteins into vesicles for transport to post-Golgi destinations. Based on these observations, we propose that Agho acts in spermatids to regulate acrosomal protein and vesicular trafficking. We are currently asking whether Agho influences trafficking of non-acrosomal cargos in spermatids. Because Agho is widely expressed, we are also investigating whether mutations affect trafficking in other cell types. In addition to elucidating the steps required for acrosome formation and function, our results should define Agho's normal function in both *Drosophila* germ line and somatic cells.

D1180C Functional consequences of a selfish X-chromosome in *Drosophila neotestacea*. *K. Pieper*[1], K. Dyer[1], R. Unckless[2]. 1) The University of Georgia, Athens, GA; 2) University of Kansas, Lawrence, KS.

Genetic conflict is ubiquitous across the tree of life and can have significant consequences for the genome. The *sex-ratio* X-chromosome (SR) of the fruit fly *Drosophila neotestacea* is a selfish sex chromosome that promotes its own transmission at the expense of the Y-chromosome. In the testes of males that carry SR, half of the sperm fail to develop through an unknown mechanism and thus no Y-bearing sperm are produced. The offspring of these males are nearly all daughters, with the only sons being sterile X0 males. SR produces this selfish phenotype equally strongly against all tested genetic backgrounds, and there are no known phenotypic effects other than halved sperm production in males. Our goals are to characterize the mechanism of the SR phenotype, identify candidates for the causal loci on the SR chromosome, and understand the downstream effects of SR. As a first step, we performed whole transcriptome sequencing of the testes and carcass (whole body minus testes) of SR-carrying males and males carrying the standard X-chromosome. We compared gene expression levels to find transcripts differentially expressed in the testes of SR males but not the carcass, which are candidates for involvement in the selfish mechanism. Transcripts that are on the X-chromosome are particularly strong candidates for the causal loci, whereas autosomal genes that are differentially expressed in the testes may be activated downstream of the SR mechanism or regulated in trans by genes located on SR. Genes that are differentially expressed in the carcass may represent previously unknown pleiotropic consequences of SR. We also examine the differential expression of genes already known to be involved in different stages of spermatogenesis to identify when the SR mechanism activates to cause developmental failure. Overall, our results quantify the functional consequences of SR, identify potential candidates for the molecular mechanism, and lay a foundation for future investigations of the evolutionary genomic consequences of genetic conflict.

D1181A 3'UTR regulation may be involved in germ cell differentiation in drosophila. *L. Shan*. IGDB, Beijing, Beijing, CN.

Alternative cleavage and polyadenylation (APA) is a widespread phenomenon that generates transcript isoforms with alternative 3'UTR. Through alteration of 3'UTRs, APA potentially regulates the stability, cellular localization, and/or translation efficiency of target mRNAs. Previous work in our lab demonstrated that Tut, Bam, and Bgcn can form a physical complex to promote germ cell differentiation in *Drosophila*. Surprisingly, *mei-P26* has alternative 3' UTR in fly testes, longer in *tut*, *bam* or *bgcn* mutant while shorter in wild type. We aim to explore the relationship between the change of 3'UTR length and germline differentiation and how differentiation-related 3'UTR is regulated. At the first step, we analyzed the 3'UTR profiles of wild type, *tut*, *bam*, or *bgcn* mutant testes by Poly(A) Site Sequencing (PAS-Seq). We found that hundreds of genes' 3'UTR were lengthened in *tut, bam,* or *bgcn* mutant testes. Our data indicate that 3'UTR length is mis-regulated in spermatogonia-enriched mutant testes, suggesting that APA may be involved in germ cell differentiation. Due to the different cell types between the wild type control and these mutant testes in PAS-Seq, we will clarify whether the change of 3'UTR in *tut, bam*, or *bgcn* mutant testes is caused by germ cell differentiating stages. Meanwhile, we will examine the 3'UTR profiles at different stage of germline differentiation to further survey the relationship between the change of 3'UTR length and germline differentiation. In order to elucidate how differentiation-related 3'UTR is regulated, the mRNA target(s) of the Tut-Bam-Bgcn complex will be identified to clarify how the Tut-Bam-Bgcn complex regulates 3'UTR.

D1182B Which parent is damaged? Can Progeny show consequences? *E. Arroyo*[1], P. Ramos[1], A. Muñoz[1], R. Camacho[2], M. Altamirano[3]. 1) Faculta de Ciencias-UNAM,Ciudad de México, México; 2) IIB-UNAM, Ciudad de México, México; 3) Fes Zaragoza-UNAM, Ciudad de México, México.

Germ cells will become eggs or sperms, as result of a prolonged process of division and differentiation, compartmentalized in time and space. The exposure of the organisms to mutagens can affect this process, modifying the type and proportion of gametes produced, damaging their integrity and their ability to contribute to the formation of the next generation. This contribution is not similar for both parents and that is why in this study we compared the effect of the mutagen Methyl-Methanesulphonate (MMS) in the germ cells from female and male of *D. melanogaster* through the ability of germ cells to produce progeny and, by using markers linked to X, was determined the distance between the white-miniature genes from classic mapping to assess the type and proportion of gametes produced that contribute to the next generation. Wild-type females w+m+/w+m+ were mated with hemizygous males wm/Y of *D. melanogaster* to obtain F_1 larvae of 72±4h that were fed food enriched with successive dilutions of MMS (0.24 µM - 1mM) or distilled water (negative control), so that the maturation of germ cells occur in the presence of the mutagen. Survival (S) of treated flies was compared to the negative control. Of flies recovered of treatment 50 virgins ♀♀ (T) and 50 ♂♂ males (T) were selected for each concentration and were mated individually with untreated flies (NT) as follows: T X T, T X NT and NT X T. The progeny was classified and counted by phenotype to calculate fertility, average amount of progeny and recombination frequency. Higher concentrations of MMS were lethal. The Fertility and amount of progeny decreased in the crosses: T XT y NT XT. The frequency of recombination between the w-m genes in the progeny of exposed individuals were modified in certain concentrations depending on the parent exposure (female or male). The progeny more affected was from T X T crosses (both exposed) but when only one parent was treated, the germ cells from F_1 males exposed were more affected than the germ cells of F_1 females treated. Probably the treatment with MMS interfered with the process of maturation of the germ cells or even, one part of the germinal cells of the parent treated were eliminated without contribute in the formation of the new individuals. Acknowledgements: Postgraduate in Biological Sciences-UNAM, CONACyT Grant. To Drosophila Stock Center Mexico by provide the biological material, and to M. en C. Yaneli Trujillo-Varela by technical support.

D1183C Spargel/dPGC-1 is involved in Insulin-TOR signaling, nutrient sensing and Oogenesis. *M. A. BASAR*, Kishana Williamson, Atanu Duttaroy. Howard University, Washington, DC.

Peroxisome proliferator-activated receptor gamma co-activator *1* (PGC-1) is a transcriptional co-activator that is expressed abundantly in tissues with high- energy demand, including the brown adipose tissue, heart, skeletal muscle, kidney, and brain. In vertebrates PGC-1 has been linked to mitochondrial biogenesis and oxidative metabolism. Spargel/dPCG-1, the fly homolog of PGC-1 also regulates the expression of mitochondrial OXPHOS genes, mitochondrial O_2 consumption and ATP production. Earlier studies claimed that spargel (srl) belongs to insulin-TOR pathway and thus facilitate nutrient sensing by TOR. Gene array data suggested that among all adult tissues *srl* mRNA is abundantly expressed in the ovary. Using an anti-srl antibody we now have proof that srl expression is strictly limited to the ovary while the rest of the body expresses negligible quantities of srl. Female germline specific knockdown of *spargel* completely shuts down the egg chamber's growth at midstage afterwards the egg chambers die through caspase-3 activation. Most interestingly, feeding excess yeast doesn't change ovarian growth when spargel is ablated, which proves that spargel serves as a critical facilitator between nutrition and growth. Our analysis reveals that germline depletion of spargel causes coagulation of Notch protein in germ cell cytoplasm and Notch mediated *E(spl)* gene activation is negatively affected in the follicle cells. Although, Notch mediated Hindsight expression kicks off earlier. Finally, we now have proof that the RNA Recognition Motif (RRM) of spargel plays an important role in healthy egg development. In summary, spargel is indispensable in coordinating ovarian growth and development.

D1184A Characterizing highly conserved genes of unknown function in the *Drosophila* female germline. *Varsha Bhargava*, Courtney Goldstein, Juliana Pineider, Kevin Gonzalez, Michael Buszczak. University of Texas Southwestern Medical Center, Dallas, TX.

(VB and CG are co-first authors)

Germ cells transfer genetic information across generations. Any change in germ line DNA is inherited by succeeding generations. Therefore germ cell DNA must be protected from both internal and external assault. An advantage of sexual reproduction stems from the ability to

generate variation by exchange of chromosomal segments during meiosis. During meiosis, hundreds of double-stranded DNA breaks are initiated at once, which if generated in most other cell types would introduce chromosomal aberrations. Germ cells, however, execute the formation of these breaks while preventing their deleterious effects from becoming pervasive throughout the genome. The mechanisms underlying the robustness of germ cells in the face of DNA damage, however, are poorly understood. We have initiated an in vivo CRISPR-Cas9 knockout screen for genes highly enriched in the *Drosophila* female germ line. Mutants from the screen were assayed for increased gamma-His2Av, a marker for DNA damage. This led to the identification of an uncharacterized but highly conserved protein that contains a Spr-T domain. We have elected to name this gene bedlam, based on its tumorous phenotype. Through experiments in yeast and in cell lines, it has been shown that other members of the Spr-T family are involved in DNA-protein adduct repair. This study aims to characterize a potentially germ line-specific, conserved DNA protein adduct repair pathway, using genetic and molecular biological approaches. Thus, characterizing bedlam function may provide key insights into DNA break repair in multicellular organisms.

D1185B Proteomic analysis of CTP synthase filaments in *Drosophila*. *A. Chakraborty*, P. Y. Wang, W. C. Lin, L. M. Pai. Chang Gung University, Tao-Yuan, TW.

De novo pyrimidine pathway utilizes CTP synthase (CTPsyn) as a rate limiting enzyme for the biosynthesis of cytidine triphosphate (CTP). In nutrient depletion conditions, CTPsyn assembles into filamentary structures. This self- assembly, higher ordered structure suggests a key regulation of metabolism in the cell. The question that allures us the most is why this metabolic enzyme needs to form filaments? Is this an easy way to tune in the production of CTP whenever required? There has been already an ongoing debate on whether this specialized structure is catalytically active or just a protein aggregate. Our lab has demonstrated that CTPsyn filaments are regulated by ubiquitination and support S phase during endocycles in follicle cells. Recently it has been reported that IMPHD2 co-localizes with the CTPsyn filaments in human cell lines. Moreover, in *Drosophila*, the DAck was also found to co-localize with the CTPsyn filaments in the germ cells where the structure maintains the integrity of cell membrane through phospholipid production. It is possible that the structure has different associated proteins at different physiological conditions which can regulate their enzymatic activity. Therefore, it would be very important to identify the structural components of these filaments and elucidate their activities. Here we use a system in which CTPsyn is tagged to a genetically engineered peroxidases, APEX2. APEX2 can oxidise the biotin-phenol to generate a very short-lived biotin-phenoxyl radical which covalently tags the endogenous proteins adjacent to the APEX2 protein. Using the above approach we have successfully labelled the CTP synthase filaments in the live *Drosophila* ovary tissues with biotin and have successfully identified the biotinylated "targets" of the filament structure in germline cells, and in the follicle cells using mass spectrometry. Currently we are examining the involvement of the candidates in CTPsyn filament formation using co-localization or knockdown approaches.

D1186C Polar cells are required for formation of a functional micropyle. *M. Giedt*, D. Harrison. University of Kentucky, Lexington, KY.

The polar cells are a pair of specialized somatic cells at each end of the developing egg chamber. While their importance in follicle cell specification and border cell migration are understood, relatively little is known about their functions in late stages of oogenesis. The micropyle is a conical structure with a central lumen at the anterior of the egg and serves as the entry point for sperm during fertilization. Its formation is known to be dependent upon the timely migration of the border cells. If border cell migration is delayed, the micropyle exhibits defects, among which are blockage of the central lumen. Here we present evidence that the polar cells have a critical role in formation of a functional micropyle. It has been described that the polar cells form an extension into the developing micropyle, but the function or mechanisms driving its formation are unknown. We observed that *upd3* mutants have an increased incidence of unfertilized eggs and failure to form the micropyle channel. The polar cell/border cell cluster still migrates to the anterior of the oocyte, but the polar cell extension misses the micropyle resulting in blockage. Using live imaging and fixed ovaries with GFP labeled polar cells, we characterized the dynamics of polar cell extension and micropyle development in wild type eggs. Results show that extension morphology during micropyle formation is highly dynamic and predictable. Furthermore, the polar cell extension forms prior to appearance of the micropyle and seems to mark the location where the micropyle will form. We are currently characterizing polar cell behavior in wild type and *upd3* mutant egg chambers and these results will be reported. While autocrine Jak/Stat activity in the polar cells is not required for process formation but, its reduction does affect process morphology and polar cell adhesion. To determine when polar cell involvement is required for micropyle formation, we are impairing polar cell functions through genetic manipulation of cytoskeletal dynamics, signaling, and viability to examine the nature and timing of polar cell formation of the micropyle channel. Results from these experiments will be reported.

D1187A Analysis of CASK gene expression and function in *Drosophila* oogenesis. *T. L. Hoffman*, C. M. Brown, R. E. Boody, C. W. Smith, M. D. Popil, S. N. Bentley, T. R. Miller, J. L. Sanford. Ohio Northern University, Ada, OH.

CASK is a Ca^{2+}/CaM-dependent serine protein kinase belonging to the family of **M**embrane **A**ssociated **Gu**anylate **K**inases (MAGUK), all of which contain PDZ (PSD-95, Dlg, Zo-1), SH3 (Src Homology 3), and GK (guanylate kinase) domains. These domains function as protein scaffolding domains at cell-cell junctions and function to cluster channels and receptors. Previous work on CASK has focused on its role in the central and peripheral nervous systems, epithelial cell junctions, and neuromuscular junctions. However, recent work has suggested a role for CASK in *Drosophila* oogenesis. Specifically, a recent RNA interference screen identified CASK as a contributor to border cell migration. Border cell migration is a process by which two specialized polar cells are carried by 8-10 surrounding "border cells" from the anterior of the egg chamber to the oocyte where they form the micropyle, the site of sperm entry in the *Drosophila* egg. This work suggests that CASK may have an important role in development of the fully mature female egg. Moreover, because border cell migration is used as a model of collective cell migration, studies of CASK protein function and binding partners can lead to novel insights into the mechanisms that regulate how cells move

en masse. As a means of establishing a general framework for future investigations into CASK's role in *Drosophila* oogenesis, CASK isoform expression and the affect of CASK knockout on fecundity and fertility were assayed. To establish which CASK isoforms are expressed in ovaries, reverse-transcriptase PCR (RT-PCR) was conducted on total fly and ovary extracts from the wild-type w1118 fly line using isoform specific primers to all seven *Drosophila* CASK isoforms (CASK-A, -B, -D, -E, -F, -G, -H). This work has confirmed the expression of CASK-A, -B, -G, -H and –E in *Drosophila* ovaries; work confirming the presence or absence of isoforms –D and –F is still underway. In order to assess a possible role for CASK in fecundity and fertility we took advantage of an available CASK knockout fly line. Fecundity was measured by counting the number of eggs laid by individual CASK knockout female flies at 24 and 48 hours (n= >30 for both wild-type and knockout fly lines). These results demonstrated that CASK knockout does not affect the rate of egg laying in female flies. Further work will assess whether CASK knockout affects fertility by measuring embryo viability. This work represents a vital first step in the characterization of CASK protein function in *Drosophila* oogenesis and the process of border cell migration.

D1188B The relationship between egg size and terminal filaments in *Drosophila melanogaster* lines selected for divergent egg size. *D. Mohan*, C. Quintanilla, C. Miles. Augustana University, Sioux Falls, SD.

Egg size is a complex, adaptive, and highly polygenic trait. Paired ovaries in *Drosophila* females consist of multiple, independent, oocyte-producing modules called ovarioles. Changes in egg size are thought to influence maternal fitness, which is correlated with ovariole number in adults. Ovariole number is reported to be determined during the larval-pupal transition phase (LP-phase) by morphogenesis of somatic structures called terminal filaments. Terminal filaments (TFs) are stacks of terminal filament cells (TFCs) that each give rise to an ovariole. Previously, we generated replicate populations of *D. melanogaster* artificially selected for large and small egg volumes (Miles et al., 2011), and inbred lines were derived from the selected populations. Adult females from multiple inbred lines from the large-egg populations had fewer ovarioles than those from small-egg lines (Jha et al., 2015). Here we use antibody staining and confocal microscopy to examine developing ovaries dissected from LP stage females from these same inbred lines. We expected that the number of TFs would predict the number of ovarioles in adults (Hodin and Riddiford, 2000). Surprisingly, we found that the number of terminal filaments was not an accurate predictor of adult ovariole number in many of these inbred lines. Several of the large-egg lines have significantly more TFs at the LP stage relative to adult ovariole numbers. We include discussion of the implications of this finding. .

D1189C Germline regulation of *Sex lethal* in *Drosophila melanogaster*. *R. Goyal*, K. Baxter, M. Van Doren. Johns Hopkins University, Baltimore, MD.

In *Drosophila*, sex-determination is under the control of the "switch" gene *Sex lethal* (*Sxl*). While in some species the sex of the soma is sufficient to determine the sex of the germline via inductive signaling, sex-determination in the *Drosophila* germline also occurs cell-autonomously via intrinsic signaling dictated by the germline sex chromosome constitution. Interestingly, when *Sxl* is expressed in XY germ cells, these germ cells are able to produce eggs upon transplantation into a female somatic gonad, demonstrating that even in the germline, *Sxl* is the "switch" and is sufficient to activate female identity [1]. In both the germline and soma the presence of two X chromosomes leads to *Sxl* expression. However, its control is different in the germline than in the soma at both, *cis*- and *trans*- regulatory levels, and we are studying how this is regulated in the germline. The DNA elements responsible for the activity of *Sxl*'s sex-specific promoter (*SxlPe*) in the germline have not been identified. To identify these elements, we are cloning different DNA fragments covering the entire region upstream of the *Sxl* transcriptional start site into an enhanced GFP (eGFP) reporter vector to test for sex-specific expression in the germline. Further, we are investigating the *trans*- acting factors that control *Sxl* expression in the germline. Based on previous studies, the X chromosome "counting genes" important for activating *Sxl* in the soma are not required in a dose-dependent manner in the germline. However, we have found that knocking down *sisterlessA* (*sisA*) specifically in the female germline results in an ovarian tumor phenotype, similar to masculinization of the germline due to loss of *Sxl* function. Encouragingly, *Sxl* expression is lowered in *sisA* RNAi ovaries. Previously, our lab has found that germ cells without SXL exhibit a derepression of *Phf7*, a male germline sexual identity gene. We are investigating if *Phf7* is expressed in *sisA* RNAi ovaries but have already found that *Phf7* RNAi rescues the ovarian tumor from *sisA* RNAi. Finally, we are searching for additional *trans*-regulators of *Sxl* through an RNAi screen of genes expressed sex-specifically in the undifferentiated germline. Our studies will further our understanding of how the 'sex' of the germline is coordinated with the 'sex' of the soma. Since a failure to match germline and somatic sex leads to defects in gametogenesis in both flies and humans, this work is important for our understanding reproductive biology and human health.

[1] Hashiyama, K., Y. Hayashi, and S. Kobayashi. "Drosophila Sex Lethal Gene Initiates Female Development in Germline Progenitors." Science 333, no. 6044 (2011): 885-88.

D1190A The novel Tudor-domain protein TDRD5P regulates male germline sexual identity. *Caitlin Pozmanter*, Shekerah Primus, Mark Van Doren. Johns Hopkins University, Baltimore, MD.

The process of sex determination in *Drosophila* is regulated by the RNA-binding protein Sex lethal (Sxl) in both the germline and the soma. While the mechanism by which SXL brings about sexual dimorphism in the soma is well studied, its function in the germline is less well understood. To identify genes that are regulated by SXL in the germline, RNA sequencing was done in a *bam* mutant background to compare *Sxl*-RNAi ovaries to control ovaries. Analysis of this expression data uncovered a previously uncharacterized gene CG15930 with a 16-fold increase in expression in *Sxl*-RNAi females. Comparison of *bam* mutant testes and ovaries also showed a 16-fold higher expression level in males suggesting that SXL functions in the female germline to repress CG15930. CG15930 contains a single tudor domain and is most closely related to the mouse *TDRD5* and the Drosophila *TDRD5* homolog *tejas*, so we have named it *tdrd5-prime* (*tdrd5p*).

To elucidate the function of TDRD5P in the male germline, we generated mutant alleles using CRISPR/CAS9 along with an HA tagged genomic

BAC construct for analysis of the TDRD5P protein. The *tdrd5p* mutant testes show several phenotypes such as hub displacement, germline loss, and a 50% reduction in fecundity indicative of an important role for TDRD5P in germline differentiation. Confocal microscopy of HA-TDRD5P testes shows an interesting localization of the protein to discrete cytoplasmic punctae near the nuclear periphery. This suggests that TDRD5P may localize to an RNA body such as the P-body or nuage similar to other tudor domain containing proteins. Since tudor domain proteins are known to function in the piRNA pathway, RT-PCR was conducted for numerous transposons in *tdrd5p* mutants and found no change in expression. We are currently conducting RNA-seq to compare *tdrd5p* mutants to wildtype to investigate whether TDR5P regulates the levels of other cellular RNAs. To determine what type of RNA body TDR5P functions in, we are conducting co-immunoprecipitation followed by mass spectrometry. Additionally, to understand the mechanism by which TDR5P functions we plan to do a series of truncations to identify potential important regions of the protein that have yet to be annotated. Based on this data we propose that the previously uncharacterized TDR5P functions to ensure the proper development of the male germline and must be repressed by SXL to allow for female germline development.

D1191B Male-specific development of the gonad stem cell niche regulated by *doublesex* and *fruitless*. *H. Zhou*, C. Whitworth, M. Van Doren. Johns Hopkins University, Baltimore, MD.

In *Drosophila melanogaster*, sexual differentiation of the male and female gonads is controlled by the key sex-specific transcription factor Doublesex (DSX). While homologs of DSX are known to control sexual development in virtually all animals, including humans, the downstream mechanism and targets for these proteins remain largely unknown. Previously, our genomic analysis of DSX identified *fruitless (fru)* as a candidate downstream target of DSX to direct male gonad development. FRU is expressed in key somatic cells of the testis stem cell system (the hub, cyst stem cells and early cyst cells) but is not found in the ovary. It is well known that *fru*, is regulated by sex-specific alternative splicing to promote male-specific expression. Surprisingly, we found that male-specific FRU expression in the gonad is not caused by TRA/TRA-2 mediated alternative splicing and, instead, DSX^M is necessary and sufficient to turn on FRU in the gonad regardless of the chromosomal sex. Our genomic analysis indicates that DSX directly binds to *fru* P3 and P4 promoters to regulates *fru* expression at the transcriptional level.

A key step in establishing gonadal sex identity is the formation of sexually-dimorphic gonad stem cell niche. We previously found that *dsx* is required at 3rd larval instar to actively maintain the male niche identity and inhibit transdifferentiation into a female niche. The temporal expression pattern of FRU correlates with this process and our genetic analysis indicates that *fru* is required to maintain male niche identity. We also found that *fru* is required in the adult for both cyst stem cell maintenance and cyst cell differentiation. We propose that *fru* functions downstream of *dsx* in the male gonad as a masculinizing factor to maintain the hub identity and cyst stem cell homeostasis.

D1193A Identification and Characterization of Novel Genes in the *Drosophila* Heart. *TyAnna Lovato*, Dylan Brown, Devon Lagueux, Richard Cripps. Univ New Mexico, Albuquerque, NM.

Drosophila is an effective model for understanding the genetic factors and pathways that direct the formation of the heart. Using fluorescent-activated cell sorting (FACS) of embryonic heart cells followed by RNA sequencing, we were able to identify novel genes expressed during embryonic heart development. In this project, we have chosen to characterize CG8147, CG9336, and CG7033. CG8147 has predicted alkaline phosphatase activity while CG7033 is a predicted chaperone protein with ATP binding capability. CG9336 currently has no known conserved functional domains. To understand the how these genes might affect heart development, we are utilizing CRISPR/Cas9 technology to generate mutant fly stocks in order to further investigate their role in development. .

D1194B Altering expression of JAK/STAT pathway components in air sac primordia of *Drosophila melanogaster*. *N. A. Powers*, A. Srivastava. Western Kentucky University, Bowling Green, KY.

JAK/STAT signaling facilitates vital developmental processes in a diverse array of mammalian and invertebrate species. One such species, *Drosophila melanogaster*, appears to be a strong candidate for investigating and modeling mechanisms for early morphogenetic changes common to both humans and fruit flies. Of particular interest are the potential roles that signaling pathways implicated in invasive growth patterns, such as JAK/STAT, serve in oncogenesis and metastasis of tumors. In *Drosophila*, progenitors of adult air sacs, which supply oxygen to flight muscles, are known as air sac primordia (ASP), and function in a manner comparable to human lungs. Because these ASP propagate into wing imaginal discs invasively, we have begun investigating possible roles that JAK/STAT signaling proteins might play in the directed morphogenesis of these structures. Our current research focuses on enhancing or suppressing expression of three components in the JAK/STAT signaling cascade of *Drosophila*: the JAK (*hopscotch*), ligand receptor (*domeless*), and STAT (*Stat92e*).

D1195C *GATAe* regulates intestinal stem cell maintenance and differentiation in *Drosophila* adult midgut. *T. Adachi-Yamada*[1], K. Takeda[1], M. Kuchiki[1], M. Akaishi[1], K. Taniguchi[1], T. Okumura[1,2]. 1) Gakushuin Univ, Tokyo, JP; 2) Waseda Univ, Tokyo, JP.

Adult intestinal tissues, exposed to the external environment, play important roles including barrier and nutrient-absorption functions. These functions are ensured by adequately controlled rapid-cell metabolism. GATA transcription factors play essential roles in the development and maintenance of adult intestinal tissues both in vertebrates and invertebrates. We investigated the roles of *GATAe*, the *Drosophila* intestinal GATA factor, in adult midgut homeostasis with its first-generated knock-out mutant as well as cell type-specific RNAi and overexpression experiments. Our results indicate that *GATAe* is essential for proliferation and maintenance of intestinal stem cells (ISCs). Also, *GATAe* is involved in the differentiation of enterocyte (EC) and enteroendocrine (ee) cells in both Notch (N)-dependent and -independent manner. The results also indicate that *GATAe* has pivotal roles in maintaining normal epithelial homeostasis of the *Drosophila* adult midgut through interaction of N signaling. Since recent reports showed that mammalian GATA-6 regulates normal and cancer stem cells in the adult intestinal tract, our data also provide information on the evolutionally conserved roles of GATA factors in stem-cell regulation.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

D1196A A functional genomics approach to identify targets of Forkhead domain transcription factors involved in cardiogenesis. *Shaad M. Ahmad*[1,2], Ye Chen[3], Xujing Wang[3]. 1) Department of Biology, Indiana State University, Terre Haute, IN; 2) The Center for Genomic Advocacy, Indiana State University, Terre Haute, IN; 3) National Heart, Lung and Blood Institute, NIH, Bethesda, MD.

While mutations in four genes encoding Forkhead (Fkh/Fox) domain transcription factors (TFs) have been linked to human congenital heart defects and eight Fkh TFs are required for proper cardiac development in mammals, little is known about the molecular mechanisms or the downstream target genes by which these Fkh TF-mediated cardiogenic functions are brought about. Our prior work identified the cardiogenic roles of two *Drosophila* Fkh genes, *jumeau* (*jumu*) and *Checkpoint suppressor homologue* (*CHES-1-like*). Mutations in these genes result in numerous heart defects, including disruptive changes in cardiac cell types and numbers, errors in cardiac progenitor cell specification and division, and defects in cardiac cell position. Furthermore, Fkh TF binding sites are significantly enriched in combination with those of other known cardiogenic TFs in the enhancers of cardiac genes. Collectively, these results indicate that these two Fkh TFs integrate diverse cardiogenic processes by regulating a large number of downstream target genes, thus raising the question of what these target genes are and what their individual functions might be during heart development. To address this question we have utilized RNA-sequencing to compare genome-wide transcriptional expression profiles of flow cytometry-purified mesodermal cells from wild-type, *jumu* loss-of-function, *CHES-1-like* loss-of-function, and *jumu* and *CHES-1-like* dual loss-of-function embryos. The putative Fkh targets identified by our analysis, i.e. genes exhibiting significant differential expression in *jumu* and/or *CHES-1-like* mutants can be divided into four classes: (i) 985 genes that are regulated only by *jumu* but exhibit no significant change in expression levels in *CHES-1-like* mutants, (ii) 318 genes that are regulated solely by *CHES-1-like*, (iii) 287 genes that are differentially expressed both in individual *jumu* mutants and in *CHES-1-like* mutants, and (iv) 541 genes exhibiting significantly altered expression levels in embryos lacking both Fkh gene functions, but not in either individual *jumu* or *CHES-1-like* loss-of-function mutants, indicating that they are regulated by the two Fkh TFs in a mutually redundant manner. Gene ontology enrichment analyses suggest multiple cardiogenic roles for these putative Fkh targets (specification of cardiac mesoderm, establishment of heart lumen, proper positioning of different heart cell types, sarcomere and myofibril formation, assembly of mitochondrial subunits and processes, cardiac progenitor cell divisions, etc.) that are being experimentally tested.

D1197B The Forkhead transcription factors CHES-1-like and Jumu mediate correct positioning of cardiac cells. *Shaad M. Ahmad*[1,2], Neal Jeffries[3]. 1) Department of Biology, Indiana State University, Terre Haute, IN; 2) The Center for Genomic Advocacy, Indiana State University, Terre Haute, IN; 3) National Heart, Lung and Blood Institute, NIH, Bethesda, MD.

The development of a complex organ requires the specification of appropriate numbers of each of its constituent cell types as well as the correct positioning of these cell types within the organ. Our previous work on *Drosophila* embryonic heart development had shown that the Forkhead (Fkh/Fox) transcription factors Checkpoint suppressor homologue (CHES-1-like) and Jumeau (Jumu) both specify cardiac progenitors by regulating the expression of the fibroblast growth factor receptor Heartless and the Wnt receptor Frizzled and determine the correct number of different cardiac cell types by regulating the subsequent division of the cardiac progenitors through a Polo-dependent pathway. Here we show that *CHES-1-like* and *jumu* are also required for the correct positioning of these cardiac cell types: loss-of-function mutations in or RNA interference knockdown of either gene results in the misalignment and incorrect locations of both cardial and pericardial cells within a hemisegment. Since defective cardiac progenitor cell divisions in *CHES-1-like* and *jumu* loss-of-function mutants frequently result in individual hemisegments having different numbers of cardiac cells than their partner across the dorsal midline, we initially examined this asymmetry as a possible cause of incorrect positioning. Our statistical analyses revealed that steric constraints imposed by the differing number of heart cells in contralateral hemisgments are not sufficient to explain all of the observed defects in heart cell positioning: statistically significant increases in the number of incorrectly positioned cardiac cells are also observed in Fkh mutants compared with wild-type embryos when only members of contralateral hemisegment pairs having the same number of each cardiac cell type are compared. In order to identify the genetic subnetworks utilized by CHES-1-like and jumu to bring about correct positioning, we next compared genome-wide transcription expression profiles of purified mesodermal cells from wild-type embryos and embryos either lacking *CHES-1-like* or *jumu* or overexpressing either of these Fkh genes. Gene ontology (GO) enrichment analysis of differential expressed genes in these Fkh mutants or Fkh-overexpressing embryos suggests that *CHES-1-like* and *jumu* could be regulating components of at least two known pathways involved in proper cardiac cell positioning: one involving Uncoordinated5 and NetrinB, and the other involving heterotrimeric G proteins and septate junction proteins. We are experimentally testing these hypotheses at present.

D1198C Improving the molecular toolkit to study muscle differentiation. *E. R. Czajkowski*[1], R. Cripps[1], A. Bryantsev[2]. 1) University of New Mexico, Albuquerque, NM; 2) Kennesaw State University, Kennesaw, GA.

During myogenesis, multinucleate muscle fibers arise from the fusion of single nucleate myoblasts. In *Drosophila*, myoblast fusion is initiated by a specific kind of myoblast called founder cells (FCs), which determine the differentiation program for the nascent muscle fibers. Much of the information about muscle fusion and general muscle development is obtained via a toolkit of genetic drivers that are used to manipulate gene expression in a cell-specific manner. Typically, genetic drivers express the yeast transcriptional factor Gal4 to activate expression from artificial transgenic constructs containing Gal4-responsive upstream activating sequence (UAS). rP298 is a FC-specific genetic driver, but its practical use is very limited because it quickly becomes inactive upon the first fusion between FCs and myoblasts. We demonstrate that by adding a self-activating transgene UAS-Gal4 to rP298, it is possible to prolong Gal4 expression beyond myoblast fusion, into more advanced developmental stages including the adulthood. The idea is that Gal4, once initiated by rP298, activates itself though the UAS promoter and maintains continuous Gal4 expression. Thus, by allowing the Gal4 gene to maintain expression in post-fused muscle fibers, we now can study those regulatory genes that only become activated in FCs and which are likely to control the muscle differentiation program.

D1199A Examining the role of Elongin B in *Drosophila* muscle development. *K. C. Dobi*[1], M. K. Baylies[2]. 1) Baruch College, New York, NY; 2) Memorial Sloan Kettering Cancer Center, New York NY.

Skeletal muscles come in a variety of shapes and sizes important for functions like running or eye blinking; however, we do not yet understand the mechanisms that generate muscle fibers with discrete morphologies. To determine how muscles with distinct shapes and sizes are formed, we have focused on the transcription elongation factor and E3 ubiquitin-ligase complex member Elongin-B. Elo-B is conserved from yeast to humans, and was recently identified in a whole-genome screen for novel factors in Drosophila muscle development. Elo-B mutants display somatic muscle defects including muscle loss and misattachment. To learn more about the role of Elo-B in muscle development, we are examining the muscle pattern of Elo-B mutants, as well as mutants for proteins that interact with Elo-B. Taken together, these experiments are providing a clearer picture of the network of gene activity required for muscle development.

D1200B Mechanisms Regulating Gonad Development and Function. *J. C. Jemc*, D. Silva, M. Bednarz, C. Lenkeit, E. Chaharbakhshi, E. Temple-Wood, A. Droste, A. Dabbouseh, S. Moqeet. Loyola University Chicago, Chicago, IL.

Fertility depends on the establishment and maintenance of interactions between germ cells and somatic cells within the gonad. During embryonic development, primordial germ cells (PGCs) and somatic gonadal precursor cells (SGPs) are specified at distal locations, and they must migrate, coalesce, and interact to form a gonad with the proper architecture. This work focuses on the characterization of the molecular mechanisms that regulate the migration of the SGPs, their interactions with each other, and their interactions with the PGCs during *Drosophila* gonadogenesis. A genetic screen resulted in the identification of a number of genes linked to the cytoskeleton genetically or molecularly that are required for gonad morphogenesis, including two transcription factors, *ribbon* (*rib*) and *longitudinals lacking* (*lola*). *rib* and *lola* encode members of the BTB (Broad complex, Tramtrack, Bric-à-Brac) family of proteins, and are required for the coalescence and compaction of SGPs and PGCs into a cohesive, spherical gonad. Both genes are expressed in the embryonic gonad, and genetic studies reveal that these genes may function in a common pathway to promote gonad morphogenesis. As BTB proteins form homo- and heteromeric complexes, we tested the ability of these proteins to interact via their BTB domains and observe both homo- and heterodimerization of the BTB domains of both proteins, suggesting that these proteins may cooperate to regulate a common set of target genes. Studies are underway to determine if these proteins function predominantly in transcriptional activation or repression and to identify the downstream targets through which they function. In addition, previous studies by *Davies et al. 2013* demonstrated a requirement for Lola in the adult testis. Therefore, we examined a potential role for Rib in homeostasis in the adult gonad. We observe expression of Rib in the germ cells and somatic cells of the adult testis. Current studies are focused on identifying the role of Rib in these cell types in the adult testis. These studies suggest that Rib and Lola cooperate to regulate early gonad morphogenesis, and that Rib may function similarly to Lola to regulate homeostasis in the adult.

D1201C Variable Effects of eRpL22 Family Paralogue Depletion on Eye Development in *Drosophila melanogaster*. *B. W. Gershman*, V. C. Ware. Lehigh University, Bethlehem, PA.

In *D. melanogaster* ribosomal protein (Rp) eRpL22-like has a tissue-specific expression pattern in the testis and eye (Kearse *et al*., 2011), compared to its ubiquitously expressed paralogue eRpL22. Consistent with a ribosomal role, these paralogues are structurally similar within the rRNA binding domain (C terminus). Yet, considerable sequence divergence within the N terminal domain of each paralogue suggests the possibility of functionally divergent roles as components of ribosomes or as proteins functioning in other pathways. The developmental significance of differential expression of eRpL22 paralogues is poorly understood. Here we focus on the requirements for eRpL22 paralogues in three stages (3rd instar larva, midpupa, adult) of eye development using an RNAi-mediated Gal4-UAS strategy for tissue-specific paralogue knockdown, followed by immunohistochemistry to determine paralogue localization and score differences in eye development phenotypes for each paralogue. eRpL22 is ubiquitously expressed, but eRpL22-like localization is tissue/cell-specific depending on developmental stage. In 3rd instar larva, eRpL22-like is detected at low levels within the cytoplasm of the eye disc proper; however, within the peripodial membrane, the cytoplasmic distribution of eRpL22-like is polarized toward the disc. In pupal stages, eRpL22-like is highly enriched within the developing inter-ommatidial bristle (IOB) complex. In adult tissue, localization of eRpL22-like shifts from the IOB complex to the ring of accessory cells surrounding each ommatidia (2° pigment, 3° pigment, IOB complex). Non-overlapping localization patterns in different cell/tissues is suggestive of specific functional roles for eRpL22 paralogues. We then compared eRpL22 paralogue localization during eye development following paralogue knockdown. *eyeless*-Gal4 induced knockdown affects early mitotic ells while GMR-Gal4 induced knockdown affects differentiated cells. *eyeless*-Gal4- mediated eRpL22 depletion results in ablation of head and eye structures. In contrast, no notable effect on gross eye morphology or the head is apparent with *eyeless*-Gal4 mediated eRpL22-like depletion. GMR-Gal4 mediated eRpL22 depletion leads to ommatidia disorganization and generation of a late onset eye lesion; however, eRpL22-like knockdown with GMR-Gal4 generates a bristle-specific defect. Notably, a different phenotype results from paralogue-specific knockdown using the same Gal4 driver, suggesting non-redundant (and specialized) roles for each paralogue during eye development. Future work will include attempts to rescue paralogue-specific knockdown phenotypes with overexpression of the cognate paralogue to determine the degree, if any, of functional redundancy in the eRpL22 family.

D1202A Organ-Specific Transcriptional Co-regulation of Growth and Form in the *Drosophila* Embryo. *R. Loganathan*[1], J. Lee[2], M. B. Wells[1], E. Grevengoed[1], M. Slattery[2], D. Andrew[1]. 1) Johns Hopkins University, Baltimore, MD; 2) University of Minnesota.

Tubular organogenesis is critically dependent on the coordinate regulation of growth and form as the size and shape of tubular tissues are the prime determinants of their functional complexity. Transcription factors, which regulate the spatiotemporal patterns of gene expression during development, affect multiple aspects of organogenesis. In this work, we describe the distinct role of Ribbon (Rib) during organogenesis of

epithelial tubes. Our investigations of the embryonic salivary gland (unbranched tubular organ) and the trachea (branched tubular organ) demonstrate that Rib co-regulates growth and form during tubular organogenesis. In *rib* null embryos, we observed remarkable changes in cell shape and size of both organs, which underlie the characteristic tube elongation defects. Notably, the changes in growth and form in the *rib* mutants occurred without effects on cell specification, proliferation, apoptosis, apicobasal polarity or junctional integrity. How does Rib selectively co-regulate growth and form in these tubular epithelia? To identify the transcriptional targets of Rib, we performed whole embryo microarray gene expression analysis comparing RNA samples (1.5X minimal fold change, $P < 0.05$) from age-matched wild-type and *rib* null embryos. Microarray gene expression analysis showed that 774 genes were potentially activated whereas 1176 genes were potentially repressed by Rib. To distinguish direct tissue-specific Rib targets, we performed ChIP-seq analysis in embryos driving *rib* expression specifically in the salivary gland and the trachea. Superposition of potential Rib targets obtained from the microarray gene expression and ChIP-seq analyses resulted in a total of 60 genes (20 activated, 40 repressed) subject to direct Rib regulation in the salivary gland. Interestingly, Rib was subject to transcriptional autoregulation during salivary gland formation. Meanwhile, 242 genes (54 activated, 188 repressed) were subject to potential direct Rib regulation in the trachea. A subset of potential Rib targets in both organs was validated by *in situ* hybridization and qRT-PCR. Intriguingly, Rib targets in both tubular organs functionally clustered around cell growth and morphogenesis, suggesting that the co-regulation of growth and form is the primary role for Rib during tubular organogenesis. Moreover, the mechanism by which Rib mediates co-regulation of growth and form is organ-specific as revealed by distinct target clusters in the salivary gland (translational machinery) and the trachea (growth factor signaling machinery). Collectively, our results suggest organ-specific mechanisms in the transcriptional co-regulation of growth and form by Rib in the *Drosophila* embryo.

D1203B The molecular process of epidermal cuticle formation in *Drosophila* larvae. *Y. Pesch*[1,2], M. Behr[2]. 1) LIMES-Institute, University of Bonn, Germany; 2) Institute of Biology, University of Leipzig, Germany.

The proper architecture of an outer body wall is fundamental to animals and plants. The insect cuticle provides exoskeletal stability and protection against wounding, desiccation, invading pathogens and other potential harms. In order to adapt to increasing body growth the insect cuticle is periodically shedded in a molting process. Molting and wounding requires organized cuticle rearrangement. It has been discussed that chitin microfibrils as a main component of the cuticle may undergo spontaneously self-assembly, but how exactly the complex architecture of the cuticle is arranged and reorganized during growth, molting and wound healing is not known.

We discovered a molecular mechanism of cuticle formation, maturation and protection in the *Drosophila* larval epidermal cuticle.

Upon cuticle synthesis chitin is secreted by the epidermal cells and forms to fibrils of various lengths at the apical cell surface. The chitin binding protein Obstructor-A (Obst-A) binds these nascent chitin-fibrils and forms a scaffold for their proper integration into the lamellar cuticle structure. The Obst-A scaffold is required to recruit and localize the deacetylases Serp and Verm for matrix assembly and maturation. Loss of *obst-A* leads to severe growth defects, impaired epidermal cuticle integrity and death before the molt to second instar larval stage. In summary, the molecular Obst-A core unit is essential to form, mature and keep an intact cuticle in insects.

For cuticle organization Chitinases process chitin-fibrils into fragments of different lengths. Surprisingly, our results indicate that Chitinases also play a role in maintaining structural and mechanical integrity of the cuticle. The Chitinase 2 is necessary for lamellar organization and for proper function of Obst-A at the apical cell surface.

All molecular mechanisms of cuticle formation are primarily concentrated at the apical cell surface in a so far completely uncharacterized region - the cuticle assembly zone. Our findings place the assembly zone in the center of cuticle organization. Due to protein sequence conservations this may hold true for insects and most chitinous invertebrates.

Finally, our data provide first evidence for a novel feedback mechanism how the cuticle assembly zone protein Obst-A regulates ecdysone signaling for larval molting and growth control.

D1204C Regulation of Air Sac Primordium Development by a Cathepsin-L in *Drosophila melanogaster*. *C. J. Fields*[1], Qian Dong[1], Breanna Brenneman[1,2], Ajay Srivastava[1]. 1) Western Kentucky University, Bowling Green, KY; 2) University of Virginia, 1335 Lee Street, Charlottesville, VA 22903.

In *Drosophila*, the adult thoracic Air Sacs supply oxygen to the flight muscles. These Air Sacs develop from a group of larval cells that form the Air Sac Primordium (ASP). The ASP is located superficially over the larval wing imaginal disc and in response to Fibroblast Growth Factor (FGF) signal, migrates and invades into the wing imaginal disc to occupy a more deep seated position. The regulation of invasive behavior during ASP development is not well understood. Data are presented that implicate a Cathepsin-L in ASP development and invasive cellular behavior. Utilizing immunohistochemistry and confocal microscopy we assessed the expression of Cathepsin-L in larval wing imaginal discs. The knockdown of Cathepsin-L in ASP was achieved by UAS-Gal4 driven RNAi and the overexpression of Cathepsin-L was performed using the UAS-Gal4 system of gene expression in *Drosophila*. We found that a single Cathepsin-L is upregulated in the ASP and downregulation of this gene results in aberrant ASP development, loss of Filopodia from ASP and suppression of invasive behavior. Furthermore, we demonstrate that overexpression of Cathepsin-L results in degradation of the Basement Membrane (BM). Our data demonstrate that a Cathepsin-L is involved in the development of ASP by regulating the invasive cellular behavior and by degrading the BM. These data are particularly significant given that Cathepsin-L is also expressed during early human lung development and invasive cellular behavior and degradation of the BM are critical events during tumor metastasis.

D1205A Novel growth factors regulate tube morphogenesis in the *Drosophila* ovary. *Sandra G. Zimmerman*, Anne E. Sustar, Celeste A. Berg. University of Washington, Seattle, WA.

During development, flat epithelia give rise to tubular structures that exhibit a spectacular variety of shapes, sizes, and functions. Although tubes are essential for multi-cellular life, relatively little is known about the genetic and molecular mechanisms that create tubes. A tractable model for tubulogenesis in *Drosophila,* one that resembles primary neurulation in vertebrates, is the formation of dorsal appendages (DAs), eggshell structures that facilitate gas exchange for the embryo. DAs are secreted by cellular tubes that form during oogenesis in the egg chamber. An egg chamber consists of one oocyte and 15 germline nurse cells surrounded by a somatic follicular epithelium: columnar cells cover the oocyte and squamous "stretch" cells cover the nurse cells. Cues from these different cell types facilitate DA formation, which occurs when two dorsal patches of columnar cells fold into tubes and elongate by migrating over the stretch cells, all in the absence of cell division or cell death. Mutations in the genes encoding the SOX transcription factor, Bullwinkle (BWK), which functions in the germline, or non-receptor tyrosine kinases SHARK and SRC42A, which function in the stretch cells, lead to DA cell adhesion defects, aberrant cell migration, open tubes, and moose-antler-like DAs. Other components of the pathway are unknown. To discover new genes that regulate tubulogenesis downstream of *bwk*, we used mass spectrometry to identify differentially expressed proteins in the stretch cells of *bwk* vs. wild-type egg chambers. Members of a novel family of growth factors, Imaginal disc growth factors (IDGFs), were upregulated in the stretch cells of *bwk* egg chambers. IDGFs are catalytically inactive, chitinase-like proteins that have human orthologs and arose through gene duplication. Due to a conserved mutation that renders them inactive as chitinases, IDGFs have acquired new functions through evolution. Using stretch-cell specific overexpression, RNAi, and CRISPR/Cas9-mediated gene deletions, we found not only that IDGF upregulation is sufficient to cause DA defects, but that IDGF expression is also required normally at optimal levels for proper DA morphogenesis. Our data suggest that IDGFs may function redundantly in this context. Although previous studies demonstrate that IDGFs interact synergistically with insulin to modulate growth and polarization of cultured cells, our data suggest that the insulin signaling pathway is not the central mode of action for IDGFs in DA formation. Determining how these novel growth factors function will be a key to understanding the genetic and molecular basis of tubulogenesis.

D1206B Dissecting the mechanism of calcium wave during *Drosophila* egg activation. *Qinan Hu*, Mariana Wolfner. Cornell University, Ithaca, NY.

Egg activation is the critical step in which the developmentally arrested oocytes shifts to begin embryogenesis. This process is characterized by a rise of intracellular free calcium levels, beginning at one region of the oocyte and sweeping across the whole egg. This Ca^{2+} rise triggers downstream events including meiosis resumption and changes in the transcriptome and proteome. Although in vertebrates and marine invertebrates fertilization triggers the Ca^{2+} rise, in *Drosophila* it is triggered prior to fertilization in the form of a wave that starts at pole of oocytes. This calcium wave is induced by physical forces that occur during ovulation. Genetic studies and inhibitor tests suggest that these forces activate mechanosensitive ion channels in the oocyte that then let in Ca^{2+} from extracellular environment. Currently we are using chemical, physical, and genetic means to identify the ion channels that introduce Ca^{2+} into the oocyte, and to determine how and where they act and are regulated.

D1207C Identifying protease targets of the *Drosophila* serine protease inhibitor Serpin42Da. *J. R. Kannangara*, M. A. Henstridge, T. K. Johnson, J. C. Whisstock, C. G. Warr. Monash University, Clayton, AU.

Serine protease inhibitors, or serpins, are a family of protease inhibitors that use a complex mechanism of conformational change in order to inhibit target proteases. Although the structural mechanism of serpin function is well characterised, the biological roles and targets of many serpins remains elusive. The genome of *Drosophila melanogaster* contains 29 serpin-encoding genes*,* however very few have been functionally characterised. Among the few characterised *Drosophila* serpins, Serpin42Da (Spn42Da) is of particular interest as it encodes 10 different isoforms that are predicted to have distinct sub-cellular localisations and different target specificity. To investigate the roles of Spn42Da to help predict target proteases we over-expressed Spn42Da transgenes in various *Drosophila* tissues. We found that over-expressing the Spn42Da-A isoform in ovarian follicle cells using *slbo*-Gal4 causes embryos to be laid with a fragile eggshell due to defects in the cross-linking of the inner-most eggshell layer, the vitelline membrane. As Spn42Da-A has previously been shown to inhibit the serine proteases Furin1 (Fur1) and Furin2 (Fur2) *in vitro*, we examined these proteases as candidate targets for Spn42Da-A. Genetic interaction experiments between *spn42Da-A* and the Furin proteases revealed that *spn42Da-A* interacts with *Fur2* in the follicle cells during eggshell biogenesis. We are currently performing additional genetic interaction experiments between *spn42Da-A* and *Fur1,* and investigating the effect of both *Fur1* and *Fur2* RNAi knockdown on eggshell biogenesis. Finally, we have also generated a *spn42Da* null mutant which we are currently testing for interactions with *Fur1* and *Fur2*. Taken together, this study will provide fundamental insight into the molecular mechanisms surrounding *Drosophila* eggshell biogenesis, and lead to a greater understanding of the serpins and proteases involved in producing this complex structure.

D1208A Differentiate or die: the role of *Dm ime4* in embryogenesis and gametogenesis. *C. F. Hongay*[1], Kangning Li[1], Jill Pflugheber[2], Aquilla Chase[1]. 1) Clarkson Univ, Potsdam, NY; 2) Saint Lawrence University, Canton, NY.

My previous work on *Dm ime4* showed its importance for oogenesis (Hongay and Orr-Weaver, PNAS 2011). Recent work in my laboratory demonstrates that this gene is crucial during embryogenesis and continues to be essential in adults for fertility. We used a balanced stock where *ime4+* can be traced with GFP, and the *ime4*$^{(hypomorph)}$ allele is unmarked. We scored under several microscope magnifications, using DAPI and other stainings, GFP(+) and GFP(-) embryos in all stages of development and found statistically significant decreases -measured as departures from the expected Mendelian ratios of GFP(-) to GFP (+) and analyzed by Chi square tests-starting at gastrulation. Furthermore, GFP(-) embryos display high levels of apoptosis, aberrant DAPI stainings, and other severe malformations. In addition, we localized *Dm ime4*

mRNA by *in situ* hybridization and *Dm* IME4 by antibody staining in wild-type embryos and found very informative and specific localizations that strongly support its role en embryogenesis.

For our gamatogenesis analyses in adult gonads, we used the "live or dye" staining system together with other stem/germ/somatic markers to determine the role of *Dm* IME4 in germ and somatic cell differentiation. Our findings are in line with recent reports on the role of *Dm ime4* orthologs (*e.g*., mttl3 mutants are embryonic lethal in mice), but with the use of hypomorphic mutants and RU-486 inducible RNAi, we are able to get detailed and valuable information on its role in Drosophila development.

Given the evolutionary conservation of *Dm ime4*, our findings will inform future studies in vertebrate systems.

D1209B An Lmx1 homolog is required for *Drosophila* ovary support structure development. *A. Allbee*, B. Biteau. University of Rochester, Rochester, NY.

Lmx1a and Lmx1b are examples of highly conserved LIM homeobox transcription factors with clearly established requirements for the patterning, differentiation and adult function of diverse tissues in mammals. Not surprisingly, these transcription factors are implicated in a wide variety of human pathologies, including Nail Patella Syndrome, Parkinson's disease, renal disease and ovarian carcinoma. However, few examples exist where precise target genes, binding partners and downstream cellular functions are known. CG32105 is a *Drosophila* homolog of both Lmx1a and Lmx1b, and has never been studied.

Using CRISPR, we have generated flies carrying a full deletion of the CG32105 open reading frame. Surprisingly, homozygous adults are viable with no obvious defects in external morphology or behavior. However, females are sterile with collapsed ovaries. Looking as early as larval stages, we show this phenotype likely arises from a primary defect in the development of ovarian support structures. Consistent with this, lineage-tracing analysis reveals that a CG32105 putative enhancer drives expression in what is possibly a common somatic ovary progenitor. Furthermore, by driving knock down and over-expression of the transcript, we find this same enhancer is sufficient to both recapitulate and rescue the knock out phenotype, respectively. Taken together, these data argue a requirement for this Lmx1 homolog in the development of *Drosophila* ovary support structures, and indicate we have the tools necessary to address function at precise temporal and cell-specific levels. Using both a candidate approach and a simple, non-biased screening approach, we expect rapid and precise dissection of the mechanisms through which this Lmx1 homolog mediates *Drosophila* ovary development. Interestingly, while *Drosophila* oogenesis is a highly studied process with major contributions to cell biology, very little is known with regards to the genetic mechanisms through which the ovary develops. We therefore anticipate contributing not only to the understanding of LIM homeobox transcription factors, but also to the understanding of this largely understudied developmental process, which could be used as a powerful, genetically tractable model in developmental biology.

D1210C Controlling reproduction through microRNAs: lessons Drosophila might have taught mammals. *J. A. Sanchez-Lopez*[1], I. Carmel[1], D. Spiller[2], M. White[2], Y. Heifetz[1]. 1) The Hebrew University of Jerusalem, Rehovot, IL; 2) The University of Manchester, Manchester, UK.

Mating induces a rapid change in the *Drosophila* female's physiology and behavior. The female increases her oviposition rate and avoids other males, and the morphology of her reproductive tissues changes rapidly. Mating-induced physiological responses are modulated by major changes in the expression profile of the female reproductive tissues. In the lower female reproductive tract (lower RT) miRNAs are involved in the regulation of this rapid response to mating. It has been demonstrated that miRNAs also regulate reproductive processes in mammals, such as mouse, pig and human. In human, for example, miRNAs regulate responses to gametes and modulate communication between the embryo and the maternal tract during implantation and pregnancy. miRNAs are highly conserved across species and thus might play a role in regulating the basic modules of reproduction which are broadly conserved.

To better understand the role of miRNAs in female reproductive success, we compared the miRNA expression profiles of unmated and mated Drosophila lower RTs with reported changes in miRNA profile of human endometrium in preparation for implantation to identify candidate miRNAs. We found two miRNAs, *let-7a* and *miR-9a,* whose expression was significantly altered in both organisms. Using a miR-sensor, we tested miRNA spatial localization *in vivo* in Drosophila females. We showed that the expression of *miR-9a* in Drosophila is reduced specifically in the spermathecal secretory cells (SSC) at 6h post-mating. This is the time when the reproductive tract is preparing for fertilization and egg laying. We then devised a miR-sensor system for use *in vitro* in mammalian cells to test *in vitro* the interaction of trophoblast spheroids (simulating the embryo) with human endometrial cells. We observed that the human endometrial cell line reacted to the presence of the trophoblast spheroids by increasing the expression of *miR-9a*. These results suggest that *miR-9a* might be an important miRNA for reproductive success as it seems to mediate the reproductive mechanisms of both species. Using our miR-sensor system, we further performed time lapse analysis to follow the interactions of the trophoblast and endometrial cells in real-time. We observed that the endometrial cells react to the presence of the trophoblast through miRNA-dependent and independent mechanisms. Understanding the role of conserved miRNAs in reproductive tissues will provide insight into mechanisms of female reproductive success.

D1211A Investigating the role of intracellular pH in epithelial stem cell differentiation. *M. Benitez*, B. Ulmschneider, B. Grillo-Hill, D. Barber, T. Nystul. University of California-San Francisco, San Francisco, CA.

Transitions in the fate or morphological state of epithelial cells are central to metazoan development, homeostasis, and tissue repair. We are using the follicle stem cell (FSC) lineage in the Drosophila ovary as a model to investigate the potential role of intracellular pH (pHi) in cellular differentiation in an adult stem cell lineage. We found that pHi increases naturally during follicle cell differentiation and that blocking this increase interferes with differentiation toward the polar/stalk cell fate. To investigate how pHi is controlled during follicle cell differentiation, we performed an RNAi screen through a collection of 20 genes that are predicted to regulate pHi. Our preliminary studies identified three genes from this collection that are required for follicle cell differentiation. We found that RNAi knockdown of CG8177, CG13384 and CG32081

prevents proper FSC development, leading to abnormal and ectopic polar/stalk cell formation. We are currently investigating how the knockdown of these genes affects follicle cell pHi. Lastly, we are investigating whether changes in pHi promote follicle cell differentiation by inhibiting Hedgehog pathway activity. Collectively, these studies will provide insight into a novel form of regulation of cellular differentiation in an epithelial stem cell lineage.

D1212B The Control of Germline Sexual Identity in *Drosophila melanogaster.* *Pradeep Kumar Bhaskar*, Raghav Goyal, Kelly Baxter, Mark Van Doren. Department of Biology, Johns Hopkins University, Baltimore, MD.

Sexual dimorphism is common throughout the animal kingdom, with males and females exhibit phenotypic characters specific for their sex. While a great deal is known about the establishment of sexual identity in somatic cells, this process is much less well understood in the germline. Germline sexual identity is critical for sex-specific development of germline stem cells and production of sperm vs. eggs. Thus, it is an essential aspect of animal sexual reproduction and human fertility. Germ cells depend on both signals from the somatic gonad as well as their own sex chromosome genotype to determine their sex. Therefore, when the "sex" the germline fails to match the "sex" of the soma, germline development is severely disrupted. How somatic signals and germ cell intrinsic cues act together to regulate germline sex determination is a key question about which little is known in any organism. We have previously identified the JAK/STAT pathway as a key male determining signal from the soma to the germline; evidence exists for a similar signal in females, but this signal has not been identified. Further, the RNA-binding protein Sex-Lethal (SXL) and the chromatin factor PHF7 have been identified as key components promoting female vs. male germline identity, respectively. We propose that the JAK/STAT pathway is a direct activator of PHF7 expression in the male germline, and that this pathway is inhibited by SXL in the female germline. Further, male-specific expression of PHF7 appears to be regulated at the levels of both transcription and translation to ensure its proper male-specific expression. Additionally we have evidence suggesting signals from the soma can affect PHF7 function, whereas SXL function in the germline seems to be exclusively regulated by intrinsic factors. We are investigating the regulation of the key germline sex determination factors PHF7 and SXL and how they are controlled by both somatic signals and germ cell intrinsic factors.

D1213C Inhibition of the RTK PVR in the hub cells of the *Drosophila* testis stem cell niche. *N. Bui*, J. Leatherman. University of Northern Colorado, Greeley, CO.

An adult stem cell is an undifferentiated cell, found among differentiated cells in a tissue or organ. The specific microenvironment of each tissue that adult stem cells reside in is called the stem cell niche. In a living organism, adult stem cells maintain and repair the tissue in which they reside. In the *Drosophila* testis niche, there are two different stem cell populations including the germline stem cells (GSCs) and the cyst stem cells (CySCs). These stem cells cluster around the hub, a group of non-dividing cells that constitute the niche. Previous research has indicated that proper communication between and within the hub cells, CySCs, and GSCs is important in maintaining stem cell self-renewal, and allowing proper cellular differentiation to achieve tissue homeostasis. We are investigating the function of a receptor tyrosine kinase (RTK) called PVR (PDGF-VEGF-related receptor) in this niche. We found that the ligand Pvf1 is secreted from hub cells, and its receptor PVR is expressed in both the hub and the cyst stem cells. We hypothesize that PVR signaling may be one mechanism by which the hub maintains expression of proper self-renewal signals during spermatogenesis. In this study we inhibited PVR in the hub cells by hub-restricted expression of either a PVR RNA interference (RNAi) transgene, or a dominant negative transgene. By comparing the experimental group with the control group, we found defects in the germline. Some testes had GSC-like individual germ cells distant from the hub where differentiation is expected. Other testes had transit-amplifying spermatogonia next to the hub. We also noticed that there was a reduction in the number of cyst stem cells surrounding the hub. Our conclusion is that inhibition of PVR in the hub leads to the improper maintenance of both stem cell populations in the testis, thus disrupting the regulation of spermatogenesis.

D1214A *Hrb27C* functionally interacts with ecdysone signaling to maintain the *Drosophila* female germline stem cell fate. *Danielle S. Finger*, Elizabeth T. Ables. East Carolina University, Greenville, NC.

Stem cells maintain an undifferentiated fate while creating new daughters destined for differentiation. Effective use of stem cells as tools for clinical therapy requires a detailed understanding of the molecular mechanisms that control their fate *in vivo*. A variety of molecular signals influence stem cell fate, including local signals from adjacent cells and long-range hormonal signals; however, little is known about how hormones promote the undifferentiated fate. Germline stem cells (GSCs) in the *Drosophila melanogaster* ovary are an ideal model to study the mechanisms of stem cell maintenance on a per-cell basis *in vivo*. GSCs are known to be directly regulated by the steroid hormone ecdysone, which is structurally and functionally similar to human sex hormones. Using a forward genetic screen, we identified *Heterogeneous nuclear ribonucleoprotein at 27C* (*Hrb27C)* as a potential downstream target of ecdysone signaling. *Hrb27C* is a member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family of RNA binding proteins that aid in the localization, maturation, and translation of newly formed RNAs. To investigate whether and how *Hrb27C* regulates GSC maintenance we used *Flippase/Flippase Recognition Target (FLP/FRT)*-mediated recombination to create homozygous *Hrb27C* mutant GSCs, and used molecular markers to track their fate. Our results demonstrated that *Hrb27C* mutants display greater GSC loss than their respective controls, indicating that *Hrb27C* is required for GSC maintenance. This loss is due to deregulation of pMAD, a component of the BMP signaling pathway that is required for repressing differentiation of GSCs. Further, non-allelic non-complementation experiments demonstrated that *Hrb27C* functionally interacts with both ecdysone signaling and BMP signaling to control GSC number. Taken together, these results suggest a model wherein *Hrb27C* mediates the effects of ecdysone signaling in GSCs. Future experiments will be aimed at determining if other hnRNPs work independently or in complex with *Hrb27C* to control GSC maintenance. This will increase our understanding of the function of *Hrb27C* and the hnRNP family in the regulation of stem cell fate, and provide insight into the mechanisms by which steroid hormones maintain stem cell fate in adults *in vivo*.

D1215B Investigating the role of neuropeptides in *Drosophila* ovary. *T. Ma*, D. Drummond-Barbosa. Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.

Reproduction depends on many factors including mating behavior, feeding behavior, and, crucially, the ability to detect and respond to changes from external stimuli. Neuropeptides are signaling molecules known to be involved in many of these processes in both mammals and other organisms such as insects. For example, neuropeptide Y (NPY) in mammals has been shown to play important roles in feeding, metabolism, reproduction, and stress response, with similar roles shown for neuropeptide F (NPF), the insect ortholog of NPY. Insulin-like peptides (ILPs) are neuropeptides produced by the central nervous system (CNS) and have been shown to directly affect germline stem cell (GSC) proliferation, GSC adhesion to the niche, niche size, cyst growth and follicle cell proliferation, and vitellogenesis in the *Drosophila melanogaster* ovary. Insulin also play crucial roles in mammalian fertility and reproduction. Other neuropeptides such as short NPF (sNPF) and prothoracicotropic hormone (PTTH) have been proposed to play important roles in oogenesis and reproduction in insects. However, much of the connection between neuropeptides and oogenesis has remained unexplored. My project aims to fill this gap by using the well-characterized *Drosophila* ovary to identify and functionally characterize neuropeptides that affect oogenesis, either directly or indirectly. Many neuropeptides and their receptors have been conserved through evolution; therefore, the results will likely have broad relevance and lead to a greater understanding of how the brain and ovary interact in humans and other mammals to control reproduction.

D1216C Cellular mechanisms underlying asymmetric sister chromatid segregation during asymmetric division of Drosophila male germline stem cell. *R. Ranjan*, X. Chen. Johns Hopkins University, Baltimore, MD.

Due to the crucial role that epigenetic mechanisms play in regulating cell identity and function, it has been a long-standing question whether and how stem cells maintain their epigenetic memory through many cell divisions. We found that during asymmetric division of Drosophila male germline stem cell (GSC), histone H3 (H3) becomes asymmetrically segregated — the “old” H3 is retained in the GSC while the “new” H3 is enriched in the differentiating daughter cell (Science, 338: 679-682). Recently, we also found that randomized H3 segregation pattern correlates with both GSC loss and progenitor germline tumor phenotypes, suggesting that asymmetric H3 inheritance is required for both GSC maintenance and proper differentiation (Cell, 163: 792–793). We propose that old and new H3 are asymmetrically deposited to sister chromatids, and mitotic machinery recognizes this asymmetry for differential segregation. To understand this question, we are using live-cell imaging to visualize spatial and temporal regulation of sister chromatids segregation. We have obtained promising data showing asymmetric microtubule activities in GSC, which first interact with the nuclear membrane at the GSC side during G2-phase, and then start “poke-in” and make a hole to interact with chromatin during G2/M phase. Interestingly, during G2 phase, centromeres cluster near the nuclear membrane at the stem cell side, with the “old” Cid (centromeric H3) enriched sister centromeres facing towards the centrosome at stem cell side, which is likely to secure attachment between old H3-enriched sister chromatids and microtubules emanating from the centrosome at the stem cell side. This occurs prior to the attachment of new H3-enriched sister chromatids to microtubules from the centrosome at the differentiating daughter cell side. Coincidentally, we also observed that sister chromatids enriched with old versus new H3 are condensed in a temporally separable manner: the old H3-enriched sister chromatids condense prior to new H3-enriched sister chromatids. We hypothesize that asymmetric microtubule activity, asymmetric centromere and differential condensation of sister chromatids act together to ensure their proper attachment followed by asymmetric segregation. By contrast, such an asymmetry in either the microtubule activity or sister chromatid condensation is not observed in symmetrically dividing progenitor germ cells. Together, we propose that the GSC microtubule has a novel function— it directs the orientation of sister chromatids. This proposed function provides an unappreciated role of the microtubule, namely, in selectively recognizing and segregating sister chromatids based on their differential epigenetic information.

D1217A HES overexpression causes neuroblast hyperplasia by repressing differentiation factors. *S. S. Magadi*[1], Nikolaou Christoforos[2], Christos Delidakis[1]. 1) FoRTH, Heraklion, Crete, GR; 2) UOC Department of Biology, Heraklion, Crete, GR.

Hes genes are immediate targets of Notch and are implicated in maintaining the undifferentiated state in many stem cell-based systems throughout the animal kingdom. Earlier work from our lab showed that larval CNS hyperplasia induced by Notch depends on the integrity of the *E(spl)* locus (harbors 7 Hes genes), although in this context we could detect activation of many other genes by Notch. The CNS defects were due to excessive accumulation of neural stem cells, or neuroblasts (NBs), at the expense of differentiated neurons and glia. In the process of dissecting the genomic networks regulated by Notch in proliferating larval NBs, we sought to discern the part of the signal that is transduced via the Hes proteins. Indeed, we could produce similar CNS hyperplasias by overexpressing only Hes proteins; the most severe defects were obtained by coexpression of E(spl)mγ and deadpan (dpn), another Hes gene that responds to Notch in this tissue. We analyzed transcriptional profiles from hyperplastic CNSs overexpressing Hes proteins individually or in combination. Different Hes overexpressions had nearly identical transcriptomes, which also had 50% overlap with Nicd overexpressing CNSs, suggesting that a large part of the Notch signal is transduced via the Hes proteins. Although Hes proteins are repressors, there is good overlap in both down- and up-regulated genes of Nicd, suggesting an extensive degree of feedforward regulation. For example, the stemness promoting factor grh and the temporal transcription factors svp and cas were amongst the common upregulated genes. We went on to characterize in more detail top downregulated transcription factor genes. Of 6 TFs studied in depth, three are primarily expressed in differentiating neurons, two are expressed in late NBs and one is expressed in intermediate neural precursors of the Type II lineages. Loss of function of most of these genes resulted in no effect suggesting functional redundancy, however overexpression or misexpression of some of these factors led to premature NB loss. At least three of these downregulated targets contain regions that are bound by Dpn by ChIP, suggesting direct regulation. We propose that the Notch-Hes axis maintains NBs in an undifferentiated state by repressing a cohort of TF genes that promote terminal differentiation.

D1218B Eyeless regulates nutrient-insensitive neuroblast proliferation in the central brain. *Conor Sipe*, Sarah Siegrist. University of Virginia, Charlottesville, VA.

The genetically tractable neural stem cell (NSC) population in the *Drosophila* central brain is ideal for investigating how organismal nutritional status affects stem cell proliferation. All neurons in the fly central brain are generated from asymmetric cell divisions of NSCs, known as neuroblasts (NBs). Most NBs enter a period of quiescence at the end of embyrogenesis coincident with declining maternal stores; upon larval feeding and reception of a nutrient-derived cue, these nutrient-sensitive NBs reenter the cell cycle and begin a second round of proliferation that continues until early pupal stages. In contrast, mushroom body (MB) NBs, a subset of central brain NBs, never enter quiescence and divide continuously regardless of developmental stage or systemic nutritional state. Both nutrient-sensitive NBs and nutrient-insensitive MB NBs reside in close proximity to one another, sharing a common brain macroenvironment. This suggests that quiescence versus proliferation decisions may be regulated in a cell-intrinsic manner. Currently, we are investigating whether the *Drosophila* Pax-6 ortholog, Eyeless (Ey) regulates NB quiescence versus proliferation in response to nutrient conditions. Ey, a paired box and homeodomain transcription factor, is expressed specifically in MB NBs and not in other central brain NBs during the embryo to larval transition. When Ey is knocked down in MB NBs, we observe that MB NBs, like other NBs, now enter quiescence when nutrients are limited. Importantly, we find that these quiescent MB NBs reactivate and re-enter the cell cycle once animals are returned to a complete diet. In addition, overexpression of Ey allows nutrient-sensitive non-MB NBs to maintain proliferation when nutrients are limited. This suggests that MB NBs utilize Ey to bypass quiescence during periods of nutrient restriction. Taken together, these results identify a novel genetic program under the control of Ey that promotes neuroblast proliferation independent of systemic nutrient cues.

D1219C Transcriptional regulation of *Drosophila* intestinal stem cells. *David Doupé*[1], Owen Marshall[2], Andrea Brand[2], Norbert Perrimon[1,3]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Gurdon Institute and Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK; 3) Howard Hughes Medical Institute, Boston, MA.

Intestinal stem cells (ISCs) in the *Drosophila* midgut have emerged as an excellent model for the study of epithelial stem cells and homeostasis. The modes of ISC fate, their ability to respond to tissue damage and the signaling pathways involved in their regulation are broadly conserved with mammalian epithelia of the intestine, epidermis and esophagus. While the extrinsic signaling pathways regulating *Drosophila* ISCs have been extensively studied over the last several years, less is known about the intrinsic determinants of stem cell fate. We have used targeted DamID to profile gene expression in undisturbed stem/progenitor cells and the major differentiated cell type (enterocytes). This allowed us to identify 53 transcription factors specifically expressed or enriched in the stem/progenitor population. Characterization of the most highly enriched TFs identified critical regulators of proliferation and differentiation with mammalian orthologs implicated in epithelial homeostasis or cancer. Target profiling by DamID reveals binding to the regulatory regions of cell cycle genes and signaling pathway components, providing possible links between intrinsic and extrinsic regulators of ISC fate.

D1220A Groucho controls proliferation and differentiation of *Drosophila* intestinal stem cells by regulating transcriptional output of multiple signaling pathways. *X. Guo*, R. Xi. National Institute of Biological Sciences, Beijing, CN.

Abstract: Groucho is an evolutionarily conserved transcriptional co-repressor and has been implicated in regulating cell proliferation and cell fate decisions in many developmental processes. But whether it has a role in intestinal stem cell lineages is unknown. From an RNAi genetic screen, we identified Groucho as a critical regulator of cell proliferation and differentiation of intestinal stem cells in Drosophila midgut. We find that depleting Groucho in ISCs leads to rapid accumulation of ISC-like cells and failure of enteroendocrine cell differentiation. It is known that Notch signaling activation is essential for ISC differentiation, but loss of Groucho does not compromise Notch pathway activation. Furthermore, ectopic expression of NiCD or the Notch target E (spl) genes fails to induce differentiation of Groucho-depleted ISCs. Together with the analysis on its role in ISC proliferation, we propose that Groucho acts as a co-repressor to regulate the transcriptional output of multiple signaling pathways: Notch signaling to promote ISC differentiation, and EGFR signaling to inhibit ISC proliferation.

D1221B Sox21a is a critical regulator of adult stem cell proliferation in the *Drosophila* intestine. *F. Meng*, B. Biteau. University of Rochester Medical Center, Rochester, NY.

The precise control of stem cell proliferation is essential to maintain tissue homeostasis. These resident stem cells can adapt their proliferative activity according to tissue demands, allowing normal tissue turnover at homeostasis condition and tissue repair by increasing their proliferation rate under various stress conditions. Although the stress-sensing signaling pathways are shared between stem cells and their differentiated progenies, stress-induced proliferative response is specifically activated in stem cells. The mechanisms underlying this stem cell-specific proliferation control are largely unknown. Here we find that the Sox family transcription factor Sox21a is specifically expressed in progenitor cells in the *Drosophila* intestine. We show that the stem cell-specific Sox21a expression is necessary for their proliferation both at homeostasis and stress conditions. Interestingly, we demonstrate that Sox21a expression level is induced upon stress exposure, which requires Ras, Jun N-terminal Kinase and Fos. Furthermore, we show that the stress-induced Sox21a expression is sufficient to induce stem cell proliferation. Surprisingly, the *Sox21a* mutant flies display no obvious developmental defects besides their shortened lifespan, highlighting a role of Sox21a in regulating stem cell proliferation specifically in adult intestine. In summary, our data show that the stem cell-specific Sox21a as an essential proliferation regulator, suggesting that stem cell-specific mechanisms are utilized to generate a unique proliferative response. We are currently investigating how Sox21a interacts with other known stress-sensing signaling pathways, as well as the mechanism(s) controlling stem cell-specific Sox21a expression. In addition, we are trying to identify Sox21a-interacting factors and transcriptional target genes to fully understand the mechanisms by which Sox21a precisely controls stem cell proliferation.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

D1222C The transcription factor Hindsight promotes enterocyte differentiation and is required for the specification of adult intestinal stem cells during the larval/pupal transition. *B. H. Reed*, B. Baechler, C. McKnight, P. Pruchnicki, N. Biro, D. Saghattchi. Univ Waterloo, Waterloo, ON, CA.

The *Drosophila* gene *hindsight* (*hnt*), homologue of mammalian *Ras responsive element binding protein-1* (*RREB-1*), encodes a C_2H_2-type Zinc finger nuclear protein that is required for several developmental processes including germ band retraction, tracheal differentiation, retinal morphogenesis, and proper growth of the follicular epithelium of the ovary. In some processes *hnt* has been identified as a direct target of the Notch signaling pathway. Given the importance of Notch signalling in the regulation of the adult intestinal stem cells (ISCs), we have investigated the regulation and function of *hnt* in this context. We show that *hnt* is expressed throughout the larval and adult midgut, and we further demonstrate that Hnt is required for ISC establishment during the larval/pupal transition. In the adult midgut we find that ISC *hnt* expression does not require Notch signalling, but is dependent on the EGFR/RAS/MAPK signalling pathway. Moreover, we find Hnt overexpression to be a potent effector of ISC loss through enterocyte (EC) differentiation. We also show that EC differentiation via Hnt overexpression can suppress ISC tumour formation associated with either the loss of Notch signalling or the activation of the EGFR/RAS/MAPK signalling pathway.

D1223A *Zfh2*, a conserved *Drosophila melanogaster* transcription factor involved in intestinal stem cell homeostasis. *S. Rojas Villa*[1], B. Biteau[2]. 1) University of Rochester, Rochester, NY; 2) University of Rochester Medical Center, Rochester, NY.

In most adult tissues homeostasis is maintained by tight regulation and crosstalk between pathways controlling self-renewal versus differentiation in adult Somatic Stem cells (SSC). Misregulation of signaling pathways regulating these processes have been linked to several conditions, such as cancer, and other age related diseases. In the last decade SSC in the *Drosophila melanogaster* intestinal epithelium have emerged as a powerful model to study these mechanisms. In this simple tissue the intestinal lineage has been well characterized and markers for every cell population have been defined. Recently our laboratory has identified *zinc-finger homeodomain 2* (*zfh2*), a highly conserved transcription factor, as an important player in the maintenance of intestinal homeostasis. Using immunohistochemistry, I have shown that *zfh2* is expressed in the *Drosophila* intestinal progenitors. Interestingly *zfh2* over-expression leads to a mild hyperproliferation phenotype. Conversely, its knock-down completely blocks stress mediated proliferation. Our preliminary data suggests that *zfh2* knock-down does not affect basal tissue turnover. In conclusion I have shown that proper expression of *zfh2* is necessary to maintain tissue homeostasis in the *Drosophila melanogaster* intestinal epithelium. We are now testing interactions with pathways known to regulate intestinal stem cell proliferation. Interestingly, mutations in *ATBF1*, the *zfh2* mammalian homolog, have been associated with different types of cancers. However nothing is known about any possible role of *ATBF1* in adult tissues under basal conditions. Characterizing *zfh2* in the *Drosophila* adult intestinal epithelium can shed light on the function of its mammalian homolog.

D1224B Injury-stimulated and self-restrained BMP signaling dynamically regulates stem cell pool size during *Drosophila* midgut regeneration. *Ai-Guo Tian*, Bing Wang, Jin Jiang. UT Southwestern Medical Center, Dallas, TX.

Many adult organs rely on resident stem cells to maintain homeostasis. Upon injury, stem cells increase proliferation, followed by lineage differentiation to replenish damaged cells. Whether stem cells also change division mode to transiently increase their population size as part of a regenerative program and if so, what is the underlying mechanism have remained largely unexplored. Here we show that injury stimulates the production of two BMP ligands, Dpp and Gbb, which drive an expansion of intestinal stem cells (ISCs) by promoting their symmetric self-renewing division in *Drosophila* adult midgut. We find that BMP production in enterocyte is inhibited by BMP signaling itself, and that BMP autoinhibition is required for resetting ISC pool size to the homeostatic level after tissue repair. Our study suggests that dynamic BMP signaling controls ISC population size during midgut regeneration and reveals mechanisms that precisely control stem cell number in response to tissue needs.

D1225C Metabolic role of GABA in the blood progenitors. *M. Shin*, J. Shim. Hanyang University, Seoul, KR.

Drosophila hematopoietic organ called Lymph gland is well known for its intrinsic and extrinsic controls in the maintenance of blood progenitors. Previous study has identified GABA as a novel systemic factor that regulates the blood progenitor maintenance through changes in cytosolic calcium concentration. GABA is released from a subset of neuroendocrine cells in the brain of which secretion is mainly controlled by the olfactory receptor neurons (ORNs). When GABA secretion is decreased, blood progenitors are differentiated as cytosolic calcium, a downstream of GABAB receptor in the blood progenitors, is down-regulated. However, whether excessive GABA influences the blood progenitor of *Drosophila* has not been described. Here, we found that constitutive activation of ORNs is enough to release excessive GABA from the brain that also interferes with the blood progenitor maintenance. Excessive systemic GABA increases the level of reactive oxygen species (ROS) in the blood that in turn results in the precocious differentiation of blood cells. Interestingly, this phenotype is rescued by changes in the TCA cycle, indicating a tight link between the systemic GABA and metabolic changes in the blood cells. Overall, our work highlights the novel function of an unconventional TCA-cycle that couples in part with a shunt pathway and its importance in the fine balance of ROS in the blood progenitors.

*This work was supported by NRF-2014S1A2A2028388 and NRF-2014R1A1A1002685.

D1226A Regulation of niche cell plasticity in the Drosophila testis. *Leah Greenspan*, Erika Matunis. Johns Hopkins University, School of Medicine, Baltimore, MD.

Stem cells are necessary to maintain homeostasis within adult tissues. These cells receive signals from their surrounding microenvironment or niche in order to function properly. Precise regulation of stem cells and their niches is vital to prevent depletion or overgrowth of the stem cell pool. Although many mammalian niches have been characterized, the *Drosophila* testis provides a unique and more accessible system to study the regulation of a stem cell niche *in vivo*. This well-defined niche consists of a cluster of non-dividing (or quiescent) somatic hub cells that signal to the attached germline and somatic stem cells. Damaging the testis or overexpressing genes that activate the cell cycle in hub cells induces hub cell divisions, but also leads to the conversion of hub cells to somatic stem cells. This change in cell fate is accompanied by the formation of new niches, characterized by the presence of ectopic hubs each supporting active stem cells. Recently I found that the highly conserved cell cycle inhibitor and tumor suppressor retinoblastoma homolog RBF is a critical regulator of hub cell maintenance. Loss of RBF in the hub is sufficient to cause hub cell proliferation, and lineage tracing reveals that RBF prevents hub cells from converting to somatic stem cells. In addition, extended knockdown of RBF in the hub also leads to ectopic niche formation. Live imaging of hub cells undergoing loss of quiescence in multiple conditions indicates that converting hub cells migrate farther distances than their wild-type counterparts. Knockdown of RBF along with its interacting partner (the cell cycle activator E2F) rescues both the over-proliferation and cell fate conversion phenotypes, suggesting that targets of E2F may drive both the proliferation of hub cells and their conversion to somatic stem cells. However, these two phenotypes may be separable. Loss of the transcription factor escargot from hub cells causes them to convert to somatic stem cells (Voog et al., Cell Reports 2014), and my preliminary data suggest that Escargot does not prevent loss of hub cell quiescence or ectopic niche formation. Thus, RBF works through the canonical cell cycle pathway to actively regulate hub cell quiescence and identity, and likely acts upstream of escargot and additional factors during this process. This work provides a model for understanding the regulation of niche cell quiescence and identity, which could be pertinent in other stem cell niches to prevent over-proliferation and metastases. .

D1227B The Role of PVR in *Drosophila* Testis Stem Cells. *Nastaran SoleimaniBarzi*, Jade Koretko, Kenneth Hammer, Judith Leatherman. University of Northern Colorado, Greeley, CO.

Adult stem cells live in different tissues and they support the tissue they reside in. The stemness behavior is regulated by the environment they reside in, called the stem cell niche. The niche environment in mammals is complex, which makes it hard to study, therefore we use the *Drosophila* testis as a simple model to study adult stem cells in their niche. In the *Drosophila* testis niche there are two populations of stem cells: germline stem cells (GSCs), which will differentiate into sperm, and cyst stem cells (CySCs) that will differentiate into cyst cells. These stem cells are physically in contact with a group of somatic cells known as the hub. As long as they stay in contact to the hub, they will remain stem cells. CySCs and their lineage play a crucial role in self-renewal and differentiation of GSCs. EGFR, a member of Receptor Tyrosine Kinase (RTK) family, is known to promote differentiation in cyst lineage. PVR (PGFG-VEGF-related receptor) is another member of RTK family that is expressed in the cyst lineage. This led us to ask the question whether PVR functions similarly to EGFR, or whether it has a distinct function. Pvf1, the ligand for PVR, is restricted to the hub, suggesting that PVR signaling is only activated in CySCs, not cyst cells. We hypothesize that PVR is one required factor in CySC self-renewal. In order to test our hypothesis we used two strategies, PVR gain of function and PVR loss of function. For gain of function we expressed a constitutive PVR transgene, which caused cyst-lineage cells to divide away from the hub, where they would normally differentiate. For loss of function we expressed PVR RNAi transgenes, a dominant negative PVR transgene, and performed mosaic analysis. Our results show a reduction in CySC number when PVR is ablated in the cyst lineage. In conclusion, PVR is required to maintain the normal cyst stem cell population in *Drosophila* testes. Future work will examine whether PVR is required to maintain normal cell division rates in these stem cells.

D1228C Reactivation of Quiescent Neuroblasts Requires Activation of PI3-kinase Signaling in Cortex Glia. *X. Yuan*, S. Siegrist. University of Virginia, Charlottesville, VA.

Precise timing of stem cell entry into and exit from quiescence is essential for tissue morphogenesis and homeostasis. *Drosophila* neuroblasts (NBs), the stem cells of the Drosophila CNS, provide a model for investigating regulation of stem cell quiescence. At the end of embryogenesis, all NBs in the central brain, except for a subset, exit cell cycle and enter a period of quiescence. NBs exit quiescence and begin proliferating in response to a nutrient-derived cue received soon after larvae consume their first complete meal. NB exit from quiescence fails if larvae consume a meal that lacks amino acids. The current model posits that amino acids are required for the synthesis and secretion of Dilp6 in glia and that local production of Dilp6 in glia is required to reactivate quiescent NBs through activation of the highly conserved PI3-kinase growth signaling pathway in NBs. We are investigating whether amino acids are also required for glia growth, morphology, and subsequent ensheathment of NBs. When animals are fed a diet lacking amino acids, we find that the amount of glial membrane and growth is reduced compared to animals fed a complete diet. This reduction in glial growth is likely caused by a reduction in levels of PI3-kinase activity in glia in response to absence of dietary amino acids. NBs fail to exit from quiescence when dietary amino acids are absent or when PI3-kinase is reduced in glia. Currently, we are investigating whether nutrition first stimulates activation of PI3-kinase signaling in cortex glia, leading to glial growth and ensheathment of NBs, and whether glial ensheathment is required for NB exit from quiescence.

D1229A Destruction of E2f1 is dispensable for stem cell proliferation. *Taylor D. Hinnant*, Elizabeth T. Ables. East Carolina University, Greenville, NC.

Growing evidence suggests that some molecular regulatory pathways dictating cell fate are associated with the cell cycle machinery; however, the molecular connections remain unclear. Because the ability of *Drosophila* ovaries to create mature oocytes depends on a well-characterized population of germline stem cells (GSCs), the proliferative ability of GSCs must be tightly regulated. GSCs therefore serve as an exceptional model for study of cell cycle control in stem cells. The GSC cell cycle has been previously characterized by monitoring the morphology of the fusome, a germline-specific organelle that changes morphology during the cell cycle. To more precisely understand GSC cell cycle regulation, we characterized the Fluorescence Ubiquitin Cell Cycle Indicator (FUCCI) system in GSCs, which utilizes degradable versions of E2f1::GFP and CycB::RFP to fluorescently label cells in each phase of the cell cycle. We found that the FUCCI system faithfully labeled GSC cell cycle phases consistent with somatic cell types, but must be used in combination with fusome morphology for most accurate cell cycle assessment. Importantly, we found that E2f1::GFP expression mirrors that of Cyclin E, previously demonstrated to control GSC fate independently of proliferation. In contrast to *Cyclin E* mutants, however, loss of *E2f1* has only minor effects on maintenance of the GSC fate. Additionally, GSC proliferation and maintenance appear to be largely independent of degradation of E2f1, as stabilizing E2f1 expression throughout the cell cycle results neither in major loss of GSCs nor obvious increases in cell death. Our data suggest that repression of E2f1 activity in GSCs may instead be more highly controlled by Rb and/or the repressor E2f2. We are currently investigating the role of *E2f2* in this context. Our data also further emphasizes that cell cycle-independent roles of Cyclin E regulate maintenance of the GSC fate. These findings provide further insight into the regulation of the balance between maintaining cell fate and proliferation in GSCs, with broad implications on other asymmetrically dividing cells.

D1230B Investigating Histone Inheritance Patterns at Specific Genomic Loci. *E. Kahney*[1], M. Wooten[2], L. Feng[3], X. Chen[4]. 1) Johns Hopkins University, Baltimore, MD; 2) Johns Hopkins University, Baltimore, MD; 3) Johns Hopkins University, Baltimore, MD; 4) Johns Hopkins University, Baltimore, MD.

Stem cells rely on epigenetic mechanisms to regulate cell identity as they undergo **asymmetric cell division (ACD)** to give rise to a daughter that self-renews and another daughter that differentiates, despite each containing identical genomes. Pioneering work from our lab developed a dual-color labeling system to study ACD in *Drosophila* male **germline stem cell (GSCs)** and found that preexisting (old) histones are preferentially retained in the self-renewed GSC while newly synthesized (new) histones are preferentially enriched in the differentiating daughter. Histone modifications play important roles in regulating gene expression and thus stem cells may maintain their identity by selectively inheriting preexisting histones. **Hypothesis:** During ACD, the self-renewing daughter inherits old histones with modifications that maintain active expression at stem cell-promoting genes and repression at differentiation genes. This allows for continuation of the stem cell state by maintaining epigenetic memory. Meanwhile, the differentiating daughter inherits new histones in these regions, erasing the epigenetic stem cell memory and allowing for differentiation to occur.

I will investigate the conservation of asymmetric histone inheritance during ACD in the *Drosophila* female germline and elucidate the epigenetic histone modifications present at genomic loci that undergo gene expression switching during differentiation. Using previously described methods, I have compared the histone inheritance patterns found in actively dividing female GSCs to those found in male GSCs. We know that during ACD in the male germline, old versus new histones are almost completely segregated between the two daughters. In contrast, my new data show that global histone inheritance patterns in actively dividing female GSCs appear symmetric, but correlation mapping can identify significant regions of non-overlapping old and new histone signal. The difference in gene expression programs for male versus female gametogenesis might underlie the global chromatin change seen during male GSC differentiation but local change during female GSC or other cell lineage differentiation, which undergo far fewer expression changes. To explore this possibility, I have created an array of fluorescent probes targeting well-characterized genes associated with promoting the stem-cell state or the differentiation program. To visualize changes in the epigenome that occur during ACD, I will add these fluorescent probes along with antibodies against old versus new histones as well as specific histone modifications in order to perform a proximity ligation assay. This method will reveal how the epigenome signature changes between the renewed stem cell daughter versus the daughter destined to differentiate in ACD.

D1231C Coordinating DNA Replication Initiation to Asymmetrically Segregate Histones H3 in the *Drosophila* Germline. *J. Snedeker*, M. Wooten, R. Ranjan, X. Chen. Department of Biology, Johns Hopkins University, Baltimore, MD 21218, USA.

One of the major mechanisms by which stem cells maintain tissue homeostasis is by the asymmetric division of stem cells such that one daughter cell remains a stem cell and another daughter cell differentiates. The loss of asymmetric stem cell division could contribute to a wide variety of diseases such as cancer, tissue degeneration, and infertility. Despite its critical importance, little is understood about the epigenetic mechanisms underlying asymmetric cell division. Previously, our lab found that preexisting (old) and newly synthesized (new) histones H3, a major carrier of epigenetic information in *Drosophila*, are segregated asymmetrically during the asymmetric division of the *Drosophila* male Germline Stem Cell (GSC), and that the disruption of proper asymmetric histone segregation leads to defects in GSC maintenance and proper spermatogenesis. We recently obtained data suggesting that old and new histones are differentially incorporated during DNA replication, wherein old histones are preferentially incorporated on the leading strand while new histones are incorporated on the lagging strand. However, this explanation alone is not sufficient for creating a global asymmetry old and new histone incorporation on the two sets of sister chromatids. If DNA replication forks fire bidirectionally, it would create a patchwork of old and new histone-enriched chromosomal domains, but not a global asymmetry. We have preliminary data suggesting that the initiation of DNA replication in GSCs is tightly regulated temporally and precisely regulated spatially. We found that GSCs have a unique nuclear architecture and replication initiation pattern: Heterochromatin is localized to a perinucleolar domain where centromeres cluster. DNA replication apparently initiates near this perinucleolar region and

progresses outwards. By contrast, in the differentiating GB daughter cell DNA replication initiates stochastically throughout the nucleus prior to the reformation of the nucleolus. This perinucleolar heterochromatin could potentially serve as a fork block for DNA replication, and thereby coordinate DNA replication fork to progress unidirectionally resulting in the establishment of global asymmetric histone incorporation onto the two sets of sister chromatids, in preparation for their asymmetric segregation during mitosis.

D1232A Histone H3 Threonine Phosphorylation Regulates Asymmetric Histone Inheritance in the Drosophila Male Germline. *Jing Xie*. Johns Hopkins University, BALTIMORE, MD.

A long-standing question concerns how stem cells maintain their identity through multiple divisions. Previously, we reported that pre-existing and newly synthesized histone H3 are asymmetrically distributed during Drosophila male germline stem cell (GSC) asymmetric division. Here,we show that phosphorylation at threonine 3 of H3 (H3T3P) distinguishes pre-existing versus newly synthesized H3. Converting T3 to the unphosphorylatable residue alanine (H3T3A) or to the phosphomimetic aspartate (H3T3D) disrupts asymmetric H3 inheritance. Expression of H3T3A or H3T3D specifically in earlystage
germline also leads to cellular defects, including GSC loss and germline tumors. Finally, compromising the activity of the H3T3 kinase Haspin enhances the H3T3A but suppresses the H3T3D phenotypes. These studies demonstrate that H3T3P distinguishes
sister chromatids enriched with distinct pools of H3 in order to coordinate asymmetric segregation of ''old'' H3 into GSCs and that tight regulation of H3T3 phosphorylation is required for male germline
activity.

D1233B Developmental Toxicity Testing of Cigarette Smoke and E-Cigarette Vapor Using *Drosophila melanogaster* Primary Embryonic Stem Cell Cultures. *T. M. Ubina*, J. Juarez, M. Gallardo, J. Pilotin, N. Bournias-Vardiabasis. California State University, San Bernardino, San Bernardino, CA.

E-cigarettes (EC) are relatively new and many of the effects they have on the body are still unknown. Given this, even less is known about the effects they have on embryonic development. Previously identified as a reliable method for teratogen testing, *Drosophila melanogaster* embryos fully develop and hatch into larvae 24 hours after oviposition which makes them attractive as a fast and reliable method of testing developmental toxicants (Bournias-Vardiabasis, 1983). When primary cultures are taken at the gastrula stage of development, the embryonic stem cells will differentiate into the beginnings of the muscular and neuronal systems *in vitro* within 24 hours and these differentiated cells, called myotubes (MT) and neuronal clusters (NC), can be observed and quantified. In this study, primary cultures of wild type *D. melanogaster* embryos were homogenized and cultured at the gastrula stage and were then exposed to conventional and e-cigarette vapors dissolved into culture media at various concentrations. From conventional cigarettes, mainstream smoke (MS) and sidestream smoke (SS) were both tested. MS smoke is the smoke that the smoker actively inhales and SS smoke is what comes from the burning end of the cigarette and is what we commonly think of as second-hand smoke. The smoke concentrations were measured in Puff Equivalents (PE) which is the amount of smoke from 1 puff of a cigarette that will dissolve into 1 mL of solution. SS smoke, MS smoke were tested at 1.0 PE/mL, 0.1 PE/mL and 0.01PE/mL. EC vapor was tested at 0.1 PE/mL and 0.01 PE/mL. The numbers of neuronal clusters and myotubes were counted 24 hours after exposure and a Student's t-test was performed. If the cell counts of either NCs or MTs were reduced enough to be deemed significant by the t-test, the solution was labeled as a developmental toxicant. MS smoke was toxic at 1.0 and 0.1 PE/ml, SS was toxic at all concentrations, EC vapor was toxic at 0.1PE/mL.

D1234C Polyploidy Associated Hypertrophy: An Alternative to Mitotic Organ Regeneration. *E. Cohen*, D. Fox. Duke University Medical Center, Durham, NC.

In numerous organs, tissue injury is repaired through compensatory proliferation, a regenerative injury response which leads to restoration of tissue morphology and mass. However, numerous tissues have been shown to respond to tissue injury by a distinctly different, non-proliferative process known as hypertrophy. Following injury, hypertrophic tissues increase the size and DNA copy number (ploidy) of remaining cells to restore lost organ mass. While hypertrophy is clearly important to repairing numerous injured organs such as the mammalian liver, this repair process does not always restore tissue morphology and function. Thus, understanding molecular differences between hypertrophy and the more regenerative compensatory proliferation process could improve efforts to regenerate organs. To approach this question, we established that the *Drosophila* pylorus undergoes compensatory proliferation when injured during juvenile (larval) stages, and then switches to a hypertrophic repair program when injury occurs during adulthood. Studying this process, we can now molecularly interrogate the regulation underlying the decision to undergo hypertrophic or proliferative injury responses. We will discuss recent work identifying the time frame in which the switch between proliferation and hypertrophy occurs. This study provides valuable new insights into conserved mechanisms of organ injury repair using a novel model system.

D1235A Chinmo is necessary and sufficient to maintain male fate in somatic cells of the adult Drosophila gonads. *Miriam Akeju*[1], Qing Ma[1], Margaret de Cuevas[1], Justin Fear[2], Brian Oliver[2], Erika Matunis[1]. 1) Johns Hopkins, Baltimore, MD; 2) National Institutes of Health, Bethesda, MD.

Sexual identity is actively maintained in specific differentiated cell types long after sex determination occurs during development. In the adult Drosophila testis, the putative transcription factor Chronologically inappropriate morphogenesis (Chinmo) is required to maintain the male identity of somatic cyst stem cells and their progeny; loss of Chinmo causes these cells to transform into female cells (Ma et al., *Dev Cell,* 2014). Overexpression of the male sex determinant DoublesexM (DsxM) partially rescues this sex transformation, and ectopic expression of the

female sex determinant DsxF enhances it, indicating that Chinmo acts together with the canonical sex determination pathway to maintain the male identity of testis somatic cells. In the adult ovary, ectopic expression of Chinmo is sufficient to induce a male identity in adult ovarian somatic cells; here, however, it acts through a DsxM-independent mechanism (Ma et al., *Development*, 2016). We conclude that Chinmo is necessary and sufficient to promote a male identity in somatic cells of the adult ovary and testis and that the sexual identity of adult somatic cells can be reprogrammed in Drosophila gonads. To gain further insight into how sex identity is maintained, we are using a genome-wide approach to identify the downstream targets of *chinmo* in adult somatic cells. By using a technique called targeted DNA adenine methyltransferase identification (TaDa)(Southall et al., *Dev Cell,* 2013), we will reveal cell type-specific gene expression differences in adult wild-type and chinmo mutant tissues.

D1236B Apoptosis in *Drosophila* hemocytes lead to nitric oxide mediated pro-inflammatory shift in the immune system and developmental function. *M. B. Arefin*[1], L. Kucerova[1], R. Krautz[1], H. Kranenburg[1,2], F. Parvin[1], U. Theopold[1]. 1) Stockholm University, Stockholm, Sweden; 2) University of Münster, Münster, Germany.

Insects have evolved an efficient innate immune system to fight both pathogens and to ensure cellular and organismal homeostasis. *Drosophila* hemocytes perform cellular reactions not only via phagocytosis and encapsulation, but they also release soluble factors (e.g., antimicrobial peptides and cytokines) to induce humoral responses. Furthermore, they engage in coagulation and wounding, and in development. Recent genome wide transcriptome studies identified several immune molecules in humoral and/or cellular pathways involved in nematode infection in *Drosophila.* To shed light on cellular immunity during nematode infection, we eliminated hemocytes by overexpressing the pro-apoptotic genes hid and grim (hml-apo larvae). Surprisingly, we find that although hml-apo larvae contain fewer plasmatocytes and crystal cells (the two hemocyte subtypes present in naïve larvae) they are resistant to nematode infections. Further characterization of hml-apo larvae shows a shift in immune effector pathways including massive lamellocyte differentiation, melanotic nodule formation, induction of Toll as well as repression of imd-signaling. This leads to a pro-inflammatory state. In addition, pupal lethality, and leg defects in adult escapers are found. Most of the phenotypes are alleviated upon administration of antibiotics and changing food source suggesting an involvement of the microbiota. We find that nitric oxide acts as a key regulator in this process. Finally we show that the substrate for nitric oxide synthase L-arginine similarly modulates the response towards an early stage of tumor progression in fly larvae.

D1237C JAK/STAT Signaling in *Drosophila* Muscles Controls the Cellular Immune Response Against Parasitoid infection. *Hairu Yang*, Dan Hultmark. Umeå University, Umeå, SE.

In *Drosophila melanogaster*, several signaling pathways, including the JAK/STAT pathway, are known to mediate blood cell activation and/or to be involved in the defense against parasitoid wasp infection. How these signals control the cellular defense is not understood for any of them. Here we show that parasitoid wasp infection activates JAK/STAT signaling in the *Drosophila* larva, not only in blood cells (hemocytes), but also in somatic muscles. In the muscles, JAK/STAT signaling is activated by the cytokines Upd2 and Upd3, which are secreted from circulating hemocytes. Deletion of the *upd2* or *upd3* genes, but not the related *os* (*upd1*) gene, reduces the cellular immune response. In addition, suppression of the JAK/STAT pathway in muscle cells reduces the encapsulation of wasp eggs and the number of circulating effector cells, called lamellocytes. These results suggest that Upd2 and Upd3 are important cytokines, coordinating the cellular immune response of *Drosophila*. Furthermore, via a JAK/STAT-dependent mechanism, muscles participate in controlling the defense against wasp infection.

D1238A E3 ubiquitin ligase Sherpa mediates Toll innate immune signaling in Drosophila. *T. Kuraishi*[1,2,3], H. Kanoh[1,4], L.-L. Tong[1], S. Kurata[1]. 1) Division of Molecular Genetics, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan; 2) Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency, Tokyo 102-0076, Japan; 3) Graduate School of Medical Sciences, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan; 4) Division of Glycopathology, Institute of Molecular Bio- membrane and Glycobiology, Tohoku Pharmaceutical University, Sendai 981-8558, Japan.

Fruit fly is a sophisticated model animal which possesses an evolutionary conserved innate immune system as represented by the Toll signaling pathway in metazoa. Despite a significant contribution for the identification of mammalian Toll-like receptors, the molecular basis of the intracellular Toll pathway in flies still remains unclear. In this study, we found a suitable cell-line for analyzing the Toll pathway and performed a comparative genome-wide RNAi screening comprised of four distinct screens. Based on the bioinformatic analyses, we identified an E3 ligase gene, Sherpa, as a novel signaling component. Sherpa deficient flies showed susceptibility to bacterial infection as well as the defects in the antimicrobial-peptide production against Gram-positive bacteria and fungi. Cell-based reporter assays indicated that Sherpa functions genetically downstream of Drosophila MyD88 (dMyd88), an adaptor protein for the Toll receptor, and upstream of Tube and Pelle, Drosophila homologues for mammalian IRAK4 and IRAK1/2. Co-immunoprecipitation assays showed that Sherpa interacts with dMyd88 and induces polyubiquitination on dMyd88 and Sherpa itself. Immunofluorescence revealed that Sherpa localizes on the plasma membrane and maintains the proper membrane localization of dMyd88-Tube signaling complex. These results suggested that Sherpa leads adaptor proteins to the plasma membrane around the Toll receptor via polyubiquitinations.

D1239B Seasonal change in *Drosophila melanogaster* innate immunity. *Emily L. Behrman*[1], Virginia M. Howick[2,3], Fabian Staubach[4,5], Alan O. Bergland[5,6], Dmitri A. Petrov[6], Brian P. Lazzaro[2], Paul S. Schmidt[1]. 1) University of Pennsylvania, Philadelphia, PA; 2) Cornell University, Ithaca, NY; 3) Wellcome Trust Sanger Institute, Hixton, UK; 4) Albert-Ludwigs University, Freiburg, Germany; 5) Stanford University, Stanford, CA; 6) University of Virginia, Charlottesville, VA.

The dynamics of adaptive response to abiotic environmental factors is well documented in a variety of systems, but the role of biotic

environment in the adaptive process is less well understood. Ubiquitous microbial communities associated with hosts can be commensal, but some microbiota result in pathogenic infection that challenges the host with consequences ranging from resource reallocation away from other functions to rapid host lethality. The immune system sits at the crucial interface between an organism's external and internal environment and this balance between self and other may be essential in adaptation to environmental heterogeneity. Therefore, immune response may be an important trait to respond to biotic variation along environmental gradients. Here, we investigate innate immunity along spatial and temporal environmental gradients in wild populations of *Drosophila melanogaster*. We find a repeated complete seasonal turnover in the microbial environment associated with wild flies from spring to fall and a corresponding change in the immune response. We simultaneously sampled populations along a latitudinal cline early and late in the season and evaluated the response to infection. Both gram+ (*Enterococcus faecalis*) and gram- (*Providencia rettgeri*) bacteria were used to probe both Toll and IMD immune pathways that are initiated by different pathogen challenges. We find opposing seasonal responses to the gram+ and gram- pathogens, suggesting a seasonal trade-off in defense that is consistent with the frequency dependent selection imposed by the seasonally changes in the bacterial composition in these populations. Whole genome resequencing in these populations identified alleles in immune genes that cycle across seasonal time. We evaluate the function of natural polymorphisms within *Thioester-containing protein 3* (*Tep3*) and among other immune genes and demonstrate non-additive interactions among alleles that contributes to the seasonal differences in immune response in natural populations; these non-additive interactions shape the frequency of the genotypes in the wild across seasonal time. Overall, the data demonstrate that the innate immune response in wild *Drosophila* has a genetic basis that varies with location, season, and pathogen; the trade-offs among innate immunity and other life history traits predict that the biotic environment may be an important underlying selective force in wild populations.

D1240C Zfh2 is an *in vivo* mediator of hypercapnic immune suppression. *J. KWON*. NORTHWESTERN UNIVERSITY, EVANSTON, IL.

Zfh2 is an *in vivo* mediator of hypercapnic immune suppression

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Patients with advanced chronic obstructive pulmonary disease (COPD), the third leading cause of death in the U.S., and other severe lung diseases often develop hypercapnia (an elevated level of CO_2 in blood and tissues), which is associated with increased risk of pulmonary infection and mortality. Previously, we reported the results of an S2 cell-based RNAi screen to identify candidate genes that modulate immune suppression by elevated CO_2 levels. Knock down of select genes reversed the suppression of *Diptericin (Dipt)* by CO_2, restoring *Dipt* expression in elevated CO_2 towards levels observed in air. We also reported preliminary results suggesting that the transcription factor Zfh2 is an *in vivo* mediator of hypercapnic immune suppression. We have now confirmed those results by showing that in *S. aureus* infection assays, flies homozygous for the *zfh2[209]* mutation show decreased mortality and decreased bacterial loads compared to *w[1118]* control flies in 13% CO_2, but not in air. Importantly, Western blotting shows that in *zfh2[209]* mutants the level of Zfh2 protein is reduced in the fat body, the major organ of immune response, but not the head or carcass (the body minus the fat body and head). Further evidence that the fat body is a critical site of action for *zfh2* was obtained by knocking down *zfh2* using the shRNA of *zfh2[13305]* and either of the fat body drivers CG-Gal4 or C754, which increased survival and decreased bacterial loads in elevated CO_2 conditions. Using qPCR and a newly developed *ex vivo* fat body culture system, we determined that reducing *zfh2* levels increased expression of the antimicrobial peptide genes *Diptericin*, *Attacin* and *Metchnikowin* in elevated CO_2 conditions, but not in air. Together these results define Zfh2 as the first identified *in vivo* mediator of hypercapnic immune suppression and the fat body as a critical site of action of hypercapnic immune suppression.

D1241A Microbiota-dependent priming of antiviral intestinal immunity in Drosophila. *Christine L. Sansone*, Jonathan Cohen, Jie Xu, Ari Yasunaga, Greg Osborn, Harry Subramanian, Beth Gold, Sara Cherry. University of Pennsylvania, Philadelphia, PA.

Many pathogens, including viruses, are orally acquired and infect cells within the gastrointestinal tract. To counter this, the gastrointestinal tract has evolved as a physical and immunological barrier. Moreover, the microflora within the intestinal tract plays a fundamental role in immunity; however, much remains unknown regarding the molecular mechanisms linking the microbiota to barrier immunity against enteric viruses. Using *Drosophila* as a model insect, we previously found that the ERK pathway controls innate immunity to oral infection within the intestinal epithelium. However, it remains unclear how ERK is activated to restrict infection. Here, we show that two signals are required to activate antiviral ERK signaling in the intestinal epithelium. One signal is dependent on the recognition of peptidoglycan from the commensals, including *Acetobacter pomorum*, which primes the NF-kB-dependent induction of the secreted factor Pvf2. However, the microbiota is not sufficient to induce; a second virus-dependent Cdk9-dependent signal is required for the production of Pvf2. We have screened a panel of transcription factors that are Cdk9-dependent and identified a transcription factor required for Pvf2 induction. These findings demonstrate that sensing of specific commensals primes inflammatory signaling required for epithelial antiviral responsiveness to restrict diverse enteric viral infections. Future studies are directed toward identifying additional antiviral pathways rapidly induced in the gut following oral challenge by performing RNA-seq.

D1242B Translational regulation by Thor drives the innate immune response. *Deepika Vasudevan*[1], Jessica Sam[1], Min-Ji Kang[2], Hyung Don Ryoo[1]. 1) New York University School of Medicine, New York, NY; 2) University of Ulsan College of Medicine, Ulsan, Korea.

The translational inhibitor Thor (*Drosophila* 4E-BP) was first identified in a screen for immune-compromised phenotypes (Bernal et al, 2000). 4E-BP inhibits the initiation factor eIF4E, which is required for the recognition of the m7G cap of transcripts to load ribosomes for translation. Most cellular transcripts are translated via a cap-dependent mechanism requiring eIF4E. However, subsets of transcripts can be translated cap-

independently via special structures in their 5'UTR. While 4E-BP has drawn interest due to its role in lifespan extension, how it is induced during infection and its functional relevance remain unexplained. In this work, we show that 4E-BP is induced by the integrated stress response pathway and is required for the efficient translation of anti-microbial peptides (AMPs) involved in pathogen clearance.

Following pathogen recognition during infection, a signaling cascade is activated culminating in the transcriptional induction and synthesis of AMPs. AMP induction in response to the pathogen Ecc15 was unaltered in 4E-BP$^{-/-}$ suggesting that AMP translation may be affected. To test if AMPs are translated cap-independently in the presence of 4E-BP, we designed a bicistronic assay with the 5'UTR of AMPs sandwiched between GFP and dsRed. Thus dsRed will only be synthesized if the 5'UTR permits cap-independent translation. The 5'UTR of the AMP drosomycin not only scored positively in this assay, but enhanced the synthesis of dsRed when the cells were challenged with Ecc15. These data suggest that while AMPs may be translated cap-independently, their efficient translation during infection requires the inhibition of cap-dependent translation by 4E-BP.

We find that the transcriptional induction of 4E-BP during infection is mediated by the integrated stress response transcription factor, ATF4. ATF4 mutants are immune-compromised similar to 4E-BP$^{-/-}$ and analysis of the 4E-BP genomic region reveals several ATF4 binding sites in an intronic element (4E-BP^{i}). A dsRed reporter driven by 4E-BP^{i} responds specifically to ATF4 but not the canonical 4E-BP transcription factor, FOXO. Mutating the putative ATF4 binding sites in 4E-BP^{i} resulted in loss of reporter activity. Reporter expression is elevated in the gut of larvae orally infected with Ecc15 in accordance with our other data. ATF4 can be activated by one of two upstream kinases, PERK, which responds to stress in the endoplasmic reticulum and GCN2, which responds to amino acid deprivation. Knockdown of GCN2 but not PERK resulted in reduced ability to clear Ecc15 in larvae. These results indicate a role for GCN2/ATF4/4E-BP in mounting an immune response.

D1243C The identification and characterization of immune responsive enhancers. *Z. Wunderlich*. UC Irvine, Irvine, CA.

To produce antimicrobial peptides and up-regulate other genes important for the immune reaction, Drosophila relies on a transcriptional response. In the course of this response, hundreds of genes are differentially regulated, but the loci of only a handful have been examined to find the cis-regulatory elements responsible for these gene expression changes. To identify immune-responsive enhancers genome wide, we infected flies with measured amounts of Gram-positive or Gram-negative bacteria and performed an ATAC-seq experiment on the dissected fat bodies of the animals 4-6 hours after infection. We compared these results to flies that had been injected with a sterile solution. We are analyzing these results to find likely immune-responsive enhancers and will refine this preliminary list with bioinformatics analysis to find regions with a preponderance of binding sites for key transcription factors, Dif, Relish, serpent and Dorsal. In the future, we plan on validating these presumptive enhancers in cell culture assays and studying how natural sequence variation in these enhancers affects the transcriptional reaction in response to infection.

D1244A Insect-*Metarhizium* interactions. *Hsiao-Ling Lu*, Jonathan Wang, Raymond St. Leger. University of Maryland, College Park, MD.

The fungus *Metarhizium anisopliae* is a natural pathogen of fruit flies. We performed a screen of 2,613 mutant *Drosophila* lines to identify host genes affecting susceptibility to *M. anisopliae* 549. In addition, we used *Drosophila* gene disruption mutants and reporter lines such as drosomycin-GFP to examine variation in *Drosophila*'s responses to GFP or RFP tagged *Metarhizium* strains with different infection strategies (e.g., quick kill via toxins vs. slow kill via invasive growth), and different lifestyles (generalist broad host range strains with different degrees of virulence to *Drosophila* and specialists with narrow host ranges that show poor infectivity to *Drosophil*a). To investigate the impact of pathogen and host variation on fungal fitness and host survival we looked at correlations between key life history traits at different steps of the infection process; lethal pathogen dose (LD50), within-host growth (fungal load), host life span (LT50 values), latent period (the lag time between inoculation and sporulation), and sporulation capacity (the total number of spores per *Drosophila* cadaver). By analyzing fungal behavior post infection we identified environmental as well as genetic determinants influencing *Drosophila* pathogen interactions. *Drosophila* mutants disrupted in immune related genes showed increased susceptibility to specialist *Metarhizium*'s adapted to other insects, but mutations had less effect on generalists. Testing isolated components of the *Drosophila* immune response demonstrated that the antifungal peptide Drosomycin did not inhibit *Metarhzium* strains alone nor in combination with cecropin A.

D1245B Investigating the mechanism of declines in male fitness due to chronic infection in *Drosophila melanogaster*. *K. B. Nichols*, S. Tener, K. Staub, M. C. Chambers. Muhlenberg College, Allentown, PA.

Environmental changes can greatly affect animal hosts, impacting both physiology and behavior. Changes that alter the fitness of the organism are most important because they determine which traits continue to subsequent generations. Work across species suggests that organisms have evolved mechanisms to detect the immunological strength of a prospective mate. Using *Drosophila melanogaster* as a model organism, we are investigating the relationship between declines in male fitness and infection with the natural pathogen *Providencia rettgeri*. If a fly survives acute infection with this bacteria, the fly is chronically infected for the remainder of its life. Little was known, however, about how chronic infection impacts male reproduction. We find that chronically infected male flies produce fewer offspring when mated to groups of virgin females. We examined the mechanism behind this reproductive defect by testing the impact of chronic infection on both mate choice and sperm replenishment.

Mate choice is influenced by the composition of a male's cuticular hydrocarbons (CHCs), which are long-chain fatty acids found on the external surface of most insects that mediate a variety of host processes including desiccation resistance, communication, and mate choice. We assessed the ability of chronic infection to influence mate choice by both determining how infection changes the CHC profile of the male fly and how infection influences selection of the infected male fly in a competitive mating assay. In addition, we determined the sperm replenishment capability of chronically infected flies by successively mating the fly with virgin females and scoring viable offspring. Throughout all of these

assays, male flies were also assessed for either pathogen load or survival to allow correlation of phenotypes with severity of infection. We are currently using genetic mutants in various pathways to determine the pathways responsible for modulating the effects during infection. Ultimately, this research will provide a mechanistic example of how sensory detection of infection status impacts male reproduction.

D1246C Functional analysis of *Drosophila* mucins during development. *Z. A. Syed*[1], D. T. Tran[2], K. G. Ten Hagen[1]. 1) Developmental Glycobiology Section, NIDCR/NIH, Bethesda, MD; 2) Biological Chemistry Section, NIDCR/NIH, Bethesda, MD.

The mucosal barrier in the gut, lungs and other vital tubular organs consist primarily of mucins and protects the underlying cells against pathogens, dehydration and physical or chemical injury. Mucins are large, high molecular weight glycoproteins that contain a central polymorphic domain composed of tandem repeats, rich in amino acids serine, threonine and proline. These highly O-glycosylated proteins form densely arrayed structures that provide mutivalency and high stoichiometric power. In *Drosophila*, twenty-three mucins and mucin-related proteins that are expressed dynamically during different stages of the life cycle were identified. To understand the role of *Drosophila* mucins during development, we used the transgenic RNA interference (RNAi) system to systematically knockdown mucins. Ubiquitous knockdown of mucins expressed in the gut resulted in larval and pupal lethality, indicating that they are essential and suggesting their requirement for protective barrier formation in these tissues. Interestingly, tissue-specific knockdown of one mucin (*Muc26B*), which is expressed in the proventriculus, showed 1st instar larva lethality along with a dramatic up-regulation of genes encoding antimicrobial peptides. Additionally, loss of *Muc26B* resulted in activation of conserved signaling pathways. Immunostaining for MUC26B showed that it is expressed in the specialized secretory cells of the proventriculus called the PR cells, which are responsible for the synthesis and secretion of the peritrophic membrane (PM). Indeed, MUC26B staining could be seen in this protective membrane in wild type flies. We therefore hypothesize that MUC26B serves as a crucial component of the PM and we are currently investigating the cellular processes and signaling cascades activated in response to the loss of MUC26B. We are generating transgenic flies that endogenously express *Muc26B*lacking mucin-domains and associated chitin-binding domains to investigate the role of MUC26B in PM formation and barrier function. These transgenic flies will aid in elucidating changes in PM structure and function upon modulation of different MUC26B domains. In addition, we will investigate the role of MUC26B glycosylation in the packaging, secretion and formation of the PM using correlative light and electron microscopy (CLEM). These studies will ultimately advance our understanding of mucins and their role in the protection of epithelial cell surfaces.

D1247A The C-terminal domain of Abelson tyrosine kinase recruits it to axons. *Han Cheong*, Adeel Siddiqui, David Pierce, Mark VanBerkum. Wayne State University, Detroit, MI.

In the embryo, Abelson tyrosine kinase (Abl) is expressed in the central nervous system (CNS), and is localized to the axons of neuronal cells where it acts downstream of axon guidance receptors such as Frazzled and Roundabout. While the Abl N-terminus is highly conserved between Abl proteins of diverse taxa, the sequence of the *Drosophila* Abl C-terminal domain (CTD) of ~1100 amino acids is largely unique, with the exception of a conserved F-actin binding domain at the tail end. Here, we demonstrate that the CTD is essential for the recruitment of Abl to axon tracts. Using the Gal4-UAS system and immunohistochemistry, we confirm that removal of the CTD prevents the remaining N-terminal region from localizing to axons, and demonstrate that the CTD alone is sufficient for localization to axons. Within the CTD, we further define a ~350 amino acid region (amino acids 1117-1492, or the "third quarter") that is sufficient to drive localization of an RFP fusion protein to axons. However, in the context of full-length Abl, removal of the third quarter does not completely prevent axon localization, although stability of the protein is decreased, resulting in overall lower levels of Abl in the axons. Within the third quarter, the two putative EVH1 binding motifs are not required for axonal localization. Surprisingly, adult lethality assays using the expression of Abl transgenes in *Abl* mutant flies indicate that a second region, not involved in axonal localization (amino acids 639-804), is essential for Abl function, and ubiquitous or neuronal expression of the N-terminus fused to this region partially rescues adult lethality despite the protein's inability to localize to axons. Bioinformatic analyses suggest that large regions of the C-terminus are disordered, and these may undergo a disorder-to-order transition during protein-protein interactions. This further suggests that the C-terminus primarily facilitates binding of Abl to target proteins. Work is underway to both identify binding targets of the CTD as well as to assess the ability of our Abl transgenes to rescue axon guidance defects in *Abl* mutant embryos. As a whole, these results identified a region that is sufficient for axonal localization (the third quarter) although in the context of full-length Abl, this region may further co-operate with other regions for localization. Our results also suggest that the C-terminus has additional essential functions besides axonal localization.

D1248B Structural and Functional Analysis of Dunc-115 Using CRISPR. *C. K. Roblodowski*[1], S. Alghambi[2], R. Gomez[2], S. Giordano[2], D. Veyg[2], C. Batista[2], R. Lakha[2], Q. He[2]. 1) Queensborough Community College, 222-05 56th Avenue, Bayside, NY 11364; 2) Brooklyn College, 2900 Bedford Avenue, Brooklyn, NY 11210.

Significant progress has been made toward a better understanding of the axon guidance mechanisms during animal development. Several guidance pathways have been revealed where signaling initiated at the surface receptor level is relayed to the interior of neurons through numerous adaptor proteins. This leads to a reorganization of the cytoskeleton and other responses. We previously showed that the *Drosophila* protein Dunc-115, a homolog of the Unc-115 from the *C. elegans*, is involved in axon projection in the CNS and the visual system. Furthermore, our data also demonstrated that Dunc-115 binds to actin filaments, implying a possible mechanism for actin reorganization via Dunc-115. Dunc-115 is alternatively spliced resulting in the formation of three isoforms, and it has not previously been possible to dissect the functions of individual isoforms. Here, we report that by using the recently available CRISPR, we have generated mutant animals where the actin binding domain (VHD) of the longest isoform is deleted. The deletion of the VHD domain has severe phenotypic defects. The results suggest that the

actin binding domain is essential and that distinct isoforms function differently. The outcome of the structural and functional analysis will be discussed.

D1249C The conserved microtubule-associated protein Mini Spindles regulates dendrite branching and self-avoidance in *Drosophila* class IV dendritic arborization neurons. *Mala Misra*[1], Noor Anvery[1], Claire Kittock[1], Hendia Edmund[2], Elizabeth Gavis[2]. 1) Colgate University, Hamilton, NY; 2) Princeton University, Princeton, NJ.

Neuronal dysgenesis is a common feature of diverse neuropsychiatric and neurodevelopmental disorders, underscoring the need for a more comprehensive understanding of the molecular mechanisms underlying neuronal morphogenesis. The dendrites of *Drosophila* larval class IV dendritic arborization (c4da) neurons provide an excellent model system to study the regulation of dendrite morphogenesis because their complex branching patterns show clear parallels to those seen in vertebrates, and their two-dimensional organization on the surface of the larva allows for easy visualization. We capitalized on these properties in designing a two-tiered genetic screen to identify genes that (1) encode dendrite-localized mRNAs, and (2) regulate dendrite morphogenesis. The goal of the screen was to connect the local regulation of protein synthesis with dynamic morphogenetic events in the dendrites. One positive candidate arising from this effort was the gene *mini spindles* (*msps*), which encodes a microtubule associated protein homologous to XMAP215-family proteins in vertebrates. Previous studies have demonstrated that Msps regulates microtubule nucleation and promotes dynamic instability. We therefore hypothesized that it might play a role in regulating cytoskeletal events important for the growth and/or retraction of dendrite branches in c4da neurons. RNAi-mediated knockdown of *msps* expression in c4da neurons resulted in a modest but significant loss of branching, supporting this initial hypothesis. Strikingly, $msps^{RNAi}$ dendrites also exhibited more than twice as many self-crossings within the receptive field as control *yw* dendrites. Some previously characterized instances of aberrant c4da self-crossing have been linked to the enclosure of dendrites by overlying epidermal cells and a consequent loss of the characteristic two-dimensional array; immunostaining for Coracle, a marker of enclosure, confirmed that self-crossing dendrites in $msps^{RNAi}$ neurons also exhibit this behavior. The localization of *msps* mRNA to dendrites and the disorganization of dendrite branching in the absence of Msps protein suggest a preliminary model in which local control of Msps expression could regulate cytoskeletal dynamics and dendrite morphogenesis in response to local signaling between the neuron and the external environment.

D1250A *Drosophila* tissue inhibitor of matrix metalloproteinases regulates synaptic development through trans-synaptic signaling. *Jarrod Shilts*, Kendal Broadie. Vanderbilt University, Nashville, TN.

The activity of extracellular matrix metalloproteinases (MMPs) can shape synaptic structure and functional neurotransmission during neural development. Dysregulation of MMPs has been causally implicated in neurodevelopmental disorders such as Fragile X syndrome (FXS). *In vivo*, MMP regulation includes the secretion of endogenous tissue inhibitors of MMPs (TIMPs). However, the general roles of MMP regulation by TIMPs at the synapse remain elusive, due both to the redundancy of the mammalian MMP/TIMP system and the nesting of TIMP genes within synapsin genes. *Drosophila* has only two MMPs regulated by a single TIMP, making it possible to dissect the entire matrix metalloproteome in a tractable genetic model. Using CRISPR/Cas9 genome editing, we have created the first precise disruption of TIMP in order to study its neurological phenotypes. We discovered that loss of TIMP results in significant overelaboration of neuromuscular synapses, resembling the synaptic abnormalities seen in FXS disease models. Using cell-targeted RNAi, we have found bidirectional contributions of TIMP for maintaining synaptic morphology. Analysis of multiple *trans*-synaptic signals has revealed a mechanism where TIMP selectively tunes BMP signaling to control synaptic expansion. We are now extending these results using engineered TIMP alleles that will allow us to visualize its expression and differentiate its functional domains. To link our genetic results with biochemical data, we have optimized and begun testing a protocol for measuring MMP activity at individual synapses. Together, these genetic, biochemical, and neurobiological approaches demonstrate an important role for TIMP in regulating synaptic development, with potential applications to neurodevelopmental disease states.

D1251B Tenectin recruits integrin at synaptic terminals and stabilizes bouton architecture at the *Drosophila* neuromuscular junction. *Qi Wang*, Tae Hee Han, Peter Nguyen, Mihaela Serpe. NICHD/NIH, Bethesda, MD.

Extracellular matrix (ECM) proteins and their receptors have important roles during synapse assembly, development and plasticity, but very little is known about the underlying molecular mechanisms. In a genetic lethality screen for candidates interacting with *neto*, an obligatory auxiliary protein at the *Drosophila* NMJ, we found *tenectin* (*tnc*). Tnc is a mucin-like ECM protein expressed in motor neurons and striated muscles in 3rd instar larvae. *tnc* mutants are partially lethal and the escapers have severe climbing and locomotor defects, suggesting that Tnc is required for NMJ development. Using genetics, histology, super-resolution imaging, electron microscopy and electrophysiology approaches, we found that *tnc* mutants have collapsed synaptic boutons, but normal synaptic contacts and postsynaptic densities. Instead, Tnc is secreted into the synaptic cleft, and binds to pre- and postsynaptic βPS-containing integrin receptors. These Tnc-βPS bridges appear to stabilize the bouton shape partly by recruiting the membrane skeleton locally. Indeed, the distribution of membrane skeleton components such as α-Spectrin, Adducin, Ank2L are disrupted at Tnc-deprived NMJs. Furthermore, Tnc-coated beads recruit βPS and α-Spectrin to specific membrane domains in S2R+ cells.

Tnc is also involved in the regulation of synaptic vesicle release. Electrophysiology recordings show that *tnc* mutants NMJs have reduced mEJPs frequency, EJPs amplitude and quantal content, and exhibit higher paired-pulse ratio (PPR). Expression of Tnc in motor neurons, but not in muscles, can rescue the synaptic accumulation of βPS integrin,α-Spectrin and Adducin and partially rescue the behavior defects of *tnc* mutants. Together, our data demonstrate that pre- and postsynaptic secreted Tnc recruits integrin receptors at NMJs, and anchors the synaptic

membrane skeleton to the ECM to ensure synaptic boutons architecture and function. Our findings extend the knowledge on how ECM proteins organize the synaptic membrane skeleton to regulate synaptic structure and function. .

D1252C Notch-mediated lateral inhibition regulates proneural wave propagation when combined with EGF-mediated reaction diffusion. *M. Sato*[1], Y. Minami[2], T. Miura[3], M. Nagayama[2]. 1) Kanazawa University, Kanazawa, Ishikawa, JP; 2) Hokkaido University, Sapporo, Hokkaido, JP; 3) Kyushu University, Fukuoka-shi, Fukuoka, JP.

Notch-mediated lateral inhibition regulates binary cell-fate choice, resulting in salt-and-pepper patterns during various developmental processes. However, how Notch signaling behaves in combination with other signaling systems remains elusive. The wave of differentiation in the *Drosophila* visual center or 'proneural wave' accompanies Notch activity that is propagated without the formation of a salt-and-pepper pattern, implying that Notch does not form a feedback loop of lateral inhibition during this process. However, mathematical modeling and genetic analysis clearly demonstrated that Notch-mediated lateral inhibition is implemented within the proneural wave. Moreover, the combination of Notch-mediated lateral inhibition and EGF-mediated reaction diffusion enables a novel function of Notch signaling that regulates propagation of the wave of differentiation.

D1253A Wnt signaling specifies progenitor zone identity in the *Drosophila* visual center. *O. I. Trush*[1], T. Suzuki[2], T. Yasugi[3], R. Takayama[3,4], M. Sato[1,2,3,4]. 1) Grad. School of Med. Sciences, Kanazawa University, Kanazawa, Ishikawa, JP; 2) Laboratory of Developmental Neurobiology, Brain/Liver Interface Med.Research Center, JP; 3) Math.Neuroscience Unit,Institute for Frontier Science Initiative, JP; 4) CREST, JST, Saitama, JP.

Neuronal diversity is essential for the formation of complex and correct neuronal circuits. During brain development, various types of neuronal populations are produced from different progenitor pools to produce neuronal diversity that is sufficient to establish functional neuronal circuits. Thus, multiple progenitor pools produce different subsets of neuronal populations to contribute to the neuronal diversity. However, the molecular mechanisms that specify the identity of each progenitor pool remain obscure. Here, we show that Wnt signaling is essential for the specification of the identity of progenitor pools in the Drosophila visual center. In the medulla, the largest component of the visual center, different types of neurons are produced from two progenitor pools: the outer proliferation center (OPC) and glial precursor cells (GPC). However, we found that OPC-neurons are produced in the GPC at the expense of GPC-neurons when Wnt signaling is suppressed in the GPC. In contrast, GPCs are ectopically formed when Wnt signaling is ectopically activated; consequently, GPC-neurons are ectopically produced in the OPC. These results suggest that Wnt signaling is necessary and sufficient for the specification of identity of the progenitor pools. We also found that Homothorax (Hth), which is temporally expressed in the OPC, is ectopically induced in the GPC by suppression of Wnt signaling and that ectopic induction of Hth phenocopies the suppression of Wnt signaling in the GPC. Since β-catenin signaling specifies progenitor pool identity in the developing mammalian thalamus, evolutionarily conserved Wnt signaling may specify the identity of progenitor pools in mammalian and fly brains. .

D1254B The large and small SPEN family proteins stimulate axon outgrowth during neurosecretory cell remodeling in *Drosophila*. *T. Gu*, T. Zhao, R. Hewes. Univ Oklahoma, Norman, OK.

Nervous system remodeling in *Drosophila* occurs during metamorphosis to support the transition from larval to adult-specific behaviors. The process of neuronal remodeling involves the pruning back of old dendritic and axonal connections followed by outgrowth of adult-specific arbors. Similar remodeling processes are seen throughout the animal kingdom in different developmental contexts, but the mechanisms underlying this morphological plasticity in each case remain unclear. Here, we found Split ends (SPEN) and its homolog, Spenito (NITO), are required for metamorphic remodeling of the CCAP/bursicon neurosecretory cells. SPEN and NITO belong to a well-conserved family of nuclear proteins with critical functions in transcriptional regulation and the post-transcriptional processing and nuclear export of transcripts. Cell-targeted overexpression of SPEN in CCAP/bursicon cells had no effect on the larval morphology or the pruning back of the CCAP/bursicon cell axons at the onset of metamorphosis. During the subsequent outgrowth phase of metamorphic remodeling, overexpression of either SPEN or NITO strongly inhibited axon extension, axon branching, peripheral neuropeptide accumulation, and soma growth. Cell-targeted loss-of-function manipulations for both *spen* and *nito* caused reductions in axon outgrowth, indicating that the absolute levels of SPEN and NITO activity are critical to support developmental plasticity in these neurons. Loss of *spen* also caused defects in central neurite pathfinding and development of boutons in peripheral axons. Although *nito* RNAi did not affect SPEN protein levels, the phenotypes produced by SPEN overexpression were suppressed by *nito* RNAi. These results suggest that SPEN and NITO function additively or synergistically in the CCAP/bursicon neurons to regulate multiple aspects of neurite outgrowth during metamorphic remodeling.

D1255C Replacement of the glial architecture in *Drosophila* central brain during metamorphosis. *T. Awasaki*, K. Kato. Kyorin University School of Medicine, Mitaka, Tokyo, JP.

Glial cells are one of the major components of the central nervous system. In the *Drosophila* central brain, different subtypes of glial cells form specific architectures. Neural networks of holometabolous insects such as *Drosophila* remodel during metamorphosis. How do the glial architectures change in response to the neural remodeling? Here we report that the larval glial architecture associating with neuropil in the brain is replaced with newly formed architecture during metamorphosis. Two subtypes of neuropil-associated glia, ensheathing glia and astrocyte-like glia, are found in both larval and adult brains. Neuropils of both larval and adult brains are wrapped by ensheathing glia and filled with astrocyte-like glia. However, we found that the larval and adult neuropil-associated glial architectures are composed of different cell population. Larval neuropil-associated glial cells change their molecular and morphological features in the early pupal stage. On the other hand, immature glial cells appear at the interface between cell-body region and neuropil in the late larval stage. As the number of immature glial cells

increases, they spread over in the interface. When these immature glial cells were genetically labeled in larval stage, labels were specifically found in both subtypes of neuropil-associated glial cells in the adult brains, indicating that these immature glial cells are precursors of adult neuropil-associated glia.

In order to understand how the new neuropil-associated glial architecture is formed over the old one, we analyzed development of the glial precursors and their lineage in detail. Using the cell tracing system combined with the cell cycle marker, FUCCI, and cell death marker, PARP::VENUS, cell proliferation and apoptosis were analyzed in the glial precursor lineage, respectively. We found that these glial precursors generate immature glial cells excessively by cell proliferation and the immature cells are distributed confluently at the interface. Then, excess cells are removed by apoptosis. Finally, survived cells differentiate into two glial subtypes and form adult neuropil-associated glial architecture. These results suggest that the adult glial architecture is established through the overproduction and elimination of immature glial cells. We would like to discuss the potential molecular mechanism regulating the adjustment of the glial precursor lineage.

D1256A Regulation of Neuron-Glia Interactions in the Developing Eye. *Victoria Hans*, Asma Patel, Jennifer Jemc. Loyola University Chicago, Chicago, IL.

Glial cells in vertebrates have numerous functions, including myelination of neuronal axons. While *Drosophila* don't have myelination to increase the efficiency of action potential transmission, a subpopulation of glia wrap around neurons to support neuron function. Previous studies have shown that when *raw* expression is reduced in glia, the result is lethality during pupal stages, leading us to ask what the role of *raw* is in glia during development. The developing eye of *Drosophila* provides an excellent system for studying the role of *raw* in glia due to its well-characterized glial subtypes and well-described development. Perineurial glia start in the brain and optic stalk, then migrate into the developing eye imaginal disc during the third larval instar, where they make contact with photoreceptor cells. Following contact with neurons, perineurial glia differentiate into wrapping glia and ensheath photoreceptor axons. Axons then extend along glia to the optic lobe of the brain. Our studies using immunohistochemistry have revealed a reduction in the number of glia in the third instar eye imaginal disc when *raw* levels are reduced in glial cells using the Gal4/UAS system to produce *raw* RNAi. While we observe a reduction in the number of glia, it is unclear if the reduction of glia is due to cell death, reduced proliferation, or defects in specification. Preliminary studies in the embryo suggest that glia are properly specified early in development in *raw* mutants, making it unlikely that glia specification is the origin of the *raw* mutant phenotype. To determine if the reduction arises from an increase in cell death we have performed immunostaining for activated Caspase, as well as acridine orange and do not observe an increase in cell death. Currently, we are using an antibody for phospho-histone H3 to determine if glial proliferation is reduced in *raw* RNAi samples relative to controls. Finally, it is possible that the reduction in glia in the eye disc arises from defects in glial migration. In order to examine this possibility, a live imaging approach is being developed to examine glial migration in cells with reduced *raw* levels. Images will be taken every 10-15 minutes over the course of a few hours to observe the glial migration process, allowing us to track the path of specific glial cells and to examine the rate at which glia are migrating. Finally, glia are important for proper axon targeting to the optic lobe of the brain. In larvae with reduced *raw* levels in glia, we observe axon mis-targeting in the brain, suggesting that Raw functions indirectly to regulate axon targeting as well. Thus, we have identified a new regulatory protein that functions in cell autonomously in glia cell non-autonomously in neurons during development.

D1257B Identification of a Novel Regulator of Glial Development . *D. Luong*, M. Davis, J. Jemc. Loyola University Chicago, Chicago, IL.

Glial cells perform numerous functions to support neuron development and function, from regulation of axon pathfinding and synapse formation to ensheathment of neurons and maintenance of ionic homeostasis. In vertebrates defective glial cells have been linked to neurological diseases such as neuropathies and schizophrenia. Our lab is interested in genes that regulate glia-neuron interactions during development. Specifically, we have found the *raw* gene to be required in glia, as reducing *raw* levels in glial cells by RNAi results in lethality. Due to the lethality observed when *raw* is removed from glia we examined how glia were being affected in the developing central and peripheral nervous system. We have observed a decrease in the size of the brain and an elongated ventral nerve cord within the central nervous system. In the peripheral nervous system, reduced levels of *raw* affect the number of glia along the nerve extension region (NER) of the peripheral nerves. Further examination of peripheral nerve structure by transmission electron microscopy reveals significant changes in nerve morphology when *raw* levels are reduced. These phenotypes suggest a defect in glial specification, proliferation or survival. While glia appear to be specified normally in *raw* mutants, we are currently examining the levels of glial proliferation and death when *raw* levels are reduced. Finally, we are interested in how impaired glial function affects neuronal output. Results of a larval crawling assay reveal that larvae exhibit reduced locomotion when Raw levels are reduced in glia as compared to controls. These studies have resulted in the identification of a novel regulator of glial development, and are likely to yield novel molecular mechanisms underlying the establishment of neuron-glia interactions during development.

D1258C Glial cell remodeling during peripheral nerve reorganization in *Drosophila*. *Aswati Subramanian*[1], Matthew Siefert[2], Soumya Banerjee[3], Kumar Vishal[4], Kayla Bergmann[1], Clay Curts[1], Meredith Dorr[5], Meredith Rataiczack[6]. 1) Miami University, Oxford, OH; 2) Children's hospital, Cincinnati, OH; 3) EPFL, Lausanne, Switzerland; 4) Kansas State University, Manhattan, Kansas; 5) University of Toledo, Toledo, OH; 6) Midwestern University, Glendale, AZ.

During development, remodeling of the larval nervous system is necessary for establishing adult specific behaviors in the fruit-fly *Drosophila melanogaster*. During metamorphosis, five pairs of abdominal nerves fuse together to form a terminal nerve trunk (TNT) as they exit the CNS. Segmental nerves defasciculate from the TNT at appropriate segmental levels to innervate the body wall. Glial cells are likely to play a significant role in nerve fusion and we are interested in examining layer specific remodeling of the nerve during metamorphosis. The most

external layer, the non cellular layer of extracellular matrix called the neural lamella, disappears during the first quarter of pupal development and appears again during the last quarter of pupal development. However, the two glial layers- the perineural glia (PG) and the sub-perineural glia (SPG) persist during this process. The innermost layer-the wrapping glia retracts its processes during early pupal development and after the third day of metamorphosis, extends its processes. Glial cell numbers across all cellular glial layers change during pupal development. Confocal microscopy analyses have been substantiated by transmission electron microscopy (TEM) to study the ultrastructural changes that take place during peripheral nerve reorganization. The present study focuses on the role of the most external layer, the PG in TNT formation. A three-fold increase in the glial population was observed during the first day of metamorphosis (25% of development), when 75% of the cells comprises of perineural glia. Induction of perineural glial cell death by targeting the cell death gene *reaper* and the Diphtheria Toxin (DT), results in eclosion defects (28% emergence and 3% emergence respectively). TNT branching pattern was defective in 100% of the experimental animals analyzed at the pharate adult stage (n=15). TEM studies are currently underway to identify the nature of ultrastructural changes in these mutants. The study of glial remodeling in *Drosophila* will lay the groundwork for future studies on the role of glia- neuron communication during TNT formation, and could lead to the establishment of a model system to study gliopathies.

D1259A Border formation between medulla and lobula-complex in the optic lobe. *C. Liu*[1], T. Suzuki[2], M. Kaido[3], R. Takayama[4], M. Sato[5]. 1) Development of neurobiology; 2) Graduate School of medical,; 3) Kanazawa university; 4) Kanazawa; 5) JP.

The fly optic lobe is composed of four ganglia; lamina, medulla, lobula, and lobula plate. Neurons in these ganglia derive from two distinct progenitor pools, the outer proliferation center (OPC) and inner proliferation center (IPC). OPC produces lamina and medulla neurons, whereas IPC generates lobula and lobula plate neurons. These neurons in each ganglion are located at specific regions to form compartments, and are never intermingled with each other at the interfaces between ganglia. However, mechanisms that inhibit cell-mixing at the borders between each ganglion have been unclear. Previously we and other group showed that cell-cell interaction through Slit-Robo signaling, an axon guidance signaling, is involved in the inhibition of cell mixing between lamina and IPC (Tayler et al., 2004) and that between medulla and IPC during larval development (Suzuki et al., in press). Since cell-mixing occurs only partially in sli or its receptors mutant larvae, additional signalings may also participate in the border formation.

Here, we show that Netrin signaling, another axon guidance signaling, regulates the border formation between medulla and IPC. The NetA and NetB ligands are expressed in IPC while their receptors Fra and Unc5 are expressed in lamina glial cells. In NetAB or fra mutants, IPC cells intruded into the medulla cortex. unc5 RNAi also caused similar phenotype. Since Fra and Unc5 are expressed in the lamina glial cells, we examined glia specific loss-of-function of Fra and Unc5, which also caused cell mixing, suggesting that glial cells play essential roles in the border formation between medulla and lobula through Netrin signaling.

D1260B Dynamic requirement for Polycomb group genes in neuroepithelial stem cell proliferation and differentiation in the *Drosophila* optic lobe. *H. Luo*[1], X. Chen[2]. 1) Hunan University, Changsha, Hunan, China; 2) Tsinghua University, Beijing, China.

The Polycomb group (PcG) genes encode conserved proteins that are required for establishing a repressive state of Hox gene expression. Emerging evidence implicates these proteins in stem cell maintenance and differentiation. Here, we examine the roles of PcG genes in *Drosophila* optic lobe development. Using loss-of-function mutant assays, we show that PcG genes have a dual requirement for neuroepithelial stem cell proliferation and differentiation. The Hox gene *Abdominal-B* is derepressed in PcG mutant cells, and ectopic *Abd-B* expression inhibits neuroepithelial cell proliferation. Also associated with the functional loss of PcG genes, JAK/STAT is ectopically activated, but Notch is inactivated; these conflicting signaling activities may account for the defects in neuroepithelial cell proliferation and differentiation. Our results demonstrate a temporal requirement for PcG genes in the proliferation and differentiation of neuroepithelial stem cells in *Drosophila* brain development.

D1261C Investigating the role of *Sox Neuro* in the development of adult nervous system. *Shweta Singh*, Ken Dawson-Scully, John R. Nambu. Florida Atlantic University, Biological Sciences, 5353 Parkside Drive, Jupiter FL-33458.

Many aspects of development are controlled through the actions of specific transcription factors that regulate gene expression patterns. The mammalian *Sry* gene, a sex determining Y chromosome was discovered in 1990 and encodes a transcription factor with single high mobility group DNA binding domain. More than 20 transcription factors in humans and 8 in *Drosophila* share a related HMG domain with at least 50% identity to that of *Sry*. These Sox (Sry box) proteins bind to the minor groove of DNA and induce 70° to 90° bends to regulate chromatin structure and transcription initiation. *Drosophila* possesses 4 highly related Group B Sox genes *SoxNeuro, Dichaete*, *Sox21a* and *Sox21b*. Previous study has shown that Dichaete has a strong role in development, affecting processes that include differentiation of specific neuronal and glial cells, segmentation, hindgut development, differentiation of imaginal discs. Dichaete is expressed in several clusters of neurons in the brain, including intermingled olfactory LNs and central complex neurons and important for the elaboration of the adult olfactory system. SoxN function is important for the formation of neural progenitor cells in *Drosophila* and evidence suggests that loss of SoxN function results in defects in the neuroblast formation. Interestingly, both *SoxN* and *Dichaete* have region-specific functions in CNS development as they both regulate dorsal/ventral partitioning of the embryonic neuroectoderm into specific columns. Evidence also suggests that SoxN and Dichaete function in a redundant manner in neuroblast formation and other developmental processes including neuroectoderm formation, central nervous system development, and sensory trichome formation. However, little is known about the expression and function of SoxN in the adult brain. This study focuses on identifying the expression patterns and function of SoxN in the development of the adult nervous system. Our data shows SoxN is expressed both in neurons and glia of the adult central brain. Interestingly, SoxN expressing neurons are both cholinergic and GABAergic neurons. Our data also suggests role of SoxN in the development of olfactory circuit formation. The result of this study will lead to a

better understanding of Sox gene functions in both conserved and specific aspects of development. Sox proteins have essential developmental functions in many species, including key roles in sex determination, segmentation, neural patterning, differentiation of neurons and glia, and formation of eyes, bone, cartilage, heart, and craniofacial structures. Furthermore, Sox gene mutations are associated with a wide array of human congenital disorders and cancer. Thus, these studies on *Drosophila* Sox genes may illuminate conserved developmental functions in mammals and are relevant for human health.

D1262A New insights into Gene Regulatory Network evolution: Neofunctionalization of the *Drosophila* midline CNS gene regulatory network in the Zika vector mosquito *Aedes aegypti* . *K. Suryamohan*[1,2], C. Hanson[3], E. Andrews[4], S. Sinha[3], M. Duman Scheel[4,5], M. Halfon[1,2,6]. 1) University at Buffalo-State University of New York, Buffalo, NY; 2) NY State Center of Excellence in Bioinformatics and Life Sciences, Buffalo, NY; 3) University of Illinois Urbana-Champaign, Champaign, IL; 4) Indiana University School of Medicine, South Bend, IN; 5) University of Notre Dame, South Bend, IN; 6) Roswell Park Cancer Institute, Buffalo, NY.

Gene regulatory networks (GRNs) ensure precise spatio-temporal control of gene expression during development. Changes in GRNs underlie the evolution of morphological novelty. We have investigated the mechanisms of GRN evolution using the dengue fever and Zika vector mosquito *A. aegypti* and the fruitfly *Drosophila melanogaster* CNS developmental paradigms. Despite substantially similar nervous system morphology, the two species show significant divergence in a set of genes coexpressed in the *Drosophila* ventral midline, including the master midline regulator *single minded* (*sim*) and downstream genes including *short gastrulation* (*sog*), *Star*, and *NetrinA* (*NetA*). In contrast to *Drosophila*, we find that in *A. aegypti* midline expression of these genes is absent or severely diminished, and instead they are co-expressed laterally in the nervous system. This suggests that in *A. aegypti* this "midline GRN" has been redeployed to a new location while being simultaneously lost from its previous site of activity, with a new GRN acquiring the original role. In order to characterize the GRNs, we employed SCRMshaw—a computational method we previously developed for supervised motif-blind and sequence alignment-free CRM prediction—to identify 6 novel CRMs from *A. aegypti* and 6 CRMs for *D. melanogaster* genes and validated them *in vivo* in transgenic *D. melanogaster*. Analysis of these validations and results from genetic perturbation experiments suggest that these changes are due to a trans-dependent redeployment of the GRN, potentially stemming from *cis*-mediated changes in the expression of *sim* in combination with one or more transcription factors in *A. aegypti*. We are continuing to identify additional relevant CRMs as well as undertaking an in-depth analysis of the expression patterns of multiple genes in this network in both species. Our results illustrate a novel "repeal, replace, and redeploy" mode of GRN evolution in which a conserved GRN undergoes neofunctionalization at a new site while its original function is co-opted by a different GRN without causing substantial changes in overall morphology and function.

D1263B Identification of developmental determinants of olfactory sensory neuron identity and connectivity. *Phing Chian Chai*, Steeve Cruchet, Richard Benton. Center for Integrative Genomics, University of Lausanne, Switzerland.

Two important features that characterize nervous system development are the generation of distinct neuronal subtypes and the establishment of precise connections between them. Previous studies in *Drosophila* and vertebrates have shown that the acquisition of specific neuronal identity requires concerted actions of multiple transcription factors during animal development. However, elucidating the molecular mechanisms that are governed by these transcription factors has been difficult, partly due to the lack of definitive molecular markers that allow unambiguous identification of neuronal fates. The olfactory system of *Drosophila* offers an excellent model to address this problem, as it is comprised of ~60 olfactory sensory neuron (OSN) subtypes; almost all of these are defined by the expression of a single, distinct ligand-specific olfactory receptor gene. These diverse OSNs arise from a seemingly homogenous population of progenitor cells located at distinct regions in the antennal imaginal disc. These properties allow us to assay for the spatial and temporal contributions of the developmental determinants that are involved in specifying and diversifying OSN fate. Using a transgenic RNAi approach, we have tested the role of 808 genes in antennal OSN specification, encoding putative transcription factors, chromatin regulators and embryonic patterning determinants. To circumvent the detrimental effects of ubiquitous gene knock-down, we induced RNAi specifically in the eye-antennal discs from the second instar larval stage onwards using a combination of an *actin-flip out-GAL4* driver and *eyeless-Flippase*. Correct OSN specification entails both the expression of a particular olfactory receptor gene and targeting of its axon to a specific glomerulus in the antennal lobe. We therefore screened for phenotypic defects upon gene knock-down by examining the expression of olfactory receptor reporters and axon projections in two different populations of OSNs, as well as the global structural integrity of antennal lobe glomeruli. Our primary screen has revealed that ~15% of the tested genes might play a role in OSN specification and/or development. In an attempt to elucidate the processes that are regulated by these genes during olfactory system development, these genes were further classified into different categories based on their RNAi phenotype (eye-antennal defects, antennal lobe malformation, ectopic structures, reduced axon density, axonal mistargeting and other defects). The majority of genes (>82%) exhibit multiple phenotypic defects upon RNAi, suggesting that these factors could regulate different functions, depending on their spatial and temporal contexts. We will present the results of this screen and our follow-up work on individual determinants of OSN identity and wiring.

D1264C The functional impact of Synaptojanin phosphorylation by the Minibrain kinase during synaptic vesicle recycling in *Drosophila*. *Karen Chang*, Liping Wang, Junhua Geng, Jooyeun Lee. University of Southern California, Los Angeles, CA.

Impaired synaptic transmission is a pathological alteration commonly found in various neurological disorders. The rapid recycling of synaptic vesicles during stimulation is essential for the sustained function of the nervous system, and clathrin-mediated endocytosis is one prominent molecular pathway in synaptic vesicle recycling. Synaptojanin (Synj), a phosphoinositide phosphatase, plays an important role in endocytosis and is responsible for the uncoating of clathrin during the process. Previously, we identified that minibrain (Mnb), homolog of the mammalian

dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), is a serine/threonine kinase required for normal synaptic growth and rapid synaptic vesicle endocytosis at the *Drosophila* neuromuscular junction. We also found that Synj is a substrate of Mnb. However, site on Synj phosphorylated by Mnb has not been identified and functional consequences underlying Mnb-dependent phosphorylation of Synj are also not well understood. Here, we show that Mnb phosphorylates Synj at Serine1029 *in vivo*. Phosphorylation of Synj at S1029 is required to maintain normal synaptic vesicle recycling *in vivo,* but is not required to sustain neurotransmission during high frequency stimulation. It is interesting to note that both Mnb and Synj are upregulated in Down syndrome (DS) and Synj is mutated in Parkinson's disease. An understanding of mechanisms that modulate Synj function will thus provide valuable insights into basic mechanisms affecting neuronal communication and lead to strategies to treat these neurological disorders.

D1265A *Drosophila* SLC22A transporter is a memory suppressor gene that influences cholinergic neurotransmission to the mushroom bodies. *Yunchao GAI*, Ze Liu, Isaac Sandoval, Ronald Davis. The Scripps Research Institue, Jupiter, FL.

The mechanisms that constrain memory formation are of special interest because they provide insights into the brain's memory management systems and potential avenues for correcting cognitive disorders. RNAi knockdown in the *Drosophila* mushroom body neurons (MBn) of a newly discovered memory suppressor gene, Solute Carrier DmSLC22A, a member of the organic cation transporter family, enhances olfactory memory expression, while overexpression inhibits it. The protein localizes to the dendrites of the MBn, surrounding the presynaptic terminals of cholinergic afferent fibers from projection neurons (Pn). Cell-based expression assays show that this plasma membrane protein transports cholinergic compounds with the highest affinity among several *in vitro* substrates. Feeding flies choline or inhibiting acetylcholinesterase in Pn enhances memory; an effect blocked by overexpression of the transporter in the MBn. The data show that DmSLC22A is a memory suppressor protein that limits memory formation by helping to terminate cholinergic neurotransmission at the Pn/MBn synapse.

D1266B Mutations in the Vesicular Acetylcholine Transporter Cause Impaired Locomotion in *Drosophila*. *Andrew Blake*, Mika Heredia, Hakeem Lawal. Delaware State University, Dover, DE.

Acetylcholinergic dysfunction is a key underlying feature in the cognitive decline associated with aging. The machinery required for presynaptic acetylcholine (ACh) release and post-synaptic signaling have been described in detail, yet the precise relationship between alterations in ACh neurotransmission and downstream changes in behavior in the nervous system remain poorly understood. The vesicular acetylcholine transporter (VAChT) is a critical element of cholinergic signaling, mediating the transport of ACh from the cytoplasm into synaptic vesicles for exocytotic release. A complete loss of *Vacht* is lethal in flies, worms and mammals. Here we hypothesize that subtle changes in *Vacht* will uncover important roles of ACh release in behavior. Using point mutations, recovered in an ethyl methanesulfonate screen, to disrupt VAChT function, we report that a series of *Vacht* mutations impair two locomotion circuits, baseline and touch response locomotion. In particular, *Vacht* mutants display a deficit in the timing of the response to touch stimulus in a manner corresponding to severity of the mutant alleles studied. We categorized the resulting allelic series as mild, moderate and severe with respect to acetylcholine release. We further report the genetic rescue of *Vacht* mutant deficits using wildtype VAChT. In addition, we conducted a pharmacological rescue study using agonists of the dopaminergic and cholinergic pathways, and found that both drugs partly rescued the locomotion deficits in the mutants. Together, this report supports a key role for acetylcholine signaling in regulating the timing of the response to a mechanical stimulus and underscores the utility of point mutations that compromise VAChT activity in vivo as tools to elucidate the complex relationship between altered ACh release and behavioral deficits.

D1267C Activation of octopaminergic neurons innervating the oviduct. *Sonali Deshpande*, Daniel Suto, David Krantz. University of California, Los Angeles, Los Angeles, CA.

Oviposition (egg-laying) in *Drosophila* requires passage of the egg from the ovary to the uterus via the oviduct, and activation of octopamine receptors in the oviduct. Additional glutamatergic and peptidergic inputs work in concert with octopaminergic signals to promote movement of the egg through the oviduct, but the mechanisms by which these pathways interact remain unclear. Studies in larger insects suggest that glutamate and proctolin cause contraction of the oviduct, whereas octopamine may promote relaxation. The opposing effects of glutamate and octopamine on contraction suggest that the descending, presynaptic neurons mediating each effect might be activated in a reciprocal pattern. To test this possibility, and more precisely determine the mechanism by which glutmatergic and octopaminergic components of this circuit interact, we have developed a preparation for live imaging of both neuronal firing in the thoracic ganglia, and muscle contractions in the oviduct. Using a genetically encoded calcium indicator, we observe activation of a single octopaminergic neuron the thoracic ganglion lasting up to 100seconds and an unexpected localization of calcium signals to muscle to defined subdomains in the oviduct musculature. Further experiments using application of exogenous octopamine and calcium imaging in glutamatergic neuron will be used to establish the precise relationship between the activity of glutamatergic neurons, octopaminergic neurons and regulation of contractions in the oviduct.

D1268A Identification of novel genes that regulate dopamine dynamics through forward genetic screens in *Drosophila*. *S. Yamamoto*[1,2], S. Deal[1], J. L. Salazar[1], M. Lagarde[1], S. Saurabh[1], E. S. Seto[2]. 1) Baylor College of Medicine, Houston, TX; 2) Texas Children's Hospital, Houston, TX.

Dysregulation of dopamine (DA) dynamics (synthesis, secretion, and metabolism) and signaling underlies a number of symptoms seen in various neurological and psychiatric diseases. Core components of DA dynamics and signaling are being extensively characterized in diverse model systems. In contrast, very little is known about the genes and cellular pathways that fine-tune the activity of this neuromodulator in vivo.

To identify novel factors that regulate DA dynamics, we have been performing a forward genetic screen in *Drosophila melanogaster*. We first screened for novel genes that affect cuticle pigmentation (a DA dependent phenotype) in adult flies using chemical mutagenesis/clonal analysis

and tissue specific RNAi. Through this primary screen, we identified 123 genes, ~85% of which are evolutionarily conserved between flies and human. We then performed a Drosophila Activity Monitor (DAM) based secondary screen to determine whether these genes exhibit a defect in basal activity upon dopaminergic neuronal knockdown. Genes that exhibit strong hypo- or hyperactivity are being prioritized for a tertiary analysis to determine whether dopaminergic neuron specific knockdown of these genes directly affect brain DA levels.

We are currently focusing on genes whose human homologs have been linked to Autism Spectrum Disorders (ASD). Interestingly, 20 of the 123 genes we isolated in our primary screen have human homologs that have been strongly linked to ASD including *gigas* (*TSC2*), *Prosap* (*SHANK1-3*), *slp1* (*FOXG1*), and *Usp7* (*USP7*). By studying the molecular function of these genes in the context of dopamine dynamics and signaling, we hope to understand how defects in these genes may contribute to some of the symptoms found in ASD patients.

D1269B Receptor-based Mapping Reveals the Architecture of a Neural Circuit that Governs a Behavioral Sequence in Drosophila. *F. Diao*, Amicia Elliott, Fengqiu Diao, Sarav Shah, Benjamin White. national institute of mental health, Bethesda, MD.

Studies in both vertebrates and invertebrates have shown that neuromodulators can reconfigure the output of multifunctional neural networks to generate multiple motor patterns. However, very few such networks have been characterized in detail and knowledge of how modulators act at different levels within a network remains poorly understood. Progress has been impeded both by the lack of complete maps of most neural networks and by incomplete knowledge of how network-wide activity translates into behavior in intact animals. Here we address these issues, using the pupal ecdysis sequence of *Drosophila* as a model, taking advantage of its essential dependence on neuromodulatory inputs and its well-defined behavioral components. Pupal ecdysis consists of three, sequentially executed motor rhythms and is governed by multiple neuroactive factors, among which Ecdysis Triggering Hormone (ETH), CCAP, and Bursicon have well-characterized roles. By manipulating neurons that express the receptors of these factors, we have succeeded in mapping the hierarchical organization of the ecdysis network. We find that three distinct subsets of ETH receptor-expressing neurons comprise individual input modules, each one essential for a different behavioral phase. Bursicon receptor-expressing interneurons form a multi-functional central pattern generator (CPG) targeted by the ETHR-expressing modules. Downstream of this CPG are CCAP receptor-expressing motor neurons, which are responsible for generating the ecdysis motor rhythms. The expression patterns of the receptors of key modulators governing the ecdysis sequence thus define the hierarchical architecture of the ecdysis network. Our results provide an example of how receptor mapping can be used to identify functional nodes in a neural circuit and characterize their organization. In addition, our work serves as an entry point to investigating the mechanisms underlying multi-level control of behavioral state by neuromodulators in a neuronal circuit.

D1270C Decision-making neurons direct downstream signaling based on input specificity in male *Drosophila melanogaster*. *J. Schweizer*, L. Eaton, L. Boisvert, A. Auge, W. J. Kim. University of Ottawa, Ottawa, Ontario, CA.

Elucidating neural networks underlying behaviors is an essential task of neuroscience. Fruit fly, Drosophila melanogaster, offer significant advantages for this purpose since they exhibit easily quantifiable, robust and genetically traceable behaviors. We previously reported two consistent, context dependent mating behaviors, called longer- and shorter-mating duration (LMD and SMD). LMD is a rival-induced, prolonged mating duration and SMD a sexual experience-mediated shortened mating. We identified specific components of the underlying neural circuits and found that neuropeptide signaling plays a key role in regulating both behaviors.

We identified four SIFamide (SIFa) producing neurons located in the pars intercerebralis, which are critical to induce both behaviors. To identify how SIFamide signaling bridges between two distinct neuronal pathways, we hypothesized that SIFa neurons act as input-specific decision makers in the context of output, which is mating duration. We first screened neurotransmitter (NT) systems to test this theory since NTs are expressed in large but discrete populations of neurons, which allows for large-scale circuit mapping studies of SIFa signaling in the context of mating duration. We also tested sexually dimorphic neuronal populations in the context of SIFa signaling because LMD and SMD are male-specific behaviors.

To test the impact of SIFa signaling on mating duration, NT-GAL4 lines were crossed with UAS-SIFaR-RNAi to knock-down (KD) SIFaR protein expression in GAL4-labeled neurons. To minimize the developmental impact of SIFaR knockdown, crosses are kept at 18°C until adults eclose, then moved to 25°C. We identified that KD of SIFaR in GAD+ (GABAergic) and Tdc2+ (octopaminergic) neurons affects SMD while a SIFaR KD in Cha+ (cholinergic) and DDC+ (dopaminergic/serotonergic) neurons induced a loss of affects both LMD and SMD. These data suggest that SIFaR signaling in GABAergic/octopaminergic neurons is specifically involved with the SMD pathway while signaling in cholinergic and dopaminergic/serotonergic neurons affects both pathways of LMD/SMD. Inactivation of SIFaR+/fru+ neurons (intersectional method) disrupts only LMD not SMD. This data suggests that fru+ neurons are specifically involved with LMD.

All these data suggest that different groups of neurons are targeted by SIFa signaling depending on the social context (input), leading to distinct behavioral outputs. We suggest that SIFaR signaling pathways could be a good model to improve our understating of how neural computations decide different behavioral outputs depending on different input signals. The study of how this decision-making is achieved can provide new models for neuronal computation and a new insight on neural network architecture.

D1271A Postsynaptic function of DEG/ENaC PPK29 at the *Drosophila* Larval NMJ. *A. Hill*[1], X. Zheng[1], X. Li[2], R. McKinney[1], D. Dickman[2], Y. Ben-Shahar[1]. 1) Washington University, St. Louis, MO; 2) University of Southern California, Los Angeles, CA.

Degenerin/Epithelial Sodium Channels (DEG/ENaCs) represent a diverse family of animal-specific ion channels that play an important role in regulating ionic gradients across epithelial barriers. Members of the family are also enriched in neural tissues. However, in contrast to epithelial tissues, their neurophysiological functions are still mostly unknown. The *Drosophila* genome encodes an exceptionally large number of DEG/ENaC subunits termed *pickpocket 1-31* (*ppk*) family. By using genetic, neurophysiological, and behavioral approaches, we demonstrate that the DEG/ENaC channel subunit PPK29 contributes specifically to postsynaptic modulation of excitatory synaptic transmission at the larval neuromuscular junction (NMJ). We show that *ppk29* is necessary in the muscle for normal response to spontaneous, but not stimulus-evoked, neurotransmitter release, without an apparent impact on gross synaptic development or morphology. In addition, mutations in *ppk29* lead to impaired coordinated movement required for stereotypic larval rolling behavior. Together, our data indicate a novel postsynaptic function for members of the DEG/ENaC family of ion channels.

D1272B Neuronal insulin signaling negatively regulates synaptic vesicle exocytosis via a FOXO-dependent mechanism. *Rebekah Mahoney*[1,2], Jorge Azpurua[1], Benjamin Eaton[1,2]. 1) UTHSCSA, San Antonio, TX; 2) Barshop Institute for Aging and Longevity, San Antonio, TX.

Altered insulin signaling within the brain has been linked to cognitive dysfunction and neurodegenerative disease. Appropriate signaling downstream of the insulin/IGF-1 receptor has been linked to a number of cell processes that could contribute to the effects of insulin signaling on brain function including maintenance of neuronal health, reduced cell stress, neuron development, and synapse function. However, a role for insulin signaling during the regulation of neurotransmission has not been demonstrated. Using a novel synaptic preparation in adult Drosophila, we have found that cell autonomous insulin signaling negatively regulates the presynaptic release of neurotransmitter via the activity of the eif-4e binding protein (4eBP), a negative regulator of protein translation. In this context, the activity of 4eBP is regulated transcriptionally by the forkhead transcription factor Foxo and not the mammalian target of rapamycin (mTOR). Furthermore, the regulation of neurotransmission by insulin signaling requires the mRNA binding protein Staufen, which is known to localize mRNAs to distinct compartments within neurons, and is blocked by the protein synthesis inhibitor cycloheximide. Our data supports the model that cell autonomous insulin signaling regulates the presynaptic release of neurotransmitter via the local translation of negative regulators of synaptic vesicle exocytosis. Analysis of candidate molecules required for the effect of insulin signaling on synaptic vesicle exocytosis will be presented.

D1273C Sleep facilitates memory by blocking dopamine neuron mediated forgetting. *Jacob Berry*, Isaac Cervantes-Sandoval, Molee Chakraborty, Ronald Davis. Scripps Research Institute, Jupiter, FL.

Early studies from psychology suggest that sleep facilitates memory retention by stopping ongoing retroactive interference caused by mental activity or external sensory stimuli. Neuroscience research with animal models, on the other hand, suggests that sleep facilitates retention by enhancing memory consolidation. Recently in *Drosophila*, the ongoing activity of specific dopamine neurons was shown to regulate the forgetting of olfactory memories. Here we show this ongoing dopaminergic activity is modulated with behavioral state, increasing robustly with locomotor activity and decreasing with rest. Increasing sleep-drive, with either the sleep-promoting agent Gaboxadol or by genetic stimulation of the neural circuit for sleep, decreases ongoing dopaminergic activity, while enhancing memory retention. Conversely, increasing arousal stimulates ongoing dopaminergic activity and accelerates dopaminergic based forgetting. Therefore, forgetting is regulated by the behavioral state modulation of dopaminergic-based plasticity. Our findings integrate psychological and neuroscience research on sleep and forgetting.

D1274A Dopamine-mediated plasticity across the mushroom body. *T. Boto*, S. Tomchik. The Scripps Research Institute, Jupiter, FL.

Dopaminergic neurons innervate multiple brain regions, including the mushroom body (MB), where they modulate learning. During olfactory classical conditioning, large subsets of dopaminergic neurons are activated, releasing dopamine across broad sets of postsynaptic neurons. These dopaminergic neurons respond strongly to the US and their activity can be sufficient as reinforcement when paired with odor delivery. However, the specific way in which dopamine alters the physiology of the MB neurons is not well understood.

Using *in vivo* calcium imaging, we studied the effect of manipulation of different subsets of dopaminergic neurons in the odor response of several mushroom body regions. Odor presentation was paired with activation of different populations of neurons with the application of an odor and odor-evoked calcium responses were compared before and after the treatment.

We previously reported that pairing the activation of dopaminergic neurons labeled by TH-Gal4 with odor exposure leads to calcium plasticity specifically in the gamma lobes of the MB (*Boto et al., 2014*), whereas activation of the neurons of the PAM cluster leads to a broader pattern of facilitation. In order to understand how dopamine affects the MB responses during differential conditioning, we paired an odor (CS+) with dopaminergic neuron activation, followed by a second odor (CS-) not associated with dopamine released**.** Our results show that pairing odor with dopaminergic stimulation generates positive change in the ratio of CS+/CS- responses: either an increase of the response to the CS+ and no change/decrease on the CS- response, or no change in the CS+ response, but a decrease in the CS- response. The specifics of this plasticity depend on the odors used for the CS+/CS-.

Using more specific drivers to study different dopaminergic cluster subdivisions, we saw that subdivisions of the TH-GAL4 driver produce distinct spatial effects on neuronal plasticity.

D1275B Scabrous mediates acute ethanol response in the *Drosophila* brain through the Notch pathway. *M. Feyder*, R. Muster, A. Ray, N. Mei, A. Mathur, E. Petruccelli, K. Kaun. Brown University, Providence, RI.

Alcohol use disorders (AUDs) take a significant social and medical toll on the world's population. *Drosophila* have proven to be a useful genetically tractable model in which to investigate the molecular and behavioral effects of alcohol. We have previously shown that flies form long-lasting memories for the intoxicating properties of ethanol and identified the Notch pathway regulator *scabrous (sca)* as a potential molecular component of ethanol reward memory. Despite evidence that *sca* mutants exhibit several Notch-dependent developmental phenotypes, the general role of Notch signaling in the adult brain as well as in response to ethanol exposure remains poorly understood. Although Sca is known to bidirectionally regulate the Notch pathway in a context- and tissue-dependent manner, the exact mechanisms by which Notch and Sca interact in the adult fly brain is unclear. We predict that Sca mediates ethanol response in the mushroom body by promoting the cleavage of the Notch intracellular domain resulting in its activation. To test this, we used western blotting to characterize Sca effects on cleavage and processing of the Notch intracellular domain in adult fly heads. Compared to wild-type flies, *sca* mutants have increased levels of membrane-associated Notch and decreased levels of cleaved Notch, suggesting that Sca acts as a positive regulator of Notch pathway activity in this context. We also found that repeated exposure to intoxicating amounts of ethanol induces differential activation of the Notch pathway in wild-type and *sca* mutant flies, using both western blotting and a GFP reporter for Notch activation, suggesting that Sca affects the acute ethanol response of *Drosophila* by altering Notch cleavage and subsequent activation. Further work will investigate downstream targets of Notch, potentially illuminating its function in long-term memory formation and alcohol reward behavior.

D1276C Effect of sound as context on appetitive and aversive conditioning in Drosophila. *F. L. Hannan*[1], E. Dautaj[1], S. J. Gjeci[1], N. Narayanan[2], P. Meade[1], E. B. McGough[1]. 1) New York Med Col, Valhalla, NY; 2) Touro College, Manhattan, NY.

Associative learning and memory tasks critically depend on the simultaneous activation of multiple sensory pathways. Such tasks are very sensitive to context, including location, color, time of day and temperature. In humans it is well known that sound can trigger recall of past events, and it has been shown that sound can enhance or interfere with word recall tasks and working memory (Balch et al., 1992; Sorqvist, 2010), and that ultrasound can affect cortical activity (Legon et al., 2014). The fruit fly, Drosophila melanogaster, has been employed extensively for studies of learning and memory, using classical and operant conditioning tasks (Brembs 2011; Davis 2011). Sound is also an important environmental stimulus in flies. Male flies "serenade" females during courtship by flapping one wing (Shorey, 1962). Males also "yell" at each other with dual wing flicks during fights (Jonsson et al., 2011). We have observed that both pleasant and agonistic sounds can interfere with a reward based olfactory association task that pairs sucrose with odors inwild type flies as well as learning and memory mutants including Nf1, amnesiac and dunce. The same sounds, however, do not affect an aversive olfactory association task that pairs electric shock with odors, nor do they affect deaf flies (mer mutants). It is well known that different circuitry and neurotransmitters are involved in aversive versus appetitive conditioning, however there are also operational differences in the learning protocols that may interfere with exposure to sound. Future studies include developing an aversive conditioning protocol using bitter tastants, to better simulate the appetitive task, and to assay the effect of sounds on appetitive long term memory. Ultimately we would like to Identify the neural circuits and biochemical pathways involved in the integration of sound context into learning and memory, using the powerful genetic tools available in Drosophila.

D1277A From image to behavior: use of MiMICs to identify a novel protein kinase required for memory formation . *P. Lee*[1,5], G. Lin[1,5], Z. Zuo[1,5], K. Schulze[5,6], F. Diao[7], B. White[7], H. Bellen[1,2,3,4,5,6]. 1) Department of molecular and Human Genetics; 2) Program in Development Biology; 3) Department of Neuroscience; 4) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, United States; 5) Baylor College of Medicine, Houston, TX, United States; 6) Howard Hughes Medical Institute; 7) Laboratory of Molecular Biology, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, United StatesUSA.

We are interested in providing a better understanding of the molecular mechanism underling memory formation. Fly biologists have uncovered the role of genes required for learning and memory formation and many of these affect the cAMP-PKA-Creb pathway, including *dnc*, *rutabaga*, *amnesiac*, *DC0*, and *creb2*. These genes are often expressed in mushroom bodies (MB) which is the seat of learning and memory formation. However, it is likely that other players are involved in learning and memory.

To identify new genes required for learning and memory in the adult fly we screened a collection of MiMIC,insertions (Venken et al., 2011; Nagarkar-Jaiswal et al., 2015) MiMICs that are inserted in coding introns were modified through recombination mediated cassette exchange (RMCE) to generate flies with an additional exon that encodes GFP. We stained adult brains of 500 MiMICs that were tagged with GFP to identify genes expressed in MB. Unfortunately, only about 15% of the stocks express detectable levels of GFP in adult brains whereas GFP expression was observed in 90% of the third insaar larval brains (Nagarkar-Jaiswal et al., 2015). We therefore also converted the MiMICs with the T2A-Gal4 "Trojan exon" casette (Diao et al., 2015). This leads to the production of the Gal4 protein in the proper spatial and temporal pattern and permits the detection of GFP (via UAS-mCD8-GFP) for nearly 100% of the Gal4 tagged genes. Using this approach, we characterized the expression pattern of 30 genes encoding protein kinases and observed that 12 were expressed in MB. To identify whether or not these protein kinases are required for learning and memory we performed the olfactory aversive conditioning assay. We removed the GFP tagged kinase in adult flies using the Degrad-FP system in MBs and restored the protein in the same flies. By this approach, we identified a novel kinase required for memory. Interestingly, the loss of function of this gene leads to a complete loss of anesthesia-sensitive and long-term memory but does not affect anesthesia-resistant memory and learning. Our preliminary data indicate that this novel kinase regulates some of the substrates that are phosphorylated by protein kinase A (PKA).

In summary, we show that the MiMIC system, coupled to the Trojan exon and DegradFP technologies, is a powerful platform to screen for genes that are required for memory formation and probably many other behavioral functions.

D1278B Differences in the cyclic-AMP-induced plasticity of the odor responses among the Kenyon cell subpopulations: a single-cell imaging study. *T. Louis*, S. Tomchik. The Scripps Research Institute, JUPITER, FL.

In *Drosophila*, the mushroom bodies (MB), a critical structure for olfactory learning and memory, are formed by three different types of Kenyon cells (KC), α/β, α'/β' and γ. They all received information from the olfactory pathway and are modulated by extrinsic dopaminergic neurons also known for their role in associative learning and memory. Recently, our lab demonstrated that the modulation of cAMP levels in KCs induced by simultaneous odor presentation and artificial activation of TH-GAL4+ dopaminergic neurons or by using an adenylate cyclase activator, forskolin, lead to an increased of odor-evoked calcium response only in the γ lobes of MB. Nevertheless, it was still unknown if the learning-related plasticity seen in the g lobes relies on an enhancement of the response in cells that responded before training or on the recruitment of new responding cells. To answer this question, we designed an *in vivo* Ca^{2+}-imaging experiment for which we recorded the odor response in the soma of each KC subtype before and after forskolin exposure. Our work shows that, at the single-cell level, forskolin exposure produces diverse effects on the odor-evoked calcium responses in individual neurons, both across KC subpopulations and within a given subpopulation.

D1279C Effect of the spontaneous mutation *agn^{X1}* in *limk1* gene on formation of medium-term memory in *Drosophila melanogaster*. *E. A. Nikitina*[1,2], O. P. Yashanova[2], E. V. Savvateeva-Popova[1]. 1) Pavlov Institute of Physiology RAS, St-Petersburg, Russia; 2) Herzen State Pedagogical University, St-Petersburg, Russia.

The field of synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD) presumed to comprise a fine cellular basis for learning and memory is connected with such genetics processes as neuron-specific transcription, epigenetic chromatin remodeling, trafficking of mRNAs from soma to the remote sites of their local translation in axons and dendrites. All these processes are result from a multilevel organization of the genetic material in the nucleus of a nerve cell. *Drosophila* is a helpful model organism to determine the sequence of events in this system of hierarchical relationships. *Drosophila* LIMK1 gene (*agnostic*) with a specific chromosome architecture around the gene capable of generating miRNAs, recapitulates many features both of Williams syndrome and of neurodegenerative disorders. LIMK1 being the key enzyme of actin remodeling. This signal cascade involving receptors of neurotransmitters – small Rho GTPases (RhoA, Cdc42 and Rac1) –LIM kinase 1 (LIMK1) - cofilin – actin – is believed to play the main role in dendrite- and synaptogenesis. Conditioned courtship suppression paradigm was used to asses learning acquisition and medium-term memory formation in *Drosophila* mutant *agn^{X1}* characterized by existence of the spontaneous mutation *agn^{X1}* in *limk1* gene harbored by the *agnostic* locus. Learning acquisition appeared to be normal in intact control. However, a failure of 3-h memory formation was observed in *Drosophila* mutant *agn^{X1}* in normal conditions. We used heat shock (HS) as a stress factor. HS led to restoration of medium-term memory in this mutant. Thus stress influences for *agn^{X1}* mutant are necessary and sufficient for restoration of ability to memory formation.

This work was supported by the grant of the Russian Foundation for Basic Research (No 15-04-07738).

D1280A The role of octopamine beta-like adrenergic receptor Octβ1R in olfactory learning and memory. *J. Sabandal*, A. Clark, K.-A. Han. The University of Texas at El Paso, El Paso, TX.

Associative learning is a fundamental form of behavioral plasticity. It is indispensable for many organisms to appropriately respond to external cues predicting danger or reward for survival. The major monoamine octopamine plays a vital role in various types of learning and memory processes. Previous studies have shown that dopamine and octopamine are key mediators of olfactory learning. While dopamine regulates both aversive and appetitive learning, octopamine is known to mediate appetitive but not aversive learning. In order to clarify whether octopamine is also important for aversive learning, we employed negatively reinforced olfactory Pavlovian conditioning. Wild-type *Canton-S* flies showed robust learning and normal short-term memory. However, the flies deficient in the octopamine receptor Octβ1R exhibited poor learning but normal short-term memory. This suggests that the octopamine's signal through the Octβ1R receptor is critical for aversive learning. Studies are in progress to identify the key neural site where Ocβ1R regulates aversive learning.

D1281B A Circuit Screen for Song Production Neurons in *Drosophila melanogaster*. *A. Hammons*, D. Pacheco, M. Murthy. Princeton University, Princeton, NJ.

The courtship song production circuit in the fruit fly *Drosophila melanogaster* provides an excellent animal model for studying decision-making and motor pattern generation that are important for mate selection in a number of species. With the small and genetically tractable nervous system of *Drosophila*, identification of the specific neurons involved in courtship song production allows interrogation of the principles underlying the transformation of complex patterns in the nervous system to a motor output. Though five neuronal classes capable of producing courtship song have been identified, previous screens for song production neurons only targeted neurons expressing the male-specific transcription factor *fruitless*. The functional properties and connectivity of these five neuronal classes also remains uncharacterized. To uncover more of the neural circuits underlying song production, we performed a neural activation screen for ventral nerve cord neurons that drive song production in headless males by expressing the temperature-sensitive ion channel TrpA1 in small subsets of neurons (labeled by the Janelia GAL4 collection). We identified seven GAL4 lines capable of song production when artificially activated. An intersectional approach where TrpA1 was restricted to neurons expressing either the *fruitless* or *doublesex* gene revealed that five of these GAL4 lines contain song production neurons that do not express either of these two genes. Chronic inactivation (using tetanus toxin light-chain (TNT), a genetically encoded protein that blocks synaptic transmission) of these candidate neurons during a courtship pair assay that measures both male and female movement while also recording song, allowed us to characterize these neurons' effect on song patterning and female responses to song. Using *in vivo* calcium imaging of candidate song neurons from this screen combined with optogenetic activation of the song

command neuron P1, we are currently examining whether or not the newly identified song neurons lie within an interconnected song circuit with those neurons previously identified.

D1282C Selection for starvation resistance impairs copulation and provides a context-dependent courtship advantage. *Pavel Masek*[1], Lauren Reynolds[3], Thomas L. Turner[4], Allen G. Gibbs[5], Alex C. Keene[2]. 1) Binghamton University, NY; 2) FAU, FL; 3) Florida State University, FL; 4) UNLV, NV; 5) UCSB, CA.

Although courtship and starvation resistance are under stringent evolutionary pressure, the relationship between these traits has been difficult to investigate experimentally. Interactions between fitness and courtship have been documented in diverse species where both male and female flies display preference for mates with improved fitness characteristics that include body size, ornamental displays and pheromonal profiles. We have generated three replicated lines of starvation resistant flies by selecting for survival on a non-caloric agar diet and were then compared to three replicated populations of fed control flies. S flies live up to 18 days without food compared to the ~4 days for F flies. Starvation resistant flies display a number of behavioral and physiological changes including reduced fecundity, suggesting a trade-off between starvation resistance and reproduction. Here we characterize the courtship behaviors of male and female flies selected for starvation resistance. While courtship duration, courtship latency, song quality and quantity does not differ between starvation resistant flies and controls, the starvation resistant flies displayed dramatically reduced copulation success. Analysis of courtship in mixed pairings revealed both male and female contributions to reduced copulation in starvation resistant flies, raising the possibility of a functional trade-off between starvation resistance and copulation efficiency. While non-selected control flies outperform starvation resistant flies in competition assays under fed conditions, the result is reversed when flies are fasted prior testing. Taken together, these findings indicate that selection for starvation resistance results in genetic changes that impair copulation without affecting courtship duration and provide an adaptive copulation advantage in the absence of food.

D1283A Postmating gene expression in the parovaria (female accessory reproductive glands) of *Drosophila melanogaster* . *C. McDonough*, S. Pitnick, S. Dorus. Syracuse University, Syracuse, NY.

Female reproductive tract secretions are putatively involved in a diverse array of reproductive events, including sperm storage and survival, ejaculate-female interactions, ovulation, postmating immune response, lubrication for egg-laying, and the production of protective egg coating. However, little is known about the source, contents or function of these secretions. We characterized mating-induced gene expression in the parovaria, a secretory gland of the female reproductive tract, using RNAseq analysis at 3 time points (premating, 6hrs, and 24hrs postmating). Genes significantly upregulated postmating included proteases, peptidases and anti-microbial genes, thereby supporting established functions of female secretions in seminal fluid processing and postmating immune response. Many of these genes have similar postmating expression patterns in other female reproductive structures, indicating a suite of redundant functions spatially distributed throughout the female reproductive tract. We also identified genes with distinct expression patterns in the parovaria, suggesting unique functions of these glands. In particular, we found an enrichment of highly expressed genes on the X chromosome, including several novel genes created through gene duplication occurring during the radiation of the *melanogaster* subgroup. Contributions of these genes to postmating changes in female physiology and fertility are currently being assessed using an RNAi knockdown approach.

D1284B Sex differences in the translatome of *Drosophila melanogaster fru P1*- expressing neurons. *N. R. Newell*[1], C. M. Palmateer[1], F. N. New[2], J. E. Dalton[1], L. M. McIntyre[2], M. N. Arbeitman[1]. 1) Florida State University, Tallahassee, FL; 2) University of Florida., Gainesville, FL.

Male and female reproductive behaviors in *Drosophila melanogaster* are vastly different, but the neurons that express sex-specifically spliced *fruitless* transcripts (*fru P1*) underlie these behaviors in both sexes. How a similar set of neurons can drive such different behaviors is an unresolved question in neurogenetics. A particular challenge is that *fru P1*-expressing neurons comprise only 2-5% of the adult nervous system, and so inferences made in studies of adult head tissue or even the whole brain are limited. To assess gene expression in the *fru P1*- expressing cells we used Translating Ribosome Affinity Purification (TRAP). This technique allows us to conduct a sensitive, cell-specific assay of gene expression by identifying the actively translated pool of mRNAs from *fru P1*-expressing neurons. Additionally, to examine the changes in gene expression across development we assay three different time-points in the fly life cycle—48hr pupae, 0-24hr adult, and 10 day adults. Here, we present results on the 0-24hr adults and find that the male and female *fru P1*-expressing neurons have a shared set of 7,967 genes identified by TRAP that form a distinct repertoire relative to those identified from TRAP analyses of all neurons in the adult head. Further, there are genes that have sex-biased TRAP abundance in *fru P1*-expressing neurons, with the majority of these genes having male-biased TRAP abundance. This suggests an underlying mechanism to generate dimorphism in behavior, with a transcript repertoire that specifies male and female behaviors present in both sexes and a large additional set of genes with expression in the male. Thus, these additional genes could invoke the male-specific behaviors by establishing cell fate in a similar context of gene expression observed in females. These results suggest a possible global mechanism for how distinct behaviors can arise in different environments from a shared set of neurons.

D1285C Comparison of mate choice in *Drosophila melanogaster* exposed to a mutagen. *Y. Trujillo Varela*[1], P. Ramos-Morales[1], SM. Villagrán[2], AJJ. Espinosa[3], C. Macías[4]. 1) Laboratorio de Genética y Toxicología Ambiental Facultad de Ciencias UNAM; 2) Departamento de Biología Comparada, Facultad de Ciencias UNAM; 3) Instituto de Investigaciones Biomédicas, UNAM; 4) Instituto de Ecología, UNAM. CdMx 04510, México.

Organisms are continually exposed to a wide variety of substances which can be harmful and may put at risk the integrity of organisms and the health of future generations. Even sublethal concentrations can alter competitive, fundamental skills for reproduction and transmission of

genetic material to subsequent generations. In this work, we use Drosophila melanogaster to determine whether exposure to the mutagen Sodium Azide modifies the possibility that males could be chosen as breeding pair, comparing male performance in massive and individual crosses. Wild type (Canton –S), third instar larvae, were fed semichronically with sodium azide (SA) [0.125- 1.13E-13], a mutagen that induces repair by recombination in Drosophila melanogaster (Gonzalez-Ramos, 1997). For the rest of the assays we used SA [3.86 E -6] because this concentration does not affect the survival of the adults. For all matings, ywf homozygous untreated females were crossed with wild-type treated males mixed with mutant no treated males for any combination of the y w f markers (X-linked). A massive crossing with a ratio of operating sexes (OSP) 1:1 was performed. Two days later, females were placed individually in vials to produce progeny. The type of the progeny obtained was used as an indicator of how many couples have the parental female and the male phenotype chosen by the female. Females take part in three matings (average). It was found that the wild-type phenotype predominates in the offspring of treated and untreated males, although it is possible that the same kind of male participates in more than one copula. The experiment was repeated but now only one male of each kind of mutant marker and the treated wild-type male were put with 8 ywf females. On these matings, the female take part in two copulas and when exposed males were used, mutant progeny was more abundant than the wild-type. Acknowledgments: To Master in Biological Sciences, UNAM-CONACyT (Grant 262619) and to Drosophila Stock Center Mexico, UNAM to provide the biological material.

D1286A A Novel Gene Controlling the Timing of Courtship Initiation in *Drosophila melanogaster*. *S. A. Zaki*[1], P. Luu[1], D. H. Tran[1,2], R. L. French[1]. 1) San José State University, San José, CA; 2) Counsyl, Inc., South San Francisco, CA.

Drosophila melanogaster is an ideal model organism to study genetic regulation of innate behaviors. Male *Drosophila* carry out a complex courtship ritual involving a specific sequence of steps, which depend on the processing of sensory cues by the CNS. *fruitless* (*fru*) is the master regulatory gene that controls sex-specific behaviors; this gene is both necessary and sufficient for all aspects of the courtship routine. We have identified a gene, *Trapped in endoderm 1* (*Tre1*), that is essential for normal courtship behavior in male flies. *Tre1* encodes an orphan G-protein-coupled receptor that is required for both cell migration and establishment of cell polarity, but has not previously been implicated in courtship. The goal of this research is to characterize the role of *Tre1* in *Drosophila* mating behavior. We found that male flies in which *Tre1*-expressing cells are feminized through expression of the female specific isoform of the splicing factor Transformer (Tra^F), display unusually rapid courtship initiation. Additionally, partial loss-of-function mutants in *Tre1* show an aberrant courtship behavior, including rapid courtship initiation. To establish whether this phenotype resulted in increased reproductive fitness, we performed fertility assays and found that male flies with feminized *Tre1*-expressing cells were at a competitive reproductive advantage, while the *Tre1* hypomorphs were at a competitive reproductive disadvantage, indicating a complex requirement for *Tre1* in courtship success. Finally, we found that *Tre1-GAL4* is expressed in a sexually dimorphic pattern in the olfactory organs and olfactory centers of the CNS: the antennae, antennal lobe, and lateral horn, suggesting a role for Tre1 in the processing and integration of sensory signals during courtship. Future experiments will involve characterization of the Tre1 signal transduction pathway, specifically, determination of the Tre1 receptor ligand and downstream targets. This will further implicate Tre1's role in *Drosophila* courtship. Additionally, to determine whether feminization of *Tre1-GAL4* expressing cells leads to a gain-of-function or loss-of-function effect, we will perform experiments driving the expression of a temperature-sensitive allele of *shibire*, and the heat-sensitive TrpA1 channel in *Tre1-GAL4* neurons. We will present the results of feminization of *Tre1-GAL4* expressing neurons, as well as the effects of mutations of *Tre1* on courtship behavior. We will also describe how feminization and mutation of *Tre1* affect the fertility of male *Drosophila*. Furthermore, we will show confocal images of the sexually dimorphic expression pattern of *Tre1-GAL4* in the CNS.

D1287B Disruption of circadian rhythm through misexpression of a frontotemporal dementia-associated mutation in circadian pacemaker neurons in *Drosophila*. *S. T. Ahmad*, C. S. Krasniak, P. J. Kavaler. Colby College, Waterville, ME.

Frontotemporal dementia (FTD) is the second most common early-onset neurodegenerative disease. One subtype of FTD causes the production of $CHMP2B^{Intorn5}$, a mutant isoform of an ESCRT-III subunit. This causes disruptions in the autosomal-lysosomal and autophagy pathways. FTD patients show a variety of neurological symptoms, including disinhibition, apathy, aggressive behavior, and circadian rhythm deficits. To investigate these circadian rhythm deficits we ectopically expressed human $CHMP2B^{Intron5}$ using the GAL4-UAS system with the driver lines *cry*-GAL4 and *pdf*-GAL4 in *Drosophila*. These drivers are specific to an important subset of circadian pacemaker neurons in the brain. Using activity monitoring, we observed moderately disrupted circadian behavior. We did not observe any cellular death phenotype through whole brain imaging. To investigate the circadian deficits we are currently examining *timeless* and *period* transcript levels to investigate possible disruption of the molecular clock. This will allow us to further describe the circadian deficits caused by $CHMP2B^{Intorn5}$ misexpression.

D1288C A large-scale forward genetic screen to understand the role of glia in locomotion, arousal, and sleep. *V. Ahmad*[1], C. King[1], M. K. Gronauer[1], B. Kottler[2], R. Faville[1], B. Zhang[1]. 1) Division of Biological Sciences, University of Missouri, Columbia, MO; 2) Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology & Neuroscience, King's College London.

In order to decipher the contribution of glial cells in motor behavior and sleep regulation and to gain insight into glia-neural interaction, we used a "cell-centric" forward genetic screen approach to identify glia subset involved in these mechanisms. We hypothesize that specific glial cells are crucial for various key endpoints related to locomotor activity, arousal and sleep by modulating the functionality of specific neuronal subsections. We conducted an unbiased genetic screen by genetically manipulating subset of glia cells within a broad glial-specific *repo*-Gal4 expression pattern using the FINGR (Flippase-induced Intersectional Gal80/Gal4 Repression) method. We perturbed the function of specific glia subsets by expressing a UAS-effector (polyglutamine, polyQ) to determine its effect on behavior, and mapped glial morphology and location by labeling the relevant subset with GFP. We assayed locomotion, arousal and sleep in flies by using a video-based platform, and the DART (Drosophila Arousal Tracking) system in a 12:12 L:D cycle. An ongoing study has screened 122 lines (male and female) and identified 27 lines

with a defect in sleep impacting daytime or nighttime sleep or both. We believe that the characterization of these sleep phenotypes should help identify specific and novel roles of subset glia modulating neurons in the regulatory mechanisms of sleep and its functional consequences.

D1289A Circadian master regulator CLOCK in the central complex of *Drosophila* mediates sexual development. *Vinodh ILANGOVAN*[1], Zeynep Orhan[2], Ramanathan Narayanan[3], Halyna Shcherbata[1], Gregor Eichele[1]. 1) Max Planck Institute for Biophysical Chemistry, Goettingen, DE; 2) Bilkent University, Ankara, TR; 3) International Max Planck Research School- Neurosciences, Goettingen, DE.

Circadian locomotor activity patterns in *Drosophila* exhibit distinct sexual dimorphism in the phase of the rhythm. Since central complex is involved in integration of visual inputs and acts as higher order centre for locomotion, we investigated the presence of peripheral circadian clocks in the central complex and the possible substrate for sexually dimorphic locomotor activity patterns. Misexpression of positive regulators of circadian clock i.e., CLOCK and CYCLE in the central complex led to an altered locomotor pattern under periodic conditions but did not change the overt rhythmicity in the absence of time cues. Evening activity peak was abolished and a reduction in total activity was observed upon inducing dominant negative version of CLOCK in the central complex. Interestingly, we found that females failed to develop when a dominant negative version of CLOCK is expressed using one of the central complex drivers. Our study identified the involvement of central complex at cellular network level and the circadian activator CLOCK at the molecular level in mediating sexually dimorphic development and locomotor behavior of male flies.

D1290B Courtship, sleep and circadian rhythm of *Drosophila melanogaster* are greatly impacted by loss of the Dα1 nicotinic acetylcholine receptor subunit. *Hang Luong*, Jason Somers, Trent Perry, Philip Batterham. Bio21 Institute, School of BioSciences, University of Melbourne, Parkville, Australia.

Nicotinic acetylcholine receptors are a major class of ligand-gated ion channels in insect nervous system. Despite being well studied as insecticide targets, their roles in insect behaviours are still poorly understood. This study identified one of the ten receptor subunits, Dα1, as a modulator of sleep and circadian rhythm in Drosophila melanogaster as well as courtship behaviour. Using ends-out gene targeting, a genomic deletion of Dα1 was generated. Null mutant flies showed decreased night sleep which was due to shorter sleep episodes. Without a cycling light-dark condition, a severe loss of both sleep and circadian rhythm was observed. Additionally, courtship behaviour was also greatly affected in mutant flies. Unsurprisingly, their lifespan was significiant reduced to almost half that of control flies. Pan-neuronal GAL4 driven expression of the wildtype allele in the null background was sufficient to revert most, but not all, of these phenotypic changes. Even though mutations in Dα1 clearly confer a high level of resistance to neonicotinoids, a main class of commercial insecticides, fitness costs associated with a complete loss of Dα1 might explain the low observed frequency of such mutations in the targeted pest specieis.

D1291C A wing damage screen identifies novel genes affecting *Drosophila* aggression. *S. Davis*, A. Thomas, L. Liu, I. Campbell, H. Dierick. Baylor College of Medicine, Houston, TX.

Aggressive behavior is widespread throughout the animal kingdom as a means to compete for territory, food, and mates. Fighting animals can benefit from these encounters but also run the risk to lose resources, and they may incur physical damage and even risk death. While aggression is part of the normal repertoire of complex behaviors for many animals, excessive aggression also occurs in many human neurological and psychiatric disorders. The molecular and neuronal mechanisms underlying this trait in health and disease remain largely unknown. The identification of novel genes that regulate aggression through a forward genetic screen has so far never been attempted in any organism because the complex nature of the behavior makes screening prohibitively time consuming. We circumvented this challenge when we discovered a positive correlation between aggression and physical wing damage in male flies that were group-housed. Using this easy-to-screen wing damage phenotype we performed the first chemical mutagenesis screen to isolate mutants with increased aggression. After screening ~1,400 EMS induced X-chromosome mutants for increased wing damage, we found 5 lines that also had increased aggression. Using whole-genome sequencing, meiotic mapping, and genomic duplication rescues, we identified the causal mutation in 2 of these strains. These novel mutants likely affect neuronal excitability and gene regulation, although the mechanisms through which they function remain unknown.. This aggression-induced wing damage screening approach can also be applied to other chromosomes to further identify novel genes regulating this behavior in flies and may also provide insight into human aggression.

D1292A Male aggression requires the function of both octopamine and glutamate in dual neurotransmitting neurons. *H. R. Morgan*[1], H. M. McKinney[2], S. J. Certel[1], R. S. Stowers[2]. 1) University of Montana, Missoula, MT; 2) Montana State University, Bozeman, MT.

How information is encoded, transformed, and processed as it is transmitted between neurons is a fundamental question in neuroscience. In the last 15 years, dual transmission, or the ability of a neuron to release *multiple* transmitters has become firmly established, yet the functional significance has been difficult to dissect. Here we demonstrate through antibody labeling and new intersectional genetic tools that a subset of octopamine (OA) neurons within the adult brain also utilizes the neurotransmitter glutamate (Glut). Eliminating OA in these neurons has previously revealed aberrant male social behaviors including a delay in the initiation of aggression and a reduction in aggressive lunge number. OA has also previously been shown to mediate the choice between reproduction and aggression as males without OA display increased levels of male-male courtship and intermix aggression and courtship behavioral patterns. With this foundation, we are investigating if both OA and glutamate signaling from OA-Glut neurons is required for male aggression and reproductive behavior using two approaches. First, we examined the behavior of males deficient for glutamate in OA neurons through RNAi interference *(tdc2-Gal4/UAS-vGlut-IR).* Preliminary results indicate males lacking glutamate in the OA-Glut dual neurotransmission neurons exhibit a reduction in lunge number similar to the loss of OA yet do not display an increase in male-male courtship as compared to transgenic controls. Second, we will use the new intersectional genetic method to

eliminate only glutamate in the OA neurons by creating a vGlut complete loss-of-function allele within the OA neuronal population and quantify the subsequent changes on male behavior. Finally, males deficient in both OA and glutamate transmission will be assessed for the same behaviors. Taken together, our results demonstrate glutamate function within OA neurons is required for sex-specific, sensory-invoked behavior and provide a platform to understand the behavioral relevance of dual transmission in any system.

D1293B Molybdenum cofactor synthase 1 (Mocs1) regulates aggressive behavior in *Drosophila melanogaster*. *M. Ramin*, Y. Li, Y. Rao. Centre for Research in Neuroscience, McGill Uni., Montreal, Quebec, CA.

Aggression is an overt behavior that can be adaptive for individual survival. It occurs when two or more individuals have a conflicting common interest for food, mating partner and territory, with the dominant animals having more access to these resources. Both genetic and epigenetic factors have been shown to play important roles in regulating the levels of aggressiveness. In this study, we utilize *Drosophila melanogaster* as a model system to study the molecular mechanisms underlying the control of aggression.

In a search for genes that are involved in modulating aggressive behavior, we found that mutations in the *Mocs1* gene encoding Molybdenum cofactor synthase 1 (Mocs1), cause a significant decrease in the levels of fly aggressiveness. Cell-type-specific knockdown shows that Mocs1 is required in neurons for the control of aggression. While *Mocs1* mutants display severe defects in aggression, they show normal pattern of sexual discrimination, male-male and male-female mating behaviors. Other behaviors such as olfactory sensation and locomotor activity also remain normal in *Mocs1* mutants. These results suggest that Mocs1 plays a specific role in regulating fly aggression. To determine the exact mechanism by which Mocs1 is involved in the control of aggression, we are taking a combination of molecular and genetic approaches to investigate the molecular networks in which Mocs1 functions, and identify the Mocs1-dependent neuronal circuits that directly control aggressive behavior. The results will be presented at the meeting.

D1294C Analysis of pruritogen induced grooming behavior in *Drosophila melanogaster*. *Ciny John*, Rodney Murphey, Ken Dawson-Scully, John Nambu. Florida Atlantic University, Jupiter, FL.

Pruritus, or itch, is an unpleasant sensation that evokes a desire to scratch. Itch can be classified as either an acute or a chronic condition. Acute itch typically results from a bite or sting and can serve as a protective mechanism against tissue damage caused by parasites. However, chronic itch can result from the dysfunction of the immune and/or nervous systems. In mammals, pruritogens, or itch inducing agents, stimulate peripheral unmyelinated c-fiber pruriceptors, which are pruritogen detecting primary sensory neurons in the skin, causing transmission of sensory input to interneurons and projection neurons of the brainstem via the spinothalamic tract. This induces the itch sensation in the brain and the subsequent scratching behavior. Itch is a primary symptom in patients with broad range of illnesses, including skin diseases and systemic and metabolic disorders like liver and kidney diseases, HIV/AIDS and brain cancer. It is also seen in psychiatric disorders such as anxiety, depression, schizophrenia and delusions of parasitosis, leading to self-injurious behavior. The current understanding of pruritus in systemic diseases is limited due to the lack of sufficient animal models that mimic clinical chronic itch conditions. Furthermore, the antihistamines that are currently available for treatment of itch are typically ineffective in treating most cases of pruritus. *Drosophila* possess the homologs of many of the genes known to be involved in the mammalian pruritic pathway. Therefore, in current study, we are developing *Drosophila* as a model organism in order to investigate pruritus and identify key mediators of this condition in an established genetic model. To analyze the effects of pruritogens on *Drosophila*, we administer pruritogens to the fly and measure changes in their grooming behavior. Our preliminary results indicate that Histamine and Compound 48/80, which induces the natural release of histamine, both evoke longer grooming bout duration, compared to the control flies. Interestingly, two other pruritic mediators, Chloroquine and cowhage, do not have a significant effect on the flies' grooming behavior, leading us to believe that Histamine and Compound 48/80 evoked a pruritic response. Histamine is one of the best studied pruritogens in mammals and *Drosophila* are known to have two Histamine receptors, Histamine-Gated chloride channel 1 (HisCl1) and Ora transientless (Ort). Both receptors have been studied for their role in photoreception and visual transduction, however, additional functions of these receptors have not been investigated. Therefore, we also set out to identify the receptor(s) responsible for the change in grooming behavior we observed, using Histamine receptor mutants. Based on our findings, we conclude that *Drosophila* can indeed be used as a model organism for investigating pruritus.

D1295A Regulation of meal size by sucrose and sweet taste. *Margaux R. Ehrlich*, Keith R. Murphy, William W. Ja. The Scripps Research Institute, Jupiter, FL.

Ingestive behavior is composed of parameters such as meal frequency and meal size. Previous studies suggest that increased meal size plays a large role in the propensity for obesity. Thus, understanding how meal size is regulated could lead to interventions to treat obesity, diabetes, and general eating disorders. The advent of novel methods for measuring *Drosophila* feeding behavior has increased its use as a model for understanding the mechanisms underlying the regulation of hunger and satiety. Meal size is thought to be dictated by oral and post-ingestive signaling; however, the interaction between the two remains unclear. We found that feeding flies increasing sucrose concentrations resulted in decreased average meal size. In contrast, increasing concentrations of sucralose, a non-nutritive but sweet tasting substance, increased meal size. Interestingly, following starvation, meal size had a non-monotonic relationship with sucrose concentration, revealing state-dependent positive regulation by sucrose. These data suggest that accumulating hunger may unequally modulate taste and post-ingestive signaling to determine meal size. Furthermore, we have identified a subset of neurons that are necessary for the regulation of meal size; when these neurons are silenced, animals are unable to regulate feeding and meal size is increased. Future studies will determine whether these neurons regulate oral or post-ingestive signaling and will further elucidate the mechanisms underlying meal size regulation.

D1296B Ir76b conductance is gated by other Ir proteins to mediate amino acid taste. *Anindya Ganguly*, Anupama Dahanukar. University of California, Riverside, Riverside, CA.

Amino acid taste has remained largely unexplored in flies. Although there is evidence that flies exhibit enhanced behavioral sensitivity to amino acids when deprived of dietary amino acids, the cellular and molecular basis for amino acid taste is not understood. Our objective was to systematically study amino acid taste and investigate mechanisms underlying their detection.Yeast is the chief source of dietary proteins and free amino acids. We found that amino acids serve as the key cues for yeast feeding preference. Flies showed a strong feeding preference for yeast extract, which was lost when amino acids were depleted from it. Individual L-amino acids were able to evoke feeding preference to varying extents in binary choice assays with sucrose, which was not observed for D-amino acids. As found previously for yeast, feeding preference for amino acids showed sexual dimorphism and dependence on mating state – mated females showed higher preference as compared to males or virgin females.We conducted an RNAi screen targeting ionotropic receptors (Irs) and identified *Ir76b*, which is widely expressed in taste neurons, to be necessary for yeast preference. *Ir76b* mutants lose their preference for yeast extract and amino acids, which was restored by expressing *Ir76b* in the mutant background using the *UAS-Gal4* system. *Ir76b* is highly conserved in insects and we found that its *Anopheles gambiae* ortholog (*AgIr76b*) could rescue behavioral defects in yeast and amino acid preference in *Drosophila Ir76b* mutants, suggesting functional conservation of this receptor across evolution.By expressing GCaMP3 and imaging amino acid-evoked changes in fluorescence, we found that a subset of tarsal *Ir76b*+ neurons responded to amino acids. Interestingly, we found a functional overlap between amino acid and sucrose-sensing neurons, but not with those that detect water, salt, bitter or acid tastants. This was surprising because Ir76b has been shown to function as an ungated salt channel. We therefore examined the role of other *Ir* genes as potential co-receptors that gate Ir76b conductance in response to amino acids. One other candidate that emerged from the RNAi screen was *Ir20a*. We tested the hypothesis that Ir20a blocks salt response of Ir76b by co-expressing the two receptors in sweet taste neurons. We found that Ir76b was sufficient to confer salt response when expressed alone, but the response was greatly reduced in the presence of Ir20a. Conversely, Ir76b alone was not capable of conferring response to a mixture of amino acids, but a response was observed when Ir76b is expressed together with Ir20a.Overall, our results suggest that Ir20a, and possibly other Irs, combine with Ir76b to form amino acid-gated receptors, and offer a simple explanation for mutually exclusive roles of Ir76b in salt and amino acid sensing taste neurons.

D1297C Contribution of altered feeding to caffeine-mediated sleep suppression. *Chenchen Su*, Erin S. Keebaugh, Mounika Vattigunta, William W. Ja. The Scripps Research Institute, Jupiter, FL.

Caffeine promotes wakefulness and reduces sleep in many organisms, including *Drosophila*. Given that taste likely affects ingestion and that caffeine is also popularly studied as a bitter tastant, aversion to caffeine might influence fly food intake. Since it is known that reduced nutrient intake also reduces sleep—an effect known as starvation-mediated sleep suppression—we hypothesized that reduced feeding might contribute to caffeine-mediated effects on sleep. We quantified the individual and combined effects of starvation and caffeine supplementation on sleep in Canton-S males using the *Drosophila* Activity Monitor system. Our results may reveal the extent to which altered food intake and pharmacology influence caffeine-mediated sleep suppression in the fly.

D1298A Evidence of pleiotropic effects in some mutants of *Drosophila melanogaster*. *T. Alonso Vásquez*, P. Ramos Morales, Y. Trujillo Varela. Facultad de Ciencias UNAM, Cuidad de México, Delegacion Tlalpan, MX.

As in other organisms, in Drosophila, the mutant genes are named and characterized in regard to the change or obvious changes that cause with respect to wild-type flies, leaving aside other issues that could be affected by the presence of this mutation, i. e., although many genes could be pleiotropic, they are considered single acting and used under this concept in tools for studying genetic terminal events and mutagenesis studies. The aim of this study is to compare the displacement (negative geotropism) of mutant and wild-type flies. We used flies showing mutations that could affect locomotion and other affecting color eyes and body color which is assumed, they are not related with locomotion activity: protruding wings (nub: nubbin), miniature wings (m: miniature); dark body color (e: ebony), white eyes (w: white); and interactions among these mutations: protruded wings-dark body (nub-e), white eyes-miniature wings (w-m), white eyes-dark body (w-e), and the wild-type flies (Canton-S, CS), (Lindsley & Grell, 1972). The flies were placed in graduate plastic tubes (pipettes), it was tapped so the fly stay at the base and the traveled distance at 5 min was recorded. Both of them, CS and nub flies traveled the same distance, the rest of the mutants showed a greater displacement ($p < 0.05$), being the displacement of e and w mutants higher than all others ($p < 0.05$). The order of the registered average displacement is: e = w> w-e> nub-e = m = w-m> CS = nub. To confirm the response, the experiment was repeated at noon and afternoon. The average distance traveled by all mutants and wild type flies, were significantly different ($p<0.05$). In addition, the average of distance traveled was different among the mutants used. At noon, the order of average distance traveled was w = e = CS> nub = m = w-e = nub-e = w-m. At afternoon, the order of average distance traveled was w> e = CS = m = w-e = nub-e > w-m = nub. The results show that some of the mutations used can affect seemingly unrelated activities, such as body or eye color and locomotion. Of all the mutants, w travels farther than wild flies regardless of the schedule of tests ($p < 0.05$). It is interesting to explore further to understand why this mutant offers an apparent advantage to the carrier flies under laboratory conditions, but not necessarily in field conditions. Acknowledges: To Banco de Moscas (Drosophila Stock Center Mexico) to provide the biological material.

D1299B An optogenetic and quantitative dissection of descending neuron control of behavioral in *Drosophila*. *J. Cande*[1], G. Berman[2], S. Namiki[1], W. Korff[1], G. Card[1], J. Shaevitz[3], D. Stern[1]. 1) Janelia Research Campus, Ashburn, VA; 2) Emory University, Atlanta, GA; 3) Princeton University, Princeton, NJ.

In most animals, the brain sends signals to local neural circuitry in the nerve chord to produce behaviors. Despite the central importance of these signals as an informational and anatomical bottleneck, little is known about how these signals are encoded at the neuronal level or how they control aspects of behavior. In insects, signals from the brain to the ventral nerve chord are carried by an estimated 350 pairs of bilaterally symmetric descending neurons (DNs). To date, only a handful of these descending neurons have known functions. In order to understand how DNs can control insect behaviors, we developed a method to identify descending interneuron function in an unbiased and systematic fashion in the model insect *D. melanogaster*. Using the red-shifted channelrhodopsin *CsChrimson*, we activated neurons in a collection of ~200 lines, most of which target single neurons out of a collection of DNs that fall into 60 distinct neuro-anatomical classes. Using a PCA and frequency analysis based technique described in *Berman et al.* (2014) we created a two-dimensional behavioral space based on the underlying postural dynamics of freely moving flies with and without red light activation. In this map, stereotyped behaviors are represented by local probability density maxima, and distinct behavioral motifs are easily distinguished. We then looked for map regions that were upregulated in *CsChrimson* activated animals. Using this technique, we were able to assign phenotypes to 90% of the DNs in our collection. We find that (1) DN control of stereotyped behaviors appears to be modular, (2) much DN function can be correlated with neuro-anatomy, and (3) the nature of this correlation hints at centralized control of locomotory activities. These findings, which are only apparent in a dataset of this size, have wide-ranging implications for how complex signals from the brain are encoded by descending neurons.

D1300C Sensing, processing, and response to heating and cooling in the *Drosophila* larva. *M. Klein*[1], A. J. Ferrer[1], B. Kaminski[1], L. Ni[2], P. A. Garrity[2], M. Berck[4], A. D. T. Samuel[4], S. V. Krivov[3], M. Karplus[4]. 1) Univ. of Miami, Coral Gables, FL; 2) Brandeis Univ., Waltham, MA; 3) Univ. of Leeds, Leeds, UK; 4) Harvard Univ., Cambridge, MA.

The ability to sense and respond to temperature is universally important for the wellbeing of motile animals. Navigation in environments with variable temperature (thermotaxis) is built from signal generation in sensory neurons, signal processing in the brain, and signal conversion to physical muscle action. Working in the *Drosophila* larva model system, we explore the response to temperature at the scales of populations, individual animals, neuronal circuitry, and molecules. We find that response to heating is independent of response to cooling at all these levels, as the two distinct sensorimotor transformations operate in parallel. Modeling and Monte Carlo simulations using empirically-derived rules for behavioral mode transitions demonstrate that realistic movement can be recovered with a small number of parameters, and show the benefit of randomness in navigation decisions. Behavioral tracking of individual larvae establishes navigation patterns and shows that thermotaxis is specifically an aversive rather than attractive response to both heating and cooling. We employ an improved device for generating rapid changes in temperature to subject larvae to random noise stimuli, then generate mathematical filters that can probabilistically predict the larvae's responses at high and low temperatures. At the cellular level, we measure *in vivo* the activity of separate cool-sensing and heat-sensing neurons and identify downstream connectivity that points towards a more complete understanding of complex brain circuits. Finally, by using the above behavioral and neurophysiological methods, we uncovered the mechanism of cooling sensation, showing that two ionotropic receptors (IRs) are required for navigation and detection in adult flies and larvae, and also identifying candidates for heat sensation in the larva. The quantitative approaches used here should also be applicable to work in other sensory modalities and other model systems seeking to characterize sensory and circuit-level steps that lead to behavior.

D1301A *D. melanogaster* flies that survive environmental insults modify some crucial behaviors for later survival. *Karla I. Martínez-Ledezma*, Patricia Ramos-Morales. Laboratorio de Genética y Toxicología Ambiental, Departamento de Biología Celular, Facultad de Ciencias, UNAM, Coyoacán, México City.*.

Sodium azide (SA) (NaN3) is well known as a potent mutagen in bacteria. In eukaryotes is a metabolic poison that traps oxygen excited, interfering with the activity of various enzymes. In Drosophila melanogaster, causes mitotic recombination in somatic cells, in response to chromosomal breakage. In addition, it has been observed that the treatment itself causes an apparent delay in the development of the larvae exposed. In this study, we evaluated whether the exposure to sublethal concentrations of NaN3 affects the development cycle (10 days at 25 °C) and tropisms such as the negative geotaxis of adult flies recovered. Wild-type, third instar larvae, were fed semichronically with serial dilutions of SA, from 0.125 mM to 1.8E-13mM. Each day, adult flies recovered were counted and separated, until all flies had emerged. Two groups were formed according to the time of hatching: 10-12 days and 13 or more days. The effect on treatment in the behavior of flies from two periods of hatching (10-12 and 13 or more days) was compared. Groups of 20 flies were put into a vial (15 ml of volume) connected through a channel of 6 mm with other vial (same volume). The frequency of flies that cross in one minute to the second vial was determined. 20 families per concentration were used. Sodium Azide treatment changes the frequency with which the flies went through the second tube. Although the response was not linear, a higher frequently of crossing to the second tube was found in flies that hatched among 10-12 days and were treated with 1.8E-12 1.5E-11 2.4E-7 of SA; In contrast, flies in group 13 or more showed a higher frequency of crossing only if was previously fed with 1.5E-11. When comparing the frequency of passage between groups (10-12 and 13 or more), differences in all concentrations were confirmed, except in the highest proven, 1.25E-1. Both of them, the treatment with SA, and the emergency period, determine the behavior of crossing on Drosophila melanogaster. These results show that exposure to genotoxins should be evaluated on several types of responses, which may be relevant to determine the real effect induced on organisms surviving to toxic exposures.
Acknowledgements: To Banco de Moscas (Drosophila Stock Center Mexico) by provide the biological material, Faculty of Sciences, UNAM.

D1302B Gut bacterial modulation of locomotion in *Drosophila melanogaster*. *C. Schretter*[1], S. Keremane[2], J. Vielmetter[2], I. Bartos[3], Z. Márka[3], S. Márka[3], S. Hess[4], S. Mazmanian[1]. 1) Division of Biology & Biological Engineering, California Institute of Technology, Pasadena, CA, USA; 2) Protein Expression Center, Beckman Institute, California Institute of Technology, Pasadena, CA, USA; 3) Department of Physics, Columbia University, New York, USA; 4) Proteome Exploration Laboratory, Beckman Institute, California Institute of Technology, Pasadena, CA, USA.

Trillions of microbes inhabit the human gastrointestinal tract and are known to influence metabolism and immunity. However, relatively little is known about how these organisms influence the behavior of their host. As gut bacteria participate in digestion, they alter the levels of macromolecules, such as carbohydrates, within the gut and produce neuromodulators as byproducts. Due to their participation in energy-availability within the host, we hypothesized that gut bacteria play a role in locomotion. We have found significant differences between the locomotive behavior of adult flies without a microbiota (axenic) and those with a full microbiota (conventional). Mono-colonization studies have revealed that specific bacteria and, in particular, a bacterially-derived molecule influences a fly's gait, speed, and activity levels. Through administering antibiotics and performing later colonization time points, we have also found that these effects are not due to changes during development. Neither mass, glucose, nor the IMD (Immune Deficiency) pathway has been found to mediate these effects on locomotion.

Our results reveal that the bacterial composition of the gut and its metabolic products are an important modulator of host locomotion. Locomotion is a behavior critical for the survival of an organism and is part of the progression of certain disease states. When combined with our results, this suggests that the gut microbiota is an important factor to consider in a variety of neural pathways and their behavioral outputs.

D1303C Ion pumps in the generation of scolopidial receptor lymph. *D. F. Eberl*, B. Zora, E. Sivan-Loukianova, M. Roy. Univ Iowa, Iowa City, IA.

Chordotonal mechanoreception in insect hearing relies on ciliated sensory neurons that express TRPN and TRPV ion channels. Under high frequency auditory stimulation, these channels may only open for short times, minimizing receptor current. This is counteracted by an elevated electrochemical gradient across the ciliary membrane, achieved by a specialized receptor lymph in the scolopale space enclosed by the scolopale cell. The receptor lymph is thought to be rich in K^+ and if it resembles the endolymph of mammalian cochlea, it may also be electro-positive. To test the roles of ion pumps and transporters in generating the receptor lymph in the *Drosophila* Johnston's organ, we expressed RNAi constructs that target a panel of ion pump and transporter genes specifically in the scolopale cells, using the *nompA*-Gal4 driver. We found that subunits of the Na/K-ATPase and the V-ATPase, primary ion pumps, are essential in the scolopale cell for hearing. Furthermore, we found evidence that some secondary transport mechanisms, such as in the cation chloride co-transporter family, which rely on the gradients generated by the primary transport mechanisms, also play a role in this cell. Our results establish a model for receptor lymph generation and help to understand the fundamental mechanisms of mechanotransduction.

D1304A Subfunctionalization and Neofunctionalization of Drosophila Odorant Binding Proteins. *J. A. Johnstun*[1,2], F. S. Haire[1,2], T. F. C. Mackay[1,2], R. R. H. Anholt[1,2]. 1) North Carolina State University, Raleigh, NC; 2) W. M. Keck Center for Behavioral Biology.

The functions of most Drosophila odorant binding proteins (Obps) remain unexplored, and many exist in tandem arrays throughout the genome. As these genes most likely arose through recent duplication, genes within a cluster likely have partially redundant or pleiotropic functions. Here, we used the CRISPR-Cas9 system to generate two knock-out lines, the first lacking the four paralogs of the *Obp56a-d* cluster, and the second lacking the single *Obp56h* gene, another possible paralog of the *Obp56* cluster. Various phenotypic tests on these knockout lines demonstrate significant functional overlap and novel pleiotropic functions. Both lines shared decreased viability in early development and development time, while the *Obp56a-d* KO line uniquely showed decreased height of pupation. The *Obp56h* KO line showed increased copulation duration and decreased aversion to 2-heptanone. Reinserting the *Obp56a-d* genes one-by-one and in various combinations in a PhiC31 integration site engineered in their original location during CRISPR-Cas9 excision will enable reconstruction of their functional evolutionary history. Supported by NIH grant GM059469.

D1305B Metabolite exchange within the microbiome influences *Drosophila* behavior. *C. N. Fischer*[1], E. Trautman[2], J. M. Crawford[2], E. V. Stabb[3], N. A. Broderick[4], J. Handelsman[1]. 1) Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, USA; 2) Department of Chemistry, Yale University, New Haven, CT, USA; 3) Department of Microbiology, University of Georgia, Athens, GA, USA; 4) Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT, USA.

Our understanding of the molecular mechanisms by which microorganisms affect animal behavior rests on single-microorganism studies. Yeasts affect *Drosophila melanogaster* olfactory behavior, yet it is unclear how bacteria or microbe-microbe interactions affect *Drosophila* behavior. The *Drosophila* microbiome consists of yeasts, acetic acid bacteria (AAB), and lactic acid bacteria (LAB), which co-occur in fruit and in the *Drosophila* intestinal tract. *Drosophila* appears to augment its resident microbiome through feeding, implicating *Drosophila* behavior in microbiome selection. Beneficial AAB and LAB emit acetic acid and lactic acid, respectively, yet acidity repels *Drosophila* via a dedicated olfactory circuit. One answer to this paradox is that *Drosophila* acquires bacterial microbiome members through a passive mechanism, whereas another posits that bacterial microbiome members actively attract *Drosophila* despite their acidity, either alone or in the context of communities. To distinguish these possibilities, we adapted a behavioral assay and measured *Drosophila* olfactory preference toward microbiome members grown individually and in communities. *Drosophila* prefers cultures of microbiome members grown in communities relative to cultures of the same microorganisms grown individually and then mixed, suggesting that *Drosophila* olfactory behavior is tuned to emergent microbe-microbe interactions. The conserved olfactory receptor *Or42b* partially mediated *Drosophila* preference for the two microorganisms grown together (yeast-AAB co-culture) to the same microorganisms grown individually and then mixed. *Drosophila* co-culture

preference correlated with three emergent properties: ethanol catabolism, a unique volatile profile, and yeast population decline. *Acetobacter* conversion of *Saccharomyces*-derived ethanol was necessary and acetic acid and its metabolic derivatives were sufficient for *Drosophila* co-culture preference, supporting the hypothesis that AAB actively attracts *Drosophila* as part of a microbial community. Additional chemical and behavioral analyses identified acetaldehyde metabolic derivatives as emergent microbial community metabolites that attract *Drosophila*. In sum, we discovered a molecular mechanism by which a multispecies community affects animal behavior. Our data support a model whereby emergent microbial metabolism cues *Drosophila* to associate with a diverse, metabolically beneficial microbiome.

D1306C ORN activity patterns in *Drosophila* larvae elicited by ecologically relevant odorants. *R. Kellermeyer*, D. Mathew. University of Nevada, Reno, Reno, NV.

Most insects locate their food source primarily through olfaction. In *Drosophila* larvae, attraction and repulsion to environmental odorants are based on the activity of only 21 olfactory receptor neurons (ORNs). While a considerable amount of information has been generated regarding the ORN responses of worms, flies, and mammals to odorants, much less is known about their role in driving behavioral output. This gap in knowledge prevents development of reliable odor coding models that can elucidate general principles of information processing, as well as instruct effective solutions for insect control. In this study, we examined the hypothesis that ecologically relevant attractive or repulsive odorants elicit specific patterns of ORN activity in the *Drosophila* larva. To measure this, a simple two-choice behavioral paradigm was used to test the behavioral response of wild type *Drosophila melanogaster* larvae to 54 odorants selected from its ecological habitat. Using this behavioral screen, a panel of 10 odorants that elicited the strongest attractive or repulsive responses in larvae was identified. This panel of odorants was then used to assess the response patterns among the 21 larval ORNs. For this, we expressed each larval odorant receptor in an in vivo expression system, the "empty neuron" system, and measured neural responses using single unit electrical recordings. At the test concentration, the panel of strong behavioral determinants elicit both excitatory and inhibitory responses from a variety of larval odor receptors expressed in the empty neuron system. Further, many of these receptor-odorant combinations exhibit varying response dynamics. Overall, our preliminary evidence suggests that ecologically relevant odorants elicit specific patterns of ORN activity. This study is significant because conserved patterns of sensory neuron activity may instruct downstream olfactory coding of behavioral valence. By comparing amplitude, temporal dynamics, and distribution of all 21 ORN responses, we aim to identify conserved patterns among sensory neuron activity elicited by attractants and repellents. The results from this study have the potential to impact development of more reliable odor coding models as well as to transform existing methods of insect control.

D1307A Receptor Basis of Serotonergic Modulation in an Olfactory Circuit. *T. R. Sizemore*, A. M. Dacks. West Virginia University, Morgantown, WV.

Animals must efficiently detect and process stimuli, then tune behavioral output according to dynamic physiological demands. To achieve this, the nervous system uses neuromodulators, such as serotonin (5-HT), to modify the excitability and synaptic efficacy of individual neurons based on the animal's current state. Neuromodulation, therefore, can result in changes in lateral interactions within the network and shape the direct output from the network. For instance, 5-HT enhances odor-evoked responses of projection neurons (PNs) and enhances pre-synaptic inhibition of olfactory receptor neurons (ORNs) by local interneurons (LNs) within the antennal lobe (AL) of *D. melanogaster*. However, the effect of a neuromodulator on an individual neuron is dictated by the cognate receptor-type expressed by that neuron. Therefore, to understand the mechanistic basis for neuromodulation, the functional identity of the neurons that express each neuromodulator receptor within a network must be determined. This will allow the separation of the effects of a neuromodulator on a single neuron from the consequences of modulating the input that a neuron receives. Therefore, we have used immunocytochemistry and T2A-GAL4 transgenic animals driving the expression of GFP to determine the neuron type within the AL of *D. melanogaster* that express the five 5-HT receptors (5-HTRs). The T2A-GAL4 lines act as a direct reporter for 5-HTR translation ("protein-trap") by decoupling GAL4 from the nascent 5-HTR mRNA during translation. Using immunocytochemistry, we determined the cell type (i.e. ORN, PN, LN, etc.) and transmitter content of each 5-HTR producing cell in the AL. Each 5-HT receptor are expressed by distinct functional populations of neurons suggesting that 5-HT targets distinct features of olfactory processing within the AL. In general, the inhibitory 5-HT receptors are expressed by inhibitory neurons including distinct subpopulations of LNs and GABAergic PNs, while the excitatory 5-HT receptors were expressed by ORNs and cholinergic PNs. This suggests that within the AL, the effects of 5-HT on olfactory processing are mediated by a combination of network-wide disinhibition and glomerulus specific enhancement. .

D1308B Mapping Chromatic Visual Pathways in *Drosophila*. *TY. Lin*[1,2], J. Luo[2], K. Shinomiya[3], CY. Ting[2], Z. Lu[3], I. Meinertzhagen[3], CH. Lee[2]. 1) National Defense Medical Center, Taipei, Taiwan; 2) NICHD, Bethesda, MD, USA; 3) Dalhousie University, Halifax, Canada.

In *Drosophila*, color vision and wavelength-selective behaviors are mediated by the compound eye's narrowspectrum photoreceptors R7 and R8 and their downstream medulla projection (Tm) neurons Tm5a, Tm5b, Tm5c, and Tm20 in the second optic neuropil or medulla. These chromatic Tm neurons project axons to a deeper optic neuropil, the lobula, which in insects has been implicated in processing and relaying color information to the central brain. The synaptic targets of the chromatic Tm neurons in the lobula are not known, however. Using a modified GFP reconstitution across synaptic partners (GRASP) method to probe connections between the chromatic Tm neurons and 28 known and novel types of lobula neurons, we identify anatomically the visual projection neurons LT11 and LC14 and the lobula intrinsic neurons Li3 and Li4 as synaptic targets of the chromatic Tm neurons. Single-cell GRASP analyses reveal that Li4 receives synaptic contacts from over 90% of all four types of chromatic Tm neurons, whereas LT11 is postsynaptic to the chromatic Tm neurons, with only modest selectivity and at a lower frequency and density. To visualize synaptic contacts at the ultrastructural level, we develop and apply a

"two-tag" double-labeling method to label LT11's dendrites and the mitochondria in Tm5c's presynaptic terminals. Serial electron microscopic reconstruction confirms that LT11 receives direct contacts from Tm5c. This method would be generally applicable to map the connections of large complex neurons in *Drosophila* and other animals.

D1309C Virtual Fly Brain 2.0 - The data integration hub for Drosophila neurobiology. *D. J. Osumi-Sutherland*[1], R. Court[2], M. Costa[3], N. Staudt[3], H. Parkinson[1], J. D. Armstrong[2], G. S. X. E. Jefferis[4], C. J. O'Kane[3]. 1) EMBL-EBI, Cambridge, UK; 2) University of Edinburgh, Edinburgh, UK; 3) University of Cambridge, Cambridge, UK; 4) MRC Laboratory of Molecular Biology (LMB), Cambridge, UK.

Advances in genetic techniques allow ever more accurate targeting of specific neurons with reagents that modulate or detect their activity. At the same time, advances in imaging technology and image processing are producing detailed maps of neuroanatomy down to the level of individual synaptic connections. As a result, there are now unprecedented opportunities to use the power of model organism genetics to functionally dissect neural circuits: inhibiting, activating or otherwise modulating the activity specific neurons and observing the effects on phenotype and circuit function. To take full advantage of these opportunities, researchers need simple, intuitive tools to search and visualize data integrated from multiple bulk datasets, identify candidate circuit elements, and find reagents to target specific neurons. These tools need to be integrated with systems for combining and viewing 3D images.

Virtual Fly Brain (VFB) fulfils this role, integrating data curated from the literature and from many bulk sources. Its search system allows users to search for neurons and neuro-anatomical structures using almost any name found in the literature. Its sophisticated query system allows users to identify neurons innervating any specified neuropil or fasciculating with any specified tract. It also allows users to query for genes, transgenes and phenotypes expressed in any brain region or neuron. Search and query results combine referenced textual descriptions with links to related structures and 3D images. VFB features tens of thousands of 3D images of neurons, clones and expression patterns, registered to a standard adult brain. Any combination of these images can be viewed together on our stack browser. A BLAST-type query system (NBLAST) allows users to find similar neurons and drivers starting from a registered neuron tracing.

We will present a new version of VFB featuring a 3D image browser and a new adult ventral nerve cord template with registered image data. We will also outline plans for the release of VFB 2.0 later this year. This will feature a fully interactive 3D browser, a new query interface with full integration of NBLAST and a standard larval brain. Drosophila neurobiologists have generated a particularly rich set of shared neuroanatomical data and genetic driver lines; our experiences with VFB should be very relevant for other model organism resources focussing on the nervous system.

D1310A Mechanism underlying inhibitory control. *E. Saldes*, Paul Sabandal, Youngcho Kim, John Sabandal, Kyung-An Han. The University of Texas at El Paso, El Paso, TX.

Mechanism underlying inhibitory control

Erick Saldes, Paul Sabandal, Youngcho Kim, John M. Sabandal, and Kyung-An Han

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The ability to suppress ongoing motor actions that are no longer appropriate is a fundamental feature of executive function supporting flexible and goal-oriented behaviors. This inhibitory control is known as response inhibition and has been studied in mammals but not in non-mammalian species. We have identified that the fruit fly *Drosophila* displays response inhibition. A go/no-go test is typically used to measure response inhibition in human subjects and requires subjects to produce a motor response when cued to do so (go) or otherwise withhold it (no-go). To study response inhibition in *Drosophila*, we developed a fly version of the go/no-go test and found dopamine as an important neuromodulator for inhibitory control. When subjected to a go/no-go test, the *fmn* mutants lacking dopamine transporter initially withheld movement upon exposure to salient stimuli but, within a minute, exhibited flying behavior. The *fmn* mutants have impaired sleep thus we examined additional sleep mutants Sh and HK. Similar to *fmn*, they also displayed impaired response inhibition. Studies are in progress to clarify the role of sleep in response inhibition and underlying mechanisms. Anomalous response inhibition is associated with numerous brain disorders but its underlying mechanisms are largely unknown. Our study may help fill the knowledge gap.

D1311B RNA-processing genes control sensory neuron function in *Drosophila melanogaster*. *A. D. Dyson*, C. Kawada, R. Stewart, M. Gaglianese-Woody, A. Bellemer. Appalachian State University, Boone, NC.

Chronic pain affects approximately 100 million Americans and generates costs of up to $600 billion per year, according to the Institute of Medicine. Characterization of molecular signaling pathways in sensory neurons is an important step toward development of more effective clinical interventions. The goal of this study is to identify genes involved in regulating the function of class IV multidendritic neurons, which are nociceptors integral to the detection of noxious thermal, mechanical, and photic stimuli. We are using Drosophila melanogaster larvae as a model system, which exhibit a distinct and quantifiable response to noxious stimuli termed nocifensive escape locomotion (NEL). Recent research has found that the transcripts of key genes necessary for nociceptor function are alternatively spliced. We have systematically knocked down putative RNA-processing genes with a previously identified role in dendrite development and/or alternative splicing by crossing a *ppk-GAL4;UAS-dicer2* fly strain with RNAi lines targeting genes of interest. Using *ppk-GAL4* as our driver limits expression to nociceptors and *dicer2* expression promotes increased efficacy of RNAi knockdown. We then tested progeny for changes in NEL latency relative to wild-type larvae using a thermal nociception assay. Nineteen genes were identified for which knockdown resulted in either a significant increase or decrease in response latency, indicating a potential defect. Follow-up assays for defects in mechanical nociception have demonstrated that select genes are required for both thermal and mechanical nociception. Additionally, our results suggest that behavioral defects do not always

correlate with defects in dendrite morphology. This study has also revealed a complex role for translation initiation factors, which will be investigated further using genetic and functional assays of multidendritic neuron structure and function.

D1312C Cellular and molecular dissection of noxious cold nociception in *Drosophila*. *A. A. Patel*[1], H. N. Turner[2], K. Armengol[3], N. J. Himmel[1], M. J. Galko[2], D. N. Cox[1]. 1) Georgia State University, Atlanta, GA; 2) University of Texas, MD Anderson Cancer Center, Houston, TX; 3) University of Maryland, College Park, MD.

Diverse organisms utilize thermoreceptors to detect and respond to noxious thermal stimuli, however the basic cellular and molecular mechanisms underlying noxious cold perception are not well understood. We developed novel global and local behavioral assays for genetic and cellular dissection of noxious cold responses in *Drosophila*. Larvae respond to near-freezing temperatures via a mutually exclusive set of singular behaviors– in particular full body contraction (CT). Class III (CIII) multidendritic (md) sensory neurons are specifically activated by cold and optogenetic activation of these neurons elicits cold-evoked behavior. Moreover, blocking synaptic transmission in CIII neurons inhibits CT behavior. Genetically, the transient receptor potential (TRP) channels Trpm*,* NompC*,* and Polycystic kidney disease 2 (Pkd2) are expressed in CIII neurons where each is required for CT behavior. We further demonstrate that these TRP channels are not generally required in the propagation of a cold signal, but rather likely play important roles in the initial cold sensing. In support of this, we show that overexpression of Pkd2 in normally cold-insensitive class IV md neurons confers cold sensitivity measured by *in vivo* cold evoked calcium responses. Thus CIII neurons are multimodal with roles in noxious cold detection and gentle touch mechanosensation. To clarify how these cell may distinguish between cold and gentle touch, we developed and implemented an optogenetic dose response assay revealing that gentle touch mediated head withdrawal behavior responses are due to low threshold activation of CIII neurons, whereas noxious cold evoked CT responses are due to high threshold CIII activation. To investigate how diverse nociceptive thermosensory stimuli are processed, we optogenetically co-activated CIII (cold) and CIV (heat) md sensory neurons. Interestingly, these studies revealed a dominant CT behavioral response with simultaneous activation of CIII and CIV neurons. Moreover, expression of the warm-activated TRPA1 channel in CIII neurons confers heat sensitivity, however, heat-mediated activation of these neurons leads to a majority of larvae exhibiting CT behavior and a dramatic declination in typical heat-induced rolling behavior. To initiate an investigation into cold nociceptive neural circuitry, we have conducted live, *in vivo* imaging of cold-evoked calcium responses throughout the larval ventral nerve cord (VNC) via GCaMP6 and CaMPARI imaging. These analyses reveal that noxious cold stimuli are transmitted down CIII axons and progress from a posterior-to-anterior direction within the VNC. Collectively, we demonstrate that *Drosophila* larvae utilize distinct cells and channels to respond to noxious cold temperatures via complex thermosensory and nociceptive circuits.

D1313A Extreme Quantitative Trait Locus Mapping for Ethanol Consumption in *Drosophila melanogaster*. *S. Fochler*[1,2], T. V. Morozova[1], T. F. C. Mackay[1], R. R. H. Anholt[1]. 1) Program in Genetics, W. M. Keck Center for Behavioral Biology, and Department of Biological Sciences North Carolina State University, Raleigh, NC, USA; 2) School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK.

Excessive alcohol consumption and alcoholism incur substantial socio-economic and health costs in our society. Identifying and disentangling the genetic and environmental factors that underlie alcohol consumption in human populations is challenging, due to genetic heterogeneity, uncontrolled environments and difficulty in precisely quantifying alcohol related phenotypes, as well as comorbidity with other addictive behaviors and psychiatric disorders. *Drosophila melanogaster* presents a powerful genetic model for exploring the genetic underpinnings of alcohol consumption because both the environment and genetic background can be controlled, alcohol consumption can be quantified precisely, and pathways associated with alcohol metabolism are evolutionarily conserved. We used an outbred Drosophila population which was generated by an advanced intercross of 37 sequenced lines of the *Drosophila melanogaster* Genetic Reference Panel, which are free of inversions, free of *Wolbachia* infection, are maximally homozygous and maximally unrelated. This advanced outbred population generates virtually unlimited numbers of unique individuals, which provides excellent statistical power for extreme QTL mapping (xQTL). We measured voluntary intake of 4% ethanol in an 8% sucrose solution using the Capillary Feeding (CAFÉ) assay. We scored 6000 males and 6000 females for alcohol consumption and obtained a distribution of ethanol intake, ranging from 0 to 3 ul per fly. We pooled three replicates of the 10% lowest and highest drinkers along with random control flies, sexes separately, for extreme QTL mapping, totaling 100 males and 100 females for each pool. We performed DNA sequencing on the extreme pools to identify differentially segregating alleles between the contrasting phenotypes. Construction of genetic networks of candidate genes and superposition of human orthologs on these networks will provide insights in the genetic architecture that underlies variation in alcohol consumption. .

D1314B Dynamics of ethanol preference in Drosophila. *A. Park*, N. Atkinson. University of Texas in Austin, Austin, TX.

Alcohol use disorder (AUD) imposes adaptations that perturb psychological and physiological characteristics of an individual that can contribute to the addicted state. Drosophila are favorably used to study alcohol-related behaviors due to their well-developed genetic toolkit, and shorter lifespans. However, previous researchers have struggled to quantify alcohol consumption in Drosophila due to their small size. In addition, the assays available currently cannot measure consumption in a temporally robust way, and rely on starvation to motivate the animals. We have developed a novel assay that measures the amount of consumed ethanol and can accurately track alcohol preference in a starvation independent paradigm. This new assay is temporally precise and better emulates natural feeding behaviors. Using this assay we have found male Canton S Drosophila naïve to ethanol are innately aversive to it as a food source (>5% ABV), however this aversion disappears when the flies are pretreated with ethanol. In addition, female Drosophila have innate attraction to ethanol (<15% ABV). Now, we are measuring ethanol preference in mutants that were selected through identifying genes that were epigenetically regulated during tolerance acquisition.

These mutants are not able to acquire functional tolerance to alcohol and testing their ethanol preference will determine if tolerance to alcohol exacerbates preference for drinking.

D1315C Aged Parents Have Less Social Offspring. *D. Brenman*, S. Long, A. Simon. University of Western Ontario, London, ON, CA.

Parental and grandparental age can alter the fitness of the progeny. For example, studies have found reduced fecundity, longevity, and memory in *Drosophila melanogaster* when parents were aged. These trans-generational effects can also be sex-biased, as aged female *Drosophila* appear to have a stronger negative influence on the longevity of their daughters, whereas aged males have some influence on the longevity of their sons.

Here we studied the effect of aging on neurodevelopment in the progeny in terms of changes to basic social behaviors such the social spacing between individuals and social avoidance of stressed flies. We also investigate the trans-generational effect on social behavior on progeny whose parents have been aged.

We have found that at older ages (30 days old, 90% survival and 50 days old, 50% survival) the distance between neighboring *Drosophila* increases, indicating that they are less social. Similarly, the social spacing between the progeny of aged *Drosophila* is also increased as compared to those with young parents. This suggests that the aging process contributes heritable change to the developing brain and therefore changes to social spacing. This also may indicate different aging mechanisms in germ cells versus somatic tissues resulting in a stronger effect in the progeny of aged parents than in the parents. Interestingly, the ability of individuals to avoid aversive stimuli is maintained throughout age and does not change the parents are aged, displaying maintenance of some social behaviors with age. We also show that it is enough to have an old father for the sons to be less social. We are currently investigating the effect of aged mothers as well.

We recapitulate the effect of old age of the parents on social spacing of their progeny by accelerating the aging process though increased metabolism (aging flies at 29C instead of the usual 25C) or via reactive oxygen species exposure (paraquat). In contrast, with delayed aging of the parents through caloric restriction, the progeny appeared more social.

Understanding the impact of aging is especially important as recently individuals with neuropsychiatric disorders like autism and schizophrenia have been linked to fathers over the age of 45. And although it is still unclear how exactly the aging process affects gametes leading to changes in neurodevelopment and thus specific social behaviors, the powerful model system *Drosophila* and its underused ability to display simple social interactions will allow us to identify the underlying mechanisms.

D1316A Elevation of Dube3a in glia, but not neurons, produces synaptic changes and susceptibility to seizure. *K. Hope*, L. Reiter. UTHSC, Memphis, TN.

Duplications of chromosomal region 15q11.2-q13.1 cause Duplication 15q (Dup15q) syndrome, characterized by a high incidence of autism spectrum disorder (ASD), seizures, and cognitive impairment. This duplication includes the *UBE3A* gene, and elevated levels of UBE3A in neurons are thought to be the primary cause of Dup15q syndrome. *UBE3A* overexpression in other cell types has been largely unexplored. Evidence from mouse models indicates that *UBE3A* is bi-allelically expressed in glial cells and maternally expressed in neurons. Duplications of the *UBE3A* locus should cause elevated UBE3A in glia as well as neurons. Here we elevated the fly homolog (*Dube3a*) in glial cells and evaluated Dup15q related phenotypes. We found that elevated *Dube3a* levels in glia results in a robust seizure susceptibility phenotype absent in animals overexpressing *Dube3a* in neurons. To identify changes in gene expression, we performed RNAseq and identified 497 differentially expressed transcripts in *elav>Dube3a* animals (neuronal expression) and 1,222 differentially expressed transcripts in *Repo>Dube3a* animals (glial expression). 854 transcripts were differentially expressed in glia compared to neurons and showed significant enrichment in synaptic transmission, substrate specific channels, and neurotransmitter synthesis. Transcript expression of the presynaptic active zone protein *bruchpilot* was reduced by 2-fold in glial versus neuronal overexpression of *Dube3a*, and a similar reduction was observed at the protein level. Interestingly we saw no change in the neuronal marker elav in the brain, indicating that the reduced expression of presynaptic proteins is not due to a loss of neurons. Furthermore, a reduction of on/off transients was observed in electroretinograms of flies overexpressing *Dube3a* in glia, indicating impaired synaptic transmission. These results demonstrate that elevated levels of Dube3a in glial cells drive changes in key neuronal functions that may underlie Dup15q seizure phenotypes. We observe a similar seizure susceptibility phenotype when human *UBE3A* is overexpressed in fly glial cells, potentially indicating a conserved mechanism between fly and human *UBE3A* in seizure production. Currently we are working to identify the causal genes mis-regulated in glia and the nature of impairment between glia and neurons that result in seizure susceptibility due to glial *Dube3a* overexpression. These studies may lead to the identification of new genes and pathways for novel epilepsy treatments in Dup15q syndrome..

D1317B Social Isolation Induced Depressive Like Behavior in *Drosophila Melanogaster*. *D. Hu*, L. Liu. Peking University, Beijing, China.

Major Depressive Disorder (MDD) is an idiopathic neuropsychiatric disorder that is highly prevalent. The exact cause and mechanism of the illness are still unclear. To help investigate MDD, we proposed utilizing *Drosophila Melanogaster* as the model organism. In order to induce depressive like behavior, a chronic stress has to be used. When social isolation was used as a stressor, wild type fruit flies exhibited locomotor hyperactivity when compared with control. Similar behavior was observed when mice was given chronic unpredictable mild stress (CUMS). The locomotor hyperactivity was found to be dependent on physical interaction among the flies. It was also found that social isolation reduced the brain serotonin content in the flies, which is concurrent with the monoamine hypothesis. To confirm this possibility, we fed the flies with serotonin or monoamine based antidepressants and found these drugs could reverse the social isolation induced locomotor hyperactivity. Since neurogenesis is theorized to be the mechanism behind MDD, we tested this possibility by performing real time PCR on related genes, such as CREB. It turned out that socially isolated flies had lower CREB expression in the brain versus socially housed flies. Epigenetics were

theorized as a possible mechanism that suppressed CREB expression. To test this, we fed several HDACi and DNMTi drugs to the flies. Certain epigenetic inhibitors were indeed capable of reversing the locomotor hyperactivity. These data suggest that Drosophila Melanogaster can be utilized in the research of MDD, and that epigenetics is causatively related to MDD. Further research into this model shows promise in the hope of procuring a treatment for MDD.

D1318C Identifying new modulators of blood cell development using *Drosophila* as a low complexity model of human myeloproliferative neoplasms. *Alessandro Bailetti*, Abigail Anderson, Elizabeth Rodkin, Erika Bach. New York University School of Medicine, New York, NY.

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders that cause over-proliferation of specific myeloid lineages. More than 95% of patients with polycythemia vera, one type of MPN, have a point mutation in the *JAK2* gene ($JAK2^{V617F}$), which generates a constitutively-active JAK2 that hyperactives the JAK/STAT pathway. In *Drosophila,* JAK/STAT signaling is conserved but simplified, with a single JAK Hopscotch (Hop) and a single STAT (Stat92E). $hop^{Tum\text{-}l}$ is a dominant, temperature-sensitive allele of *hop* that hyperactivates the JAK/STAT pathway. Similar to $JAK2^{V617F}$-bearing MPN patients, $hop^{Tum\text{-}l}$ mutants have dramatically increased number of myeloid-like cells, which leads to melanotic tumors. The $hop^{Tum\text{-}l}$ tumor phenotype is dependent on JAK/STAT signaling, and reducing one copy of *Stat92E* in $hop^{Tum\text{-}l}$ flies strongly suppresses the tumor burden. Based on this dominant modification of the tumor burden by reduction in *Stat92E*, we hypothesize that reducing the dose of genes involved in JAK/STAT signaling or in hematopoiesis will enhance or suppress the tumor incidence or burden in $hop^{Tum\text{-}l}$ flies. Given the conservation of the pathway, our screen may identify genes that modify the $hop^{Tum\text{-}l}$ phenotype and that have human homologs which may be involved in MPNs.

We conducted a dominant deficiency screen of the right arm of the second chromosome. We tested 90 deficiencies in the Bloomington kit, which together uncovers 98.5% of 2R euchromatin. We have identified 11 enhancer and 8 suppresser deficiencies. A deficiency that uncovers *Enhancer of Polycomb [E(Pc)]* significantly enhances melanotic tumor burden and larval/pupal lethality in $hop^{Tum\text{-}l}$ animals. This enhancement was recapitulated by heterozygosity for *E(Pc)* alleles. To test if *E(Pc)* has a role in blood development, we depleted *E(Pc)* from hematopoeitic tissue. *E(Pc)* depletion promoted differentiation of lamellocytes, which are absent in control animals. Additionally, larval hemolymph bleeds of *E(Pc)* depleted animals revealed microtumors and small melanotic tumors, that are not present in in control larvae. E(Pc) is a highly conserved protein and regulates transcription through the Polycomb Group (PcG) factors. Recently, E(Pc) also was shown to function as part of the Tip60 complex, a chromatin remodeling complex. To further characterize the *E(Pc)* phenotype, we are first assessing if heterozygosity for PcG and Tip60 complex genes also enhance the tumor burden in $hop^{Tum\text{-}l}$ animals. Second, we are hematopoietically depleting PcG or Tip60 complex genes in control larvae to assess if their depletion also leads to blood cell abnormalities. Our current data support the model that E(Pc) represses genes necessary for lamellocyte differentiation.

D1319A Genetic analysis of invasive pathways engaged by the EcR-coactivator protein Taiman. *P. K. Byun*, K. H. Moberg. Emory University, Atlanta, GA.

The transformation of non-motile epithelial cells to a migratory state plays a significant role in normal development and diseases such as cancer. During *Drosophila* oogenesis, a specialized group of cells termed border cells (BCs) acquire the ability to detach from their host epithelium and migrate through surrounding cells to the posterior end of the oocyte. The steroid receptor transcriptional co-activator *taiman* (*tai*) plays an important role in promoting this motility process, but its downstream transcriptional targets remain poorly defined. Here we introduce a novel, pathogenic model of Tai-driven tissue invasion that allows for rapid genetic screening for elements of the Tai-induced transcriptional program. Overexpression of *tai* in non-motile pupal wing cells causes these cells to invade through adjacent thoracic cuticle and into internal tissues, leading to a high-penetrance adult phenotype of wing tips embedded into the thorax. Using this phenotype as the basis for a dominant-modifier screen led to identification of alleles of genes in the Hippo pathway as modulators of Tai-driven tissue invasion. Our published work shows that Tai and Yki (the downstream effector of the Hippo pathway) bind together and co-regulate target genes (Zhang et al, 2015). In support of this model, a version of Tai that cannot bind to Yki also cannot efficiently drive invasion of wing cells into the thorax. Genetic and transcriptomic analysis has identified candidate Tai-target genes involved in promoting wing cell invasiveness, including the extracellular serine protease *gastrulation defective* (*gd*) and the actin-remodeling complex protein *Arpc3B*. Data on *gd* and *Arpc3B* as putative transcriptional targets of Tai and Yki, and the roles of the Gd and Arpc3B proteins in driving the invasive process will be presented.

D1320B Understanding the mechanism of RIOK2 function in Glioblastoma. *A. S. Chen*, R. D. Read. Emory University, Atlanta, GA.

Glioblastoma multiforme (GBM), a tumor derived from glia and glial progenitor cells, is the most aggressive and prevalent form of primary brain cancer and is incurable. Amplification, mutation, and/or overexpression of the EGFR receptor tyrosine kinase and activating mutations in components of the PI3K pathway are common in GBM tumors, although the pathways that act downstream of EGFR and PI3K to drive tumorigenesis remain poorly understood. To better understand the underlying biology of tumorigenesis, we use a *Drosophila melanogaster* GBM model in which malignant neoplastic tumors arise from glial progenitor cells overexpressing activated oncogenic versions of EGFR and PI3K. This *Drosophila* model was used in kinome-wide genetic screens that identified Right-Open-Reading-Frame-2 (RIOK2), an atypical serine-threonine kinase, as a possible driver of EGFR-PI3K-dependent GBM. Subsequent studies in patient-derived GBM cell cultures suggest that RIOK2 promotes GBM cell proliferation and survival in response to oncogenic EGFR and PI3K signaling, and that, interestingly, these effects are dependent on RIOK2 catalytic kinase activity. Our data strongly implicate RIOK2 in driving tumorgenesis; however, little is understood about RIOK2 function or downstream targets. Preliminary immunoprecipitation experiments of RIOK2 from patient-derived GBM cell cultures coupled with proteomics identified several RIOK2 binding proteins and potential substrates. We conducted subsequent experiments in *Drosophila* since *Drosophila* possess orthologs for all of the RIOK2 binding proteins, and the functions of *Drosophila* orthologs of many of these proteins are

evolutionary conserved. Using our *Drosophila* GBM model, we conducted a blind screen of all of our candidate RIOK2 substrates, and we discovered that RNAi against several mRNA-binding proteins drastically reduced aberrant glial cell proliferation and invasion similar to RIOK2 knock-down. These RNA binding proteins are involved in stabilizing and promoting the translation of their target mRNAs, several of which are known drivers of GBM that are overexpressed in response to oncogenic EGFR and PI3K signaling. Preliminary analysis of human GBM cells showed that, upon RIOK2 knockdown, protein levels were reduced for several of these target mRNAs. Based on our preliminary results all together, we hypothesize that RIOK2 drives tumorigenesis by modulating the activity of RNA-binding proteins involved in stabilizing and promoting the translation of their target mRNAs, and that this promotes the translation of target mRNAs that drive tumor cell proliferation and survival. Our study uses *Drosophila* as a model organism to better understand the mechanisms of GBM tumorigenesis and to gain insight into the biology of novel pathways that drive GBM.

D1321C An RNAi-mediated genetic screen identifies genes that promote tumour progression in a living epithelium. *Z. Cornhill*, A. Couto, N. Mack, B. Canales Coutino, U. Nagarajan, M. Georgiou. University of Nottingham, Nottingham, GB.

The complex process by which cancer cells invade local tissue and metastasise is responsible for approximately 90% of cancer related deaths. The cell biological events that underlie this transition to malignancy are driven by invariable alterations within the genome, however relatively little is known about the genetic determinants involved. If identified, novel genes which perturb the rate of tumour progression could become potential targets for future therapeutic intervention.

Using a novel in vivo system, it is possible to characterise the behaviour of transformed cells during the early stages of tumour development and follow these cells in real time, thus improving our understanding of the critical events that initiate cell proliferation, tumour cell invasion and metastasis. Using *Drosophila* as a model organism it is possible to generate neoplastic tumours within the dorsal thorax whereby clones of transformed cells are homozygous mutant for a specific tumour suppressor gene. By specifically labelling these transformed cells with GFP, their behaviour can be observed in high temporal and spatial resolution within the living epithelium. RNAi technology can also be employed to simultaneously knock-down expression of an additional gene specifically within the mutant tissue. This forms the basis of a large-scale screen for novel genes that may promote tumour progression in this epithelium.

So far we have screened through more than 400 genes, the majority of which have previously been implicated in cancer but remain uncharacterised. We have observed a wide range of phenotypes, with genes affecting cell proliferation, invasion, cell shape, actin organisation, junction integrity and epithelial multilayering. By setting 'thresholds' for particular phenotypes 'hits' have been identified which drastically enhance tumour progression, and these genes are in the process of being fully characterised to further our understanding of their role in tumour progression.

D1322A Genetic and mathematical dissection of tumor heterogeneity that triggers cancer progression. *M. Enomoto*[1], H. Naoki[2], D. Takemoto[1], T. Igaki[1,3]. 1) Lab. of Genet., Grad. Sch. of Bio., Kyoto Univ., Kyoto, Japan; 2) Imaging Plat. for Spat.-Temp. Info., Grad. Sch. of Med., Kyoto Univ., Kyoto, Japan; 3) PRESTO, JST.

Tumor heterogeneity plays an important role in cancer progression. However, the mechanisms of how clonal diversity is generated within the tissue and how such diversity causes tumor progression are poorly understood. Here, we found in *Drosophila* eye imaginal disc that oncogenic cell clones expressing Ras^{V12} or Src normally result in benign tumors but both develop into metastatic tumors when interact with each other. We found that Notch is upregulated in Src-activated clones while the Notch ligand Delta is upregulated in Ras-activated clones, leading to trans-activation of Notch signaling in Src-activated cells nearby Ras clones. Elevated Notch signaling in Src-activated cells causes tumor invasion cell-autonomously, while at the same time induces metastatic invasion of neighboring Ras-activated cells *via* upregulation of secreted growth factor Upd. Furthermore, we developed a mathematical model for cell population dynamics in the epithelium, in which Ras- and Src-activated clones exhibit proliferation and apoptosis depending on intercellular signaling between them. Through the simulation, we found that inter-clonal cooperation of Ras- and Src-activated tumors leads to expansion and persist activation of Notch and JAK-STAT signaling throughout the tissue, which generates highly heterogeneous cell populations with distinct oncogenic activities. This heterogeneity could cause symbiotic malignant tumors observed in the *Drosophila* imaginal discs.

D1323B Modeling the effects of the *Helicobacter pylori* virulence protein CagA on induced pathogenesis in *Drosophila*. *Tiffani A. Jones*, Karen Guillemin. University of Oregon, Eugene, OR.

Healthy gut microbiota, the complex consortia of resident microbes colonizing the human GI tract, influence aspects of health and normal development. An altered, or dysbiotic, microbial community can promote pathologic inflammation or hyperplasia, which can lead to a variety of diseases including cancer. Cancer-causing pathogens, such as *Helicobacter pylori,* can be associated with dysbiotic microbiota; however, whether dysbiotic communities contribute to *H. pylori* pathogenesis is unknown. Currently, many aspects of *H. pylori*-associated pathology are thought to be due to the action of the virulence protein CagA. CagA is translocated into host epithelial cells where it mimics a mammalian scaffold protein, alters cellular polarity, and manipulates host-signaling pathways that promote cell proliferation. We hypothesize that CagA also contributes to *H. pylori* pathogenesis by inducing microbial dysbiosis that contributes to cell proliferation and altered immune signaling.

Current animal models of *H. pylori* pathogenesis rely on infection with CagA positive strains to investigate the effects of CagA on host cell proliferation and disease progression. These models are limited because they cannot accurately disentangle the effects of the virulence protein from that of *H. pylori* or other microbes in the community. Using a transgenic *Drosophila* model of CagA expression within the gut epithelium we can genetically dissect the effects of CagA on the host epithelium from that of the pathogen or other microbial contributions. We have found that expression of CagA within host intestinal stem cells causes high rates of cell proliferation in the midgut epithelium. Our transgenic

model also revealed that the gut microbiota promotes some of the pathological proliferation, as flies derived germ free have lower levels of cell proliferation than conventionally reared flies. Notably, the gut microbiota in CagA transgenic flies differs from that of wild-type flies, such that the microbiota is sufficient to induce both overexpression of an anti-microbial peptide and cell proliferation in wild-type flies. This demonstrates the pro-proliferative capacity of the CagA transgenic fly microbiota.

This model of CagA expression establishes that a bacterial virulence factor alters the gut microbial community, which exacerbates cell proliferation and immune phenotypes previously associated with *H. pylori* infection. This reveals a previously unrecognized role for the surrounding gut microbes in *H. pylori* pathogenesis, which provides valuable new insights into the mechanisms by which interactions between a pathogen, its host, and the microbiota contribute to disease.

D1324C Novel inhibitor of cdk5 signaling axis suppresses self-renewal properties of glioblastoma stem cells and induces apoptosis. *Subhas Mukherjee*[1], Monica Chau[1], Carol Tucker-Burden[1], Changming Zhang[1], Jun Kong[2], Renee Read[1], Daniel Brat[1]. 1) Emory University School of Medicine, Atlanta, GA; 2) Emory University, Atlanta, GA.

Cancer stem cells exert enormous influence on neoplastic behavior, in part by governing asymmetric cell division and the balance between self-renewal and multipotent differentiation. Growth is favored by deregulated stem cell division, which enhances the self-renewing population and diminishes the differentiation program. Mutation of a single gene *Brain Tumor (brat)* in *Drosophila*, leads to disrupted asymmetric cell division resulting in dramatic neoplastic proliferation of neuroblasts. To uncover mechanisms relevant to deregulated cell division in human glioma stem cells, we developed a novel adult *Drosophila* brain tumor model in which *brat-RNAi* is driven by the neuroblast specific promoter inscuteable. Suppressing Brat in this population led to accumulation of actively proliferating neuroblasts and a lethal brain tumor phenotype. *brat-RNAi* also caused *Drosophila* eye-overgrowth when driven by the eye-specific promoter *ey-GAL4*, providing a valuable screening phenotype. Cdk5 signaling is critical for neurogenesis and has been found to be hyperactive in neurodegenerative diseases. Paradoxically, many cancers also show abnormal activation of cdk5 signaling that drives uncontrolled proliferation, suggesting a unique therapeutic strategy where suppressing CDK5 can kill cancer cells and save dying neurons at the same time. In a screen of kinases that might oppose the actions of *brat* mutation on stem cell proliferation, we found that genetic suppression of Cdk5 in *Drosophila* partially reversed the eye over growth phenotype following Brat knock down. There is almost 79% identity between *Drosophila* Cdk5 and human CDK5. Additionally, IDH1 wild type glioblastomas in human show significant upregulation of *CDK5* mRNA compared to its lower grade glioma counterparts. We translated our findings from *Drosophila* to patient-derived glioblastoma neurosphere cultures and *in vivo* xenograft tumors in mice, where we further demonstrated that a novel pharmaceutical suppressor of cdk5 signaling axis was capable of suppressing self-renewal properties and glioma stem cell viability by inducing apoptosis. Together, our results demonstrate a previously unappreciated role of the cdk5 signaling in glioma stem cell maintenance and survival and that suppressing cdk5 could be a novel therapeutic approach to specifically eliminate glioma stem cells.

D1325A Investigating novel roles for HELQ and BLM helicases in *Drosophila melanogaster*. *B. T. Sands-Marcinkowski*, M. McVey. Tufts University, Medford, MA.

In *Drosophila melanogaster*, the conserved 3' → 5' DNA helicases Bloom (BLM) and HELQ (DmMus301) have important roles in the maintenance of genomic stability, specifically in the repair of DNA double-strand breaks during homologous recombination. The Bloom helicase has both Holliday Junction dissolvase and D-loop/Rad51 filament disruptase activity, which both promote genomic stability by suppressing promiscuous crossover events and inappropriate strand invasion (recombination between regions of homeology, etc). DmMus301 mutants are deficient in both meiotic and mitotic break repair, and its human orthologue, HELQ, is involved in recombinational repair of double strand breaks. In this study, a synthetic larval-stage lethality between *blm* and *mus301* mutants was identified and analyzed. A *mus301* mutant lacking the helicase domain was combined with two different *blm* mutant alleles. The blm^{N1} mutant consists of a deletion which interrupts the helicase domain; the blm^{N2} mutant has a smaller deletion within the second exon, and does not interrupt the helicase domain. Crosses generating blm^{N1}*, mus301* and blm^{N2}*, mus301* double mutants produced both viable and synthetically lethal double mutant isolates for each combination of alleles. Interestingly, the timing of the lethality occurred at an earlier larval stage in the blm^{N2}*, mus301* double mutants, consistent with a dominant negative effect with the helicase-possessing BLM protein. We hypothesize that a third mutation on the *mus301* chromosome is responsible for the synthetic lethality and are currently using whole genome sequencing to identify this mutation. Taken together, our data suggest there are novel roles for the BLM and HELQ helicases outside of their functions in homologous recombination repair. Furthermore, the identification of a third mutation which causes the observed larval lethality in *blm, mus301* mutants will provide additional insight about their roles in maintaining genomic integrity.

D1326B A novel genetic screen in *Drosophila* designed to discover secreted factors that drive glioblastoma initiation and progression. *N. N. Shah*, R. D. Read, C. C. Rowe. Emory University, Atlanta, GA.

Glioblastomas (GBMs) are highly proliferative, invasive primary brain tumors derived from glia and glial progenitor cells, and are incurable with current treatments. GBMs often possess mutations that activate epidermal growth factor receptor tyrosine kinase (EGFR) and Pi-3 kinase (PI3K) signaling pathways. Studies show that additional factors and mechanisms in the tumor and the tumor microenvironment cooperate with EFGR and PI3K mutations to drive tumorigenesis, although the identity of these factors remains unclear. To address this problem, we sought to identify proteins that, when overexpressed and secreted into the tumor microenvironment, contribute to tumor initiation and progression in glial cells transformed by constitutive co-activation of EGFR and PI3K signaling. To test the activity of secreted factors on GBM initiation and progression, we used a GBM model in the fruit fly *Drosophila melanogaster* in which co-activation of EGFR and PI3K drives neoplastic transformation of glial progenitor cells. In the *Drosophila* GBM model, tumorous glia arise *in vivo* in the central nervous system in live

Drosophila larvae, and create large easily visualized tumors that kill their hosts in 5-6 days. Thus, this GBM model system allows us to view the effects of overexpressed secreted factors on tumorigenesis in an intact brain microenvironment. Using this *Drosophila* GBM model, we performed an enhancer/suppressor screen wherein individual overexpression constructs for nearly every secreted and transmembrane gene in the *Drosophila* genome were tested using the *Gal4-UAS* system for their phenotypic effects on tumor growth, tumor progression, over-all brain size, and host survival. Thus, both the cell-autonomous and cell-nonautonomous effects of the tested secreted factors were phenotypically assessed in our screen. From this screen, we identified several genes as candidate enhancers. Using immunohistochemistry and fluorescent microscopy to stain and image whole dissected brains from *Drosophila* larvae, we analyzed the phenotypes caused by overexpression of the candidate enhancer genes on neoplastic glia transformed by co-activation of EGFR and PI3K. The results of our screen and the phenotypes of candidate enhancers will be presented. Our findings could reveal novel genes that, if evolutionarily conserved in humans, may drive GBM initiation and progression in the brain.

D1327C Investigating the role of inflammatory cytokines on tumor progression and metastasis in a *Drosophila* cancer model. *K. SNIGDHA*, I. WAGHMARE, A. SINGH, M. KANGO-SINGH. UNIVERSITY OF DAYTON, DAYTON, OH.

Cancer cells are surrounded by the Tumor microenvironment (TME) - a unique milieu generated by the interactions between the normal cells surrounding the tumor cells. The TME supports the survival and proliferation of tumors. Current models suggest that cancer cells induce inflammation, and the TME responds by activation of an anti-inflammatory response. The core inflammatory pathways like Jun N-terminal Kinase (JNK), Tumor Necrosis Factor (TNF), Toll-like Receptor (TLR) and Immune Deficiency pathway (IMD) are conserved in *Drosophila* and likely participate in an evolutionarily conserved interaction amongst the TME and cancer cells. However, little is known about the molecular mechanisms underlying this interaction, and it is unclear if the inflammatory response is required for maintenance and progression of the tumor. We co-activated oncogenic *Yki* activity in *scribble* mutant epithelial cells by “flp-out” or GAL4 mediated drivers to model aggressively growing tumors in *Drosophila* epithelia. Our preliminary data showed that the key inflammatory pathway like TNF is induced in the neighboring normal cells whereas TLR and JNK pathways are upregulated in tumor cells. Using genetic interaction and epistasis approaches we have analyzed the effect of modulating levels of TLR and inflammatory cytokines on tumor growth and progression. Our research will help understand the interactions between inflammatory pathways and tumor progression in an *in vivo* model, and if targeting cytokine production in the TME or cancer cells is a useful strategy to suppress the growth and spread of cancer.

D1328A Development of new colon cancer models in *Drosophila* by a targeted genetic screen of cancer pathways. *N. Underwood*, J. Farrar, J. Ahlander. Northeastern State University, TAHLEQUAH, OK.

Drosophila melanogaster is emerging as a useful system to model cancer. Using a luciferase reporter gene, tumor growth in *Drosophila* gut tissue in response to oncogene activation can be quantified. Our goal is to develop new *Drosophila* models of colon cancer. We performed a targeted screen of genes in cancer pathways to identify candidates that show excessive tissue growth when expressed in adult gut tissue. Cancer genes affecting the PI3 kinase and receptor tyrosine kinase signaling pathways produced significant tissue growth relative to negative controls. Genes affecting G1 and G1/S phases of the cell cycle also produced a significant increase in tissue growth, whereas genes affecting G2 and M phases had little effect. Candidate genes from this study will be used for future experiments to understand why cells transformed by one cancer pathway or another may respond differently to chemotherapies.

D1329B Hipk promotes tumorigenesis through JAK/STAT signaling. *E. M. Verheyen*, J. A. Blaquiere, N. B. Wray. Simon Fraser Univ, Burnaby, BC, CA.

Signal transduction pathways are crucial for coordinated development and growth of multicellular organisms. Dysregulation and mutations of components in these pathways can often lead to tumourigenesis. The evolutionarily conserved Homeodomain-Interacting-Protein-Kinase (Hipk) is a potent growth regulator and elevated levels of Hipk in Drosophila lead to tumour-like masses resembling those found with activated JAK/STAT signaling. A point mutation similar to those seen in human blood cancers in the Drosophila Janus kinase (called hop) causes constitutive activation of the JAK/STAT pathway and results in blood cell tumours in larval and adult stages. We investigated whether Hipk causes tumours through JAK/STAT. First we show that elevated Hipk in blood cells phenocopies effects seen with the hyperactive form of Hop. Furthermore, Hipk induces enhanced proliferation of hemocytes. We find that reduction of Hipk can suppress the tumorigenic effects of activated Hop. RNAi against Hipk in hemocytes can suppress both the timing of lethality and the tumour load in activated hop flies. Furthermore, we find that Hipk is required for endogenous JAK/STAT pathway activity, since homozygous mutant tissue shows markedly reduced expression of a STAT reporter. To investigate the mechanism underlying this interaction, we performed a proximity ligation assay (PLA) between Hipk and STAT92E, the Drosophila STAT. Cells co-expressing Hipk and STAT92E show a robust PLA signal indicating an interaction. We are currently confirming this through in vitro and in vivo binding studies. Our work shows that Hipk is required for JAK/STAT signaling during normal development and in fly blood cancer.

D1330C A Wingless dependent Jun kinase-Yorkie signal amplification loop promotes tumor growth in *Drosophila* epithelial tumor models. *I. Waghmare*[1], A. Singh[1,2,3], M. Kango-Singh[1,2,3]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Programs, University of Dayton, Dayton OH; 3) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH.

Cell-cell signaling interactions are of immense significance to both normal, and tumor cells. Alterations in these signaling interactions cause critical changes in cell behavior, and may underlie diseases like cancer where cells are capable of unrestrained proliferation. Studies in *Drosophila* imaginal discs have identified key cell-cell interactions like cell competition, and key molecular signals (Wingless, Dronc, Jun N-terminal Kinase, Yorkie) that regulate these intercellular interactions. These signals also play important roles during compensatory proliferation- a response that cells employ to restore tissue homeostasis. However, if these intercellular interactions are involved in the aggressive growth of cancers remains poorly understood. Using the well-established *in-vivo* clonal models in flies, we found that Ras^{V12}, $scrib^{-}$ cells behave like 'supercompetitor winner cells'. Furthermore, Wg, Dronc, JNK, and Yki are all upregulated in Ras^{V12}, $scrib^{-}$ cells, and downregulation of these signals leads to reduction in tumor growth suggesting that these signals are required for the growth of Ras^{V12}, $scrib^{-}$ tumors. We identified that Yki, and JNK activities are simultaneously induced in Ras^{V12}, $scrib^{-}$ cells, and JNK-Yki form a self-reinforcing positive feedback loop downstream of Wg and Dronc that plays a key role in promoting tumorigenesis in Ras^{V12}, $scrib^{-}$ cells. The signal amplification loop is important for aggressive tumor growth, as activation of this loop does not occur in instances where oncogenes are individually activated or when apical-basal polarity is lost. We have thus identified a novel molecular network in which Wg dependent activation of Dronc controls a JNK-Yki mediated positive feedback signal amplification loop that promotes tumor growth. Our findings are important because tumor cell specific molecular networks may generate key insights into signaling interactions during oncogenic cooperation, and therefore provide a powerful model for reconstructing key biologically meaningful changes in signaling pathways that drive growth, and altered signaling in cancer cells in flies and humans.

D1331A Diet rescues lethality in a model of a human deglycosylation disorder. *Clement Y. Chow*, Katie G. Owings. University of Utah, Salt Lake City, UT.

Autosomal recessive loss-of-function mutations in N-Glycanase 1 (*NGLY1*) cause *NGLY1* deficiency, the only known human disease of deglycosylation. Patients with *NGLY1* deficiency display developmental delay, movement disorder, hypotonia, and alacrima. NGLY1 is a conserved component of the endoplasmic reticulum associated degradation (ERAD) pathway. ERAD is responsible for degrading misfolded proteins that accumulate in the ER lumen under ER stress conditions. NGLY1 deglycosylates misfolded proteins in the cytoplasm as they are translocated from the ER lumen for degradation. While little is known about the pathogenesis underlying *NGLY1* deficiency, it is thought that loss of NGLY1 activity results in accumulation of highly N-glycosylated misfolded proteins in the cytoplasm, acting as a 'sink' for free UDP-GlcNAc. In turn, this might deplete the circulating pool of free UDP-GlcNAc in the cell, resulting in disease. We hypothesized that restoring the levels of UDP-GlcNAc in the cells might rescue some of the phenotypes associated with *NGLY1* deficiency. We used ubiquitous RNAi knockdown of *Pngl* (*Drosophila* ortholog of *NGLY1*) to model complete loss of NGLY1 activity seen in human patients. We show that supplementing the normal diet with GlcNAc can rescue lethality associated with loss of Pngl activity. When raised on normal food, without GlcNAc supplementation, we observed significant lethality, with eclosion of only 18% of expected *Pngl* knockdown adults. *Pngl* knockdown lethality occurs throughout larval and pupal development. When diet is supplemented with 100 ug/ml of GlcNAc, we observed significant rescue of the developmental lethality, raising the adult *Pngl* knockdown eclosion rate to nearly 70%. We also demonstrate that genetic alterations in the ERAD and cytoplasmic heat shock pathways can influence the lethality of *Pngl* knockdown. Finally, we provide evidence that GlcNAc rescue of lethality likely functions through the ERAD and cytoplasmic heat shock pathways. These data provide a plausible pathophysiology for *NGLY1* deficiency. More importantly, our study points to a potential therapy through a simple diet supplement.

D1332B Neuronal *Nhe3* (Solute Carrier Family 9, Subfamily A) depletion causes neurodevelopmental and behavioral defects in *Drosophila*. *Monique van der Voet*, Benjamin Harich, Michaela Fenckova, Barbara Franke, Annette Schenck. Radboud university medical center, Donders Institute for Brain, Cognition and Behaviour, Nijmegen, NL.

Members of the solute carrier family 9 (SLC9A1–9) are connected to a spectrum of neurodevelopmental disorders. Mutations in SLC9A6 cause syndromic X-linked intellectual disability Christianson-type, with microcephaly, epilepsy, and ataxia, and shows phenotypic overlap with Angelman syndrome. Mutations in SLC9A9 causes autism with seizures, and variants in the gene are associated with Attention Deficit Hyperactivity Disorder-related deficits.

The *Drosophila* homolog of human *SLC9A6* and *SLC9A9* is *Nhe3*. The protein codes for a Na^{+}/H^{+} hydrogen exchanger that is highly expressed in the brain. We have dissected *Drosophila* larvae to study the structure of synapses at the neuromuscular junction and dendritic branching of the class IV dorsal dendritic arborization C (ddaC) neurons. In addition we have performed behavioral characterization in adults, namely habituation and locomotor activity profiling.

We observe strong neurodevelopmental defects and behavioral aberrations, consistent with the neurodevelopmental defects observed in humans. We want to exploit this *Drosophila* model of early onset cognitive disorders to gain mechanistic insight in the neuronal defects and development clinical applications[1].

Relevant review: [1]van der Voet M, Nijhof B, Oortveld MA, Schenck A. *Drosophila* models of early onset cognitive disorders and their clinical applications. Neurosci Biobehav Rev. 2014 doi: 10.1016/j.neubiorev.2014.01.013.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

D1333C Functional screen of autism candidate genes using *Drosophila* studies *in vivo*. *M. F. Wangler*[1,2,3,4,5], Oz Kanca[1], Sathiya Manivannan[1], Xi Luo[1], Ning Liu[1], Matthew Lagarde[1], Yu-Hsin Chao[1], Hsiao-Tuan Chao[2,4,5], Shinya Yamamoto[1,2,3,4,6]. 1) Department of Molecular and Human Genetics, Baylor College of Medicine (BCM), Houston, TX, 77030; 2) Texas Children's Hospital, Houston, TX, 77030; 3) Program in Developmental Biology, BCM , Houston, TX, 77030; 4) Texas Children's Neurological Research Institite, Houston TX 77030; 5) Department of Pediatrics, Baylor College of Medicine, Houston, TX, 77030; 6) Department of Neuroscience, BCM, Houston, TX, 77030.

Autism spectrum disorders (ASDs) are a highly heritable developmental nervous system disorders with varying degrees of impaired social interaction, defects in verbal and non-verbal communication and repetitive behavior. Symptoms typically begin before age 3 years and are accompanied by cognitive deficits. There is significant locus heterogeneity as evidenced by a number of Mendelian disorders with ASD as a feature. In addition, several large genomic sequencing studies have been undertaken in thousands of families with as ASD proband. The incomplete functional annotation of the majority of genes in the human genome leads to problems in interpreting the results of these studies as the functional impact of variants is usually not known. Studies in model organisms such as *Drosophila* have consistently contributed to our understanding of gene function including genes involved in neuronal development, neuronal function and maintenance. We are conducting a functional screen in *Drosophila* to analyze ASD candidate genes. We examined whole-exome sequencing data from 2,500 families with a proband with ASD from the Simon's Simplex collection (SSC). Of these we focused on 2,437 human genes with *de novo* missense or in-frame indels in ASD cases. We focused on missense and in-frame alleles to study variants with unknown impact on gene function. Of these 1,931 genes (79.2%) are conserved in Drosophila. The ASD candidate genes are cross-referenced to independent genomic data from non-Autism cohorts in order to aid in prioritization. From a group of priority genes we have selected 62 *Drosophila* Mi[MIC] lines with insertions in introns between coding exons for functional screens. These lines are currently being converted to loss-of-function strains using the versatile artificial exonic element to generate a 2A-Gal4 line. These strains will be scored for viability, longevity, and a number of basic behavioral phenotypes. Strains with strong phenotypes will be studied for nervous system expression, knockdown and the human reference and ASD-related variant forms are being tested to determine the functional effect of the variants of interest. This synergy between human genomics and a versatile functional analysis pipeline in *Drosophila* should allow stronger links between the growing list of ASD candidate genes and the wealth of conserved neurobiology in model organisms.

D1334A Genome-wide association study to examine insulin resistance in a *Drosophila* model. *K. Slater*, B. Wininger, J. Stamm. University of Evansville, Evansville, IN.

Dietary factors can alter *Drosophila* metabolism and induce insulin resistance in a similar fashion as in humans. *Drosophila* therefore provides a simple and genetically tractable model for identifying genetic factors associated with human diabetes. We are carrying out a genome-wide association study to identify genomic regions that contribute to the development of insulin resistance, using the *Drosophila* Genetic Reference Panel (DGRP), a collection of ~200 fully sequenced inbred *Drosophila* lines.

Mated females from each line were fed either a normal diet or a high sugar, insulin resistance-inducing diet, and weight gain, fertility, and lifespan were measured under each dietary condition. We have completed the analysis for 30 lines, and have identified several lines in which the high sugar diet had an extreme effect on these traits. We are currently verifying these results with metabolite assays, and continuing our analysis of additional DGRP lines to identify correlations between particular genomic regions and increased or decreased susceptibility to insulin resistance. Our efforts will lead to increased understanding of the genes underlying insulin resistance and type II diabetes.

D1335B Enteroendocrine cell-derived Act-β enhances AKH action and contributes to hyperglycemia under a high sugar diet. *W. Song*[1], D. Cheng[1], S. Hong[2], B. Sappe[1], Y. Hu[1], N. Wei[1], M. O'Connor[3], P. Pissios[2], N. Perrimon[1]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Division of Endocrinology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; 3) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.

Insulin and glucagon play fundamental and counteractive roles in carbohydrate homeostasis. In addition to insulin resistance, enhanced glucagon action also contributes to hyperglycemia in the context of high caloric diet. In contrast to the wealth of knowledge regarding insulin action regulation, little is known about the physiological regulation of glucagon signaling. To identify new regulators of glucagon signaling, we established a conserved glucagon-induced hyperglycemia model in Drosophila overexpressing fly glucagon encoded by Akh (Adipokinetic hormone). We combined in vivo RNAi screen and genetic validation and identified the activin type I receptor, Baboon (Babo), and one of its ligand, Activin-β (Actβ), as important regulators of glucagon/AKH signaling. Deficiency of Babo or Actβ significantly impaired AKH action in the fat body which is equivalent to the liver, and abolished hyperglycemia associated with high-sugar diet in Drosophila. Importantly, Actβ production in enteroendocrine cells was significantly increased and promoted fat body AKH action and elevated glycemic level in response to high-sugar diet. Finally, we found that in mouse primary hepatocytes Activin A treatment potently enhances glucagon signaling as well as glucagon-induced glucose production. In summary, we have identified an evolutionarily conserved mechanism whereby activin signaling enhances glucagon action to impact hyperglycemia and/or diabetes pathogenesis.

D1336C The Spen family of RNA-binding proteins coordinate energy balance in the fatbody. *T. Reis*, K. Hazegh. Univ Colorado Medical School, Aurora, CO.

Hereditary components are responsible for 40-70% of variation in body weight and obesity, yet we currently know only ~5% of the underlying causal genes. In order to develop new treatments for obesity, we must develop a mechanistic understanding of the pathways in which the other 95% of predisposing alleles normally function. We previously developed a novel, buoyancy-based assay for body fat in *Drosophila* larvae and used it in an unbiased forward genetic screen to identify *split ends* (*spen)* as a new fat regulator. Spen is an RNA-binding protein previously

implicated in transcriptional control of conserved signaling pathways. Interestingly, despite being fat, larvae in which Spen is depleted from the fat body (FB) are sensitive to starvation, suggesting that these animals are incapable of using their excess fat stores. Consistent with this phenotype, metabolomics and RNAseq demonstrate metabolic alterations in Spen-depleted FBs indicative of a defect in mobilization of energy stores, and utilization of other metabolites (proteins and carbohydrates) as primary sources of energy. We further find that Spen function is necessary and sufficient to promote fat depletion in the FB, and that another Spen family member, Spenito (Nito), plays an opposing role in fat storage. FB overexpression of an N-terminal Spen fragment containing the RNA Recognition Motifs (RRMs) causes a dominant-negative high-fat phenotype, whereas overexpression of a C-terminal fragment lacking the RRMs but containing a nuclear localization signal and the conserved Spen paralog and ortholog (SPOC) domain had no effect on larval buoyancy. Thus, the RRMs are required for the ability of overexpressed full-length Spen to deplete fat stores, and when overexpressed alone may sequester important Spen-associated RNAs into non-functional complexes. We propose that Nito, which contains RRMs and a SPOC domain but is much smaller than Spen, may act as a negative regulator of Spen function. Finally, we find that the mammalian Spen ortholog also regulates fat storage in cultured adipocytes. No other study has implicated Spen in the regulation of metabolism or body fat control. Our work provides a new direction for understanding metabolic disease as well as a molecular handle to generate novel mechanistic insights into the causes of obesity.

D1337A Cardiomyocyte regulation of systemic lipid metabolism by the apoB-containing lipoproteins in *Drosophila*. *Hui-Ying Lim*[1,2,4], Sunji Lee[1], Hong Bao[1], Weidong Wang[3,4]. 1) Aging and Metabolism Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma; 2) Department of Physiology, University of Oklahoma Health Science Center, Oklahoma City, Oklahoma; 3) Department of Medicine and Harold Hamm Diabetes Center, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; 4) Corresponding authors.

Lipid dysregulation is a key contributor to the pathogenesis of obesity and its related disorders. The heart has emerged as an important organ in the regulation of systemic lipid homeostasis; however, the underlying mechanism remains poorly understood. Here, we show that *Drosophila* cardiomyocytes control systemic lipid metabolism by expressing *mtp* and *apoLpp*, genes so far known to be essential for apoB-lipoprotein biosynthesis only in the fat body (insect adipose tissue and liver). We found that on normal food diet, knockdown of *mtp* or *apoLpp* in the fat body or cardiomyocytes decreases systemic triglyceride (TG) levels, suggesting equal contribution of the cardiomyocyte- and fat body-derived apoB-lipoproteins in the regulation of whole-body lipid metabolism. Unexpectedly, on high fat diet (HFD), knockdown of *mtp* or *apoLpp* in the cardiomyocytes, but not in fat body, protects against the gain in systemic TG levels, suggesting that cardiomyocyte-derived apoB-lipoproteins are the predominant regulators of systemic lipid metabolism in response to HFD. We further found that HFD significantly up-regulates *mtp* and *apoLpp* expression in the cardiomyocytes, but paradoxically down-regulate their expression in the fat body, culminating in higher Mtp and apoLpp levels in the cardiomyocytes than in fat body and possibly underlying the differential systemic metabolic effects of apoB-lipoprotein inhibition in the cardiomyocytes versus the fat body. Our findings reveal a novel and significant function of heart-mediated apoB-lipoproteins in the control of systemic lipid homeostasis.

D1338B Investigating Mitochondrial Respiratory Chain Disorders(MRCD) in *Drosophila*. *K. Ohnuma*[1], Y. Sato-Miyata[1], Y. Kishita[2], M. Kohda[2], K. Murayama[3], A. Ohtake[2], Y. Okazaki[2], T. Aigaki[1]. 1) Tokyo Metropolitan University, Tokyo, Japan; 2) Saitama Medical University, Saitama, Japan; 3) Chiba Children's Hospital, Chiba, Japan.

Mitochondrial respiratory chain disorders (MRCD) are rare diseases that affect multiple organs with varying severity. MRCD can be caused by mutations in mitochondrial or nuclear genes. At present, approximately 250 nuclear genes have been identified as candidates for MRCD. However, the pathogenic mechanisms of MRCD are not fully understood. Model organisms provide unique opportunities to study molecular basis of human diseases. The fruit fly, *Drosophila melanogaster* is one of the best model organisms, in which one can utilize advanced knowledge and techniques of genetics, and a large number of resources, such as mutant stocks, and cDNA collections. Here, we established *Drosophila* models of MRCD through knocking down the orthologs of 13 human MRCD candidate genes identified in Saitama Medical University. We used the Gal4/UAS system-mediated transgenic RNAi to produce knockdown (KD) flies for each candidate gene, and analyzed MRCD-related phenotypes. Among the candidate genes (MRCD1-13), MRCD11-KD flies exhibited remarkable phenotypes: reduced body size and severe defects in locomotor activity, respiratory dysfunction and short lifespan. In addition, biochemical analysis revealed that they have symptoms of lactic acidosis and mitochondrial respiratory chain complex I defects which were similar to symptoms of the MRCD patient. These results suggested MRCD11 gene is a causative gene for MRCD. MRCD11-KD flies should be useful as a model to investigate the role of the gene and to understand the molecular pathogenesis of MRCD.

D1339C Untargeted metabolomics elucidates the role of diet and triglyceride storage in *Drosophila melanogaster* larvae. *Vishal Oza*, Laura Reed. Department of Biological Sciences, University of Alabama, Tuscaloosa, AL.

Untargeted Metabolomics has been used to identify altered metabolic pathways in disease state. Here we employ ***Drosophila*** as a model organism to evaluate various aspects of Metabolic Syndrome (MetS), a complex disease that increase the risk for heart disease and diabetes. The prevalence of MetS has been attributed to the westernized dietary habit and sedentary lifestyle. Our previous studies have established, diet as one of the important contributors to metabolic phenotypes. Using untargeted metabolomics we have isolated and identified global metabolites in ***Drosophila*** larvae. We then employed Random Forest algorithm to obtain important metabolites that differed between the High fat (HFD) and normal (ND) diet as well as between reaction norm phenotypes (flies that store more triglyceride on normal diet and flies that store less triglyceride on normal diet). We found that in flies fed on HFD had an upregulation of the omega fatty acid oxidation pathway which is an alternative to the more common beta fatty acid oxidation. Furthermore, there was no correlation observed between triglyceride storage phenotype and fatty acids, indicating diet played more important role over triglyceride phenotype. In conclusion, although untargeted

metabolomics allows for elucidation of global metabolic profile, the lack of ***Drosophila*** specific metabolites and pathways database hinders the use of ***Drosophila*** as a model organism for metabolomics studies.

Keywords: Untargeted metabolomics, *Drosophila melanogaster*, *Drosophila* metabolomics.

D1340A The power of food: how diet during development programs adult lifespan. *Irina Stefana*[1,2], Paul C. Driscoll[1], Fumiaki Obata[1], James I. MacRae[1], Alex P. Gould[1]. 1) Francis Crick Institute, Mill Hill Laboratory, London, United Kingdom NW7 1AA; 2) Current address: Dept. of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford, United Kingdom OX1 3QX.

The developmental origins of adult health and disease are well established in human populations and mammalian models. For example, malnutrition during early life predisposes the future adult to an increased risk of metabolic disease. Despite much research in this area, the molecular mechanisms underlying this process of nutritional programing remain poorly understood. We have therefore established a *Drosophila* model for programing of adult metabolism and lifespan by the diet during development. We show that larval diet can program long-lasting perturbations of metabolism in adult flies. Importantly, larval diet can also dramatically increase median lifespan by up to 145%, a larger change than that obtained with adult dietary restriction (DR). Genetic manipulations suggest that the altered size of Insulin producing cells – analogous to β-cell mass in mammals – can explain only ~10% of this programed lifespan increase. We find, instead, that the programing mechanism depends upon endogenous compounds deposited onto the substrate, which alter the lifespan of both same- and opposite-sex adults. This reveals, for the first time, the existence of a transferrable component in the regulation of lifespan between same-sex animals. Biochemical, NMR, mass spectrometry and genetic approaches identify these compounds as a specific class of lipids that impact negatively upon lifespan in a dose-dependent manner. Importantly, the production of these toxic lipids by adults is programed by diet during development. We also find that the lifespan-extending effect of decreased Insulin signaling correlates with increased resistance to toxic lipids. Together, these findings establish *Drosophila* as a powerful new integrative physiology model for uncovering new mechanisms underlying the nutritional programing of metabolism and lifespan.

D1341B Investigating the effect of acute injury on gene expression in the embryonic *Drosophila* nerve cord. *S. Benjamin*, H. Mistry. Widener University, Chester, PA.

In humans, spinal cord injuries can have long-term physical effects due to critical cellular and molecular changes that occur during the acute phase of injury. To better understand the cellular and molecular mechanisms underlying these critical and complex injuries, we are using the *Drosophila* embryonic nerve cord as a model to assess the effect of acute injury on gene expression. We anchored late stage embryos on coverslips and injured the ventral nerve cord using a glass needle. Control embryos were similarly treated, but not injured. Experimental and control embryos were harvested and total RNA was extracted from each sample. High-quality RNA thus generated was sequenced and analyzed. These data show that the expression levels of 700 genes changed significantly in the injured embryos in comparison to the uninjured embryos. The differential gene expression changes thus revealed are likely characteristic of the acute phase of trauma. We have functionally categorized these genes using specific gene ontology terms. We will first analyze genes pertinent for the regulation of cellular processes involved in axon guidance, synapse growth, and the mitotic cell cycle.

D1342C Actin isoforms in *Drosophila* muscle function. *T. Dohn*, R. Cripps. University of New Mexico, Albuquerque, NM.

Actins are critical components of sarcomere structure and muscle function. Mammals and *Drosophila* each have six actin genes that exhibit distinct expression patterns spatially and temporally. Although the actins are highly conserved sequences they maintain distinct biochemical properties and affinities within the cell. Regardless, some of the actin isoforms can partially compensate for each other, making it unclear what the role of specific actin isoforms are during muscle formation and development. Therefore, using CRISPR technology we created two *Drosophila* actin mutants to determine the role of each actin in muscle development and function. *Actin57B* is a predominantly embryonic muscle actin isoform and *Actin79B* is the predominant actin isoform expressed in the *Drosophila* tergal depressor of the trochanter (TDT or jump) muscle. Mutations of each gene were created leading to null mutants. Through viability assays and structural analysis of embryonic muscles we determined that depletion of *Actin57B* results in an embryonic lethal phenotype with disrupted sarcomere structure. Although there is an increase in *Actin87E* mRNA by *in situ* hybridization, this increase is insufficient to rescue the sarcomere structural defects in *Actin57B* embryos. *Actin79B* deficient flies were structurally and functionally normal, showing a normal jumping ability despite the loss of the predominant jump muscle actin. This rescue of jump muscle function is due to compensation by low levels of *Actin88F* expression in the jump muscles. Double mutants deficient in both *Actin79B* and *Actin88F* show deficiencies in TDT muscle function. Therefore, *Actin57B* and *Actin79B* have distinct roles in muscle structure organization, however, *Actin57B* deficiency cannot be compensated for by increased levels of embryonic *Actin87E* while *Actin79B* deficiency is completely rescued by low levels of *Actin88F.* This demonstrates that secondary actin genes will expand their expression domain to compensate for deficiencies in other actin isoform, however, this genetic expansion does not necessarily translate to functional compensation.

D1343A Developmental causes of Nemaline Myopathies. *Aaron N. Johnson*, Brenna Clay. Univ Colorado, Denver, Denver, CO.

Congenital myopathies are a heterogeneous collection of disorders defined by neonatal muscle weakness, or hypotonia, and many myopathies can progress to extreme conditions in which patients require clinical interventions for proper respiration and mobility. Over 23 distinct heterozygous mutations in *Tropomyosin 2* (*TPM2*) have been identified in patients with Nemaline Myopathy (NM), yet the mechanisms by which these mutations contribute to the disease phenotype remain largely unknown. The current clinical hypothesis is that *TPM2* NM alleles are gain-of-function mutations that disrupt sarcomere contractility in otherwise normal muscle. Based on our previous developmental

studies, we hypothesized that *TPM2* NM alleles also disrupt multiple aspects of myogenesis prior to sarcomere assembly. We expressed 9 human *TPM2* NM alleles in *Drosophila* embryonic body wall muscles and assayed muscle development. Each *TPM2* NM allele disrupted myogenesis, and multiple alleles caused myotubes to attach to the wrong tendon or to no tendon at all. These results strongly support our hypothesis that the NM disease etiology is due in part to defects in myogenesis. This is the first study to systematically characterize *TPM2* NM alleles in an *in vivo* system, and highlights the utility of using *Drosophila* genetics to functionally annotate human disease alleles. Our studies will also be a foundation for studying *TPM2* NM alleles during mammalian myogenesis.

D1344B Using *Drosophila* to uncover the molecular mechanisms underlying Pontocerebellar Hypoplasia. *Vafa Bayat*[1], Xue Yang[1,2], Zhihao Wu[1], Yen-Chi Wu[1], Hannes Vogel[1], Bingwei Lu[1]. 1) Stanford University, Stanford, CA; 2) Peking University, Beijing, China.

The molecular and cellular pathology of the neonatal/fetal disease Pontocerebellar Hypoplasia (PCH) is poorly understood. PCH is a rare congenital disorder displaying autosomal recessive inheritance characterized by hypoplasia and atrophy of the cerebellar cortex, dentate nuclei, pontine nuclei and inferior olives. A growing number of mutated genes have been implicated in this disease. Several hypotheses have been proposed regarding the relationships between these recently identified genes and the potential mechanisms, but evidence is lacking. Molecular genetic analysis in model organisms such as *Drosophila* has the potential to make up for the deficits in our understanding of PCH pathogenesis and offer more in-depth mechanistic insights. We have obtained and are generating mutants using imprecise excision and CRISPR in all of the TSEN complex genes, which have all been found to be mutated in this disease. Our data is supportive of a neurodegenerative process taking place in these mutants and our hypothesis that dysregulation of levels of key proteins implicated in other neurodegenerative diseases is present and are testing which of these are pathogenic using genetic interactions with RNAi lines, loss of function lines and overexpression. Determining which pathways and proteins are involved in the pathogenesis will also give clues to potential treatments, which are presently only supportive in nature.

D1345C Activation of lipophagy protects neurons from neurodegeneration caused by sphingolipid imbalance. *Y. Chang*[1], W. Jung[1], C. Liu[1], Y. Yu[1], W. Lien[1], H. Chao[2], C. Kuo[2], H. Ho[3], C. Chan[1]. 1) Graduate Institute of Physiology, College of Medicine, National Taiwan University, Taipei, Taiwan; 2) School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan; 3) Department of Anatomy, School of Medicine, Tzu-Chi University, Hualien, Taiwan.

Sphingolipids are essential membrane components of the neuron; hence their levels need to be tightly regulated. Infertile crescent (Ifc) is an evolutionarily conserved dihydroceramide (DHC) desaturase which converts DHC to Ceramide (Cer) for the *de novo* synthesis of Cer in *Drosophila*. While the imbalance of Cer, a bioactive sphingolipid, has been associated with several neurodegenerative diseases, the neuronal function of its precursor DHC remains unknown. To investigate the role of *ifc*, we generated *ifc* knockout flies (*ifc*-KO). Sphingolipidomic analysis showed that loss of *ifc* resulted in increased DHC. Prolonged light stimuli to the *ifc*-KO eye led to activity-dependent degeneration of photoreceptors. Clonal analysis of *ifc*-KO photoreceptors revealed the accumulation of lipophagic structure and the increased H2DCF signals upon light stimuli, suggesting that DHC accumulation may activate lipophagy and induce the production of reactive oxygen species (ROS). However, it remains to be determined whether the degeneration is attributed to lipophagic cell death or the ROS insults. Reduction of *ifc* led to the increase of Atg8/LC3 puncta in the acidified compartment and elevation of lysosomal proteases, indicating the activated lipophagy can promote subsequent lysosomal function. *ifc*-dependent neurodegeneration can be partially rescued by an antioxidant AD4, indicating that ROS is at least partially responsible for the degeneration. In addition, both ROS elevation and lipid accumulation in *ifc*-KO was suppressed by treating with the autophagy inducer Rapamycin, suggesting that enhanced lipophagy plays a protective role in *ifc*-dependent neurodegeneration. Conversely, lipophagy can be downregulated by AD4, indicating ROS insults lead to the feedback upregulation of protective lipophagy. In summary, loss of *ifc* results in DHC accumulation and ROS generation, the latter of which subsequently activates lipophagy to protect against neurodegeneration. These findings support our hypothesis that DHC is bio-active and lipophagy can be protective, highlighting their potential as therapeutic targets for regulating sphingolipid homeostasis.

D1346A Knockdown of the Sleep and Circadian Rhythm-Regulating Protein Insomniac Exacerbates Decreased Longevity in a *Drosophila* model of Alzheimer's Disease. *Thomas S. Finn*, Tyler Dewitt, Sarah Deleon, Jeremy C. Lee. University of California, Santa Cruz, Santa Cruz, CA.

Alzheimer's Disease (AD) is the leading cause of dementia in the elderly population, currently affecting over 5 million Americans. AD is characterized by an accumulation of extracellular plaques composed of Amyloid-beta (Aβ) peptides as well as accumulation of intracellular neurofibrillary tangles (NFTs). Recently, it was reported that patients with AD who also suffer from low sleep quality, have increased Aβ deposition in the brain compared to patients with AD that experience normal sleep patterns. Experiments in mice have shown that sleep is associated with a 60% increase in the interstitial space of the brain and greatly increases the rate at which Aβ is cleared (Xie, *et al.*, 2013). In a recent Genome Wide Association Study, the KCTD2 locus in humans was further implicated in AD (Boada, *et al.*, 2013). Interestingly, homologs of this locus are present in many animal models, such as the *insomniac* locus in *Drosophila*. Loss-of-function mutations of *insomniac* result in a severe sleep deprivation and dysfunction in circadian regulation (Stavropoulos & Young, 2011; Pfeiffenberger & Allada, 2012).

We have conducted experiments to test the effects of sleep and/or circadian dysfunction on AD-like pathology in a *Drosophila* model of AD. Our central hypothesis is that inducing sleep deprivation/circadian dysfunction, as a result of knocking down Insomniac, will accelerate and increase Aβ deposition, resulting in more severe AD-like pathology. To test this hypothesis, we are using a *Drosophila* AD model that expresses human Aβ42 in the CNS under an *elav*-Gal4 driver. These flies exhibit decreased longevity as compared to control flies; they also exhibit abnormal behavior and deposition of Aβ aggregates in the brain. In these AD model flies, we have knocked down Insomniac levels by also expressing shRNA that targets *insomniac* mRNA. We find that the severity of AD-like pathology, as measured by decreases in longevity, are

significantly exacerbated by knocking down Insomniac levels; i.e. the AD model flies in which Insomniac is knocked down have a shorter mean lifespan than AD model flies which express wild type levels of Insomniac. This exacerbation of the longevity defect appears to be an interaction between the Insomniac knockdown and the effects of Aβ, and is not simply an additive effect on longevity. Control flies only expressing the Insomniac shRNA showed no deviation in their survivorship compared to wild-type flies. We are currently conducting experiments to assess whether the knockdown of Insomniac also exacerbates other aspects of AD-like pathology in our flies, including effects on behavior, as measured by the RING assay, and on levels of Ab deposition in the brain.

D1347B Role of a soy protein Lunasin in Aβ42 mediated neurodegeneration in Alzheimer's Disease. *N. Glenn*[1], A. Sarkar[1], A. Giaquinto[1], M. Moran[1], G. Jones[2], A. Srivastava[2], M. Kango-Singh[1,3,4], A. Singh[1,3,4]. 1) Department of Biology, University of Dayton, 300 College Park Drive, Dayton OH; 2) Department of Biology and Biotechnology Center, Western Kentucky University, 1906 College Heights Blvd, Bowling Green, KY; 3) Premedical Program, University of Dayton, 300 College Park Drive, Dayton OH; 4) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH.

Alzheimer's Disease (AD) is a neurodegenerative disease caused by a number of factors. One of the leading factors behind the onset of AD is the accumulation of amyloid plaques in the brains of affected individuals. These plaques are formed with amyloid precursor protein (APP) is processed incorrectly and cleaved to be 42 amino acids long (Aβ42) instead of 40 (Aβ40). These two extra amino acids cause the protein to become hydrophobic in nature and form plaques which aggregate around neurons in the brain. This aggregation induces oxidative stress on the neurons which then leads to cell death. Due to the conserved genetic properties of the *Drosophila melanogaster*, fruit fly, visual system with that of humans we have developed a Drosophila eye model. In this model the Aβ42 protein is misexpressed in the developing photoreceptors of the fly eye which results in extensive cell death of the photoreceptor neurons and produces a highly reduced eye field in the adult fly. Our aim is to understand the function of a soy protein called Lunasin in Alzheimer's disease. It has been shown that Lunasin acts as an anti-inflammatory within the somatic cells. Inflammation is also one of the characteristic of AD. Therefore, we investigated the effects of Lunasin on Aβ42 accumulation mediated neurodegeneration. We found that Lunasin can rescue the Aβ42 mediated neurodegeneration in the retinal neurons as well as the axonal targeting from the retina to the brain. We are looking into the mechanism of this Lunasin mediated rescue.

D1348C Exploring the nature of mitochondrial fragmentation and loss in a Parkin loss-of-function *Drosophila* Model of Parkinson's disease. *S. Gutierrez*, J. Cackovic, K. Pearman, G. Call, L. Buhlman. Midwestern University, Glendale, AZ.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by dopaminergic neuron loss in the substantia nigra. Loss-of-function mutations in *PARK2* are the leading cause of autosomal recessive juvenile parkinsonism (AR-JP). An AR-JP *Drosophila melanogaster* model exhibits pathologies associated with loss-of-function mutations of the *parkin* (*park*, the *Drosophila PARK2* ortholog) gene including swollen mitochondria and mitochondrial dysfunction. Preliminary results from our lab show that mitochondrial networks have smaller volumes and more fragmentation in dopaminergic neurons in heterozygous *Drosophila park*^{25} (loss-of-function allele) flies, suggesting that *park*^{25} causes pre-mitophagic fragmentation. Disruptions in oxidative phosphorylation increase reactive oxygen species (ROS) production, which can lead to oxidative stress, mitochondrial fragmentation and aberrant mitochondrial morphology. Disruptions in mitochondrial respiration have been observed in Parkin loss-of-function models as well as in patients. Although Parkin-mediated mitophagy has been described in a number of cell lines and in different physiological conditions, contradicting ideas about the relationship between Parkin and mitophagy persist. An *in vivo* assessment of mitophagy would help determine how Parkin affects mitochondrial function and thus provide further insight in the role of mitochondrial pathology in the etiology of PD. To assess ROS production, we are utilizing a MitoTimer construct that consists of a mitochondrial-targeted DsRed variant that shifts from green to red fluorescence upon oxidation. Using confocal microscopy we are measuring this fluorescent ratio in TH-Gal4, UAS-MitoTimer PPL1 dopaminergic neurons in control (*park*$^{+/+}$), heterozygous (*park*$^{+/-}$) and homozygous (*park*$^{-/-}$) flies. To measure mitophagy in the PPL1 neurons, we are analyzing co-localization of mCherry-Atg8a and mitoGFP constructs driven by TH-Gal4 in these same *park* genotypes. We hypothesize that the decrease in mitochondrial network volume we have detected in Parkin loss-of-function flies is due to higher rates of oxidation and subsequent increases in mitophagy. Our results, which will be presented at the meeting, will provide insight on the dose-dependent effect of *park* mutation toxicity within the mitochondria of dopaminergic neurons and shed light on morphology and mitophagy. This will promote a better understanding of the mechanisms of degeneration caused by Parkin loss-of-function.

D1349A The influence of misregulation of inositol trisphosphate receptor on a *Drosophila* model of MJD. *D. Hahn*, J. Warrick. Richmond Univ., Richmond, VA.

Spinocerebellar ataxia 3, more commonly known as Machado Joseph Disease (MJD), is a fatal, hereditary disease characterized by muscle control loss and neuronal degeneration. Inositol Trisphosphate Receptor (IP3R) expression is a crucial component of the calcium-mediated signaling mechanism in cells and has been linked to other similar neurodegenerative diseases. Thus we investigated the effects of IP3R on the progression of MJD. Using a *Drosophila* model for MJD, IP3R expression was first upregulated and found to increase neurodegeneration in flies over the course of seven days as compared to control models. IP3R expression was then downregulated via RNAi which was found to decrease neurodegeneration after seven days. Based on these results, it has been concluded that IP3R is a significant factor of the overall MJD progression mechanism. Future experiments will be conducted to accurately describe the role of IP3R signaling in MJD pathology.

D1350B Use of a *Drosophila* model of Alzheimer's Disease to screen GPCR ligands for potential AD therapeutics. *K. A. Innamorati*[1], S. D. Mhatre[2], P. T. Nguyen[1], D. R. Marenda[1]. 1) Drexel University, Philadelphia, PA; 2) Stanford University, Stanford, CA.

Alzheimer's Disease (AD) is a progressive, neurodegenerative disease characterized by global cognitive decline and neuronal death. As of 2015, 5.3 million Americans are suffering from AD, but only five drugs are available for palliative treatment. A major challenge for AD drug discovery is the lack of animal models for the disease. One popular model, *Drosophila melanogaster*, is valued for a variety of reasons, including quick generation times- making it a good model of aging. Our lab has created a transgenic *Drosophila* model of AD which displays amyloid-containing puncta in adult brains, motor coordination difficulties, and memory deficits that can be rescued with gamma secretase inhibitor L-685,458. The model also displays altered external morphology, including melanotic plaques on the proboscis and abdomen, and crumpled wings. Using this model, our lab performed a drug screen of 264 GPCR-binding ligands. Results were organized into enhancers and suppressors of external morphology. Both Memantine (Namenda) and Donepezil (Aricept), palliative drugs available for AD treatment, scored as suppressors on our model. From our screen, we will study new compounds for the treatment of Alzheimer's Disease.

D1351C Gene interaction network in *Drosophila* reveals connections between Huntington's disease and FTD/ALS. *K. Kannan*, P. Zhang. Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT.

Nucleotide repeat expansions are a common characteristic of Neurodegenerative disorders such as Huntington's disease (HD), spinocerebellar ataxia, Fragile X syndrome and Frontotemporal dementia/amyotrophic lateral sclerosis (FTD/ALS). The mechanisms by which these repeats confer toxicity remain largely elusive. Using a sensitized genetic screen in the *Drosophila* eye, we report a series of modifiers that interact with the toxicity of both the expanded CAG repeats (HD) and the expanded GGGGCC repeats (FTD/ALS), with synthetic lethality as a readout. Synthetic lethal interactions represent strong interactions that could indicate a functional relationship between genes. Our data suggests that common functional modules exist between HD and FTD/ALS.

D1352A Role of Dpp signaling pathway in promoting survival of retinal neurons in Aβ42 mediated neurodegeneration. *J. Kleppel*[1], A. Sarkar[1], J. Little[1,2], J. Kirwan[1,3], M. Kango- Singh[1,4,5], A. Singh[1,4,5]. 1) Department of Biology, University of Dayton, OH; 2) The Ohio State University, School of Biomedical Engineering, Columbus, OH; 3) Drexel University, School of Biomedical Engineering, Science, and Health Systems, PA; 4) Premedical Program, University of Dayton, Dayton, OH; 5) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH.

Alzheimer's disease is a progressive neurodegenerative disorder with no known cure to date. One cause of Alzheimer's neuropathy is the generation of Amyloid-beta-42 (Aβ42) aggregates that trigger cell death by unknown mechanisms. Using a transgenic *Drosophila* eye model misexpressing human Aβ42, we observed the AD-like neuropathy. In a forward genetic screen we have identified Decapentaplegic (Dpp), a morphogen, as one of the genetic modifiers of Aβ42 mediated neurodegeneration. Dpp acts as the ligand for the *Dpp* pathway, which exhibits suppression of retinal neuron's cell death. The Dpp signaling pathway involves several key components. We examined the Dpp signaling pathway and its members in modifying Aβ42 mediated neuropathy. We have demonstrated that upregulating Dpp signaling pathway, by misexpressing Dpp (using *UAS dpp*) and Thickveins (using *UAS tkv*) can rescue Aβ42 mediated neurodegeneration. The number of dying cells marked with TUNEL staining was also reduced and the axonal targeting was restored from the retina to the brain, which was marked by 24B10 staining. We will test the role of Dpp signaling in Aβ42 plaques mediated neurodegeneration. Furthermore, we will analyze, if these modifiers act independent and/or parallel of each other or whether they have a linear relationship in triggering neurodegenerative response due to accumulation of Aβ42. The results from our studies will be presented.

D1353B Role of sarah/nebula in Aβ42-induced neurological impairments in *Drosophila*. *J. Lee*, S. Lee, S. Bang, K. Cho. Konkuk university, Seoul, KR.

Expression of the Down syndrome critical region 1 (DSCR1) protein, an inhibitor of the Ca(2+)-dependent phosphatase calcineurin, is elevated in the brains of individuals with Down syndrome (DS) or Alzheimer's disease (AD). Although increased levels of DSCR1 were often observed to be deleterious to neuronal health, its beneficial effects against AD neuropathology have also been reported, and the roles of DSCR1 on the pathogenesis of AD remain controversial. Here, we investigated the role of *sarah* (*sra*; also known as nebula), a *Drosophila DSCR1* ortholog, in amyloid-β42 (Aβ42)-induced neurological phenotypes in *Drosophila*. We detected *sra* expression in the mushroom bodies of the fly brain, which are a center for learning and memory in flies. Moreover, similar to humans with AD, *Aϐ42*-expressing flies showed increased Sra levels in the brain, demonstrating that the expression pattern of DSCR1 with regard to AD pathogenesis is conserved in *Drosophila*. Interestingly, overexpression of sra using the *UAS-GAL4* system exacerbated the rough-eye phenotype, decreased survival rates and increased neuronal cell death in *Aϐ42*-expressing flies, without modulating *Aϐ42* expression. Moreover, neuronal overexpression of *sra* in combination with Aβ42 dramatically reduced both locomotor activity and the adult lifespan of flies, whereas flies with overexpression of *sra* alone showed normal climbing ability, albeit with a slightly reduced lifespan. Similarly, treatment with chemical inhibitors of calcineurin, such as FK506 and cyclosporin A, or knockdown of calcineurin expression by RNA interference (RNAi), exacerbated the *Aϐ42*-induced rough-eye phenotype. Furthermore, *sra*-overexpressing flies displayed significantly decreased mitochondrial DNA and ATP levels, as well as increased susceptibility to oxidative stress compared to that of control flies. Taken together, our results demonstrating that sra overexpression augments Aβ42 cytotoxicity in Drosophila suggest that *DSCR1* upregulation or calcineurin downregulation in the brain might exacerbate Aβ42-associated neuropathogenesis in AD or DS.

D1354C The expression and role of Apolipoprotein D in Aβ42-induced Alzheimer's disease *Drosophila* model. *S. Lee*, K. S. Cho. Konkuk university, Seoul, Seoul, KR.

Apolipoprotein D (ApoD) is a member of the lipocalin family which is secreted from subsets of neurons and glial cells. Previously, it has been reported that the expression level of ApoD is strongly up-regulated in the brain of Alzheimer's disease (AD) patients, and that ApoD has a protective function against oxidative stress in several animal models. However, the role(s) of ApoD in the pathogenesis of AD is still not understood. Here, we demonstrated the role of Glial Lazarillo (GLaz), a gene for a *Drosophila* ApoD homolog, in the AD-like phenotypes of *Aβ42*-expressing flies. *GLaz* overexpression mitigated the Aβ42-induced phenotypes such as rough eyes, locomotive defect, reduced survivability and neuronal cell death. In addition, increased ROS level and sensitivity to oxidative stress in the AD model flies were significantly reduced by pan-neuronally overexpressed *GLaz*, without alteration of the amount of Aβ42 accumulations. Taken together, our results suggest that ApoD plays a protective role against the AD-like phenotypes of AD model flies by inhibiting oxidative stress, and that ApoD can be a potential genetic marker and therapeutic target of AD.

D1355A Genetic variation and mechanisms of paraquat susceptibility in *D. melanogaster*. *Pamela Lovejoy*[1], Kate Foley[1,2], Anthony Fiumera[1]. 1) Binghamton University, Binghamton, NY; 2) Tufts University, Boston, MA.

It is estimated that over a billion pounds of pesticides are applied each year in the United States. Many of these pesticides have potentially detrimental effects on non-target organisms, including humans. Paraquat is a commonly used herbicide in the U.S. that is known to increase oxidative stress in *Drosophila melanogaster* and many other organisms. Increased oxidative stress of the mitochondria leads to dopaminergic cell death and loss of motor ability, which results in a model of Parkinson's disease. The response of *D. melanogaster* to common pesticides, such as paraquat, can be informative of their effect on other non-target organisms. The purpose of this work is to map the genetic basis for natural variation affecting low-dose paraquat susceptibility in *D. melanogaster*. In order to quantify the naturally occurring variation in susceptibility to paraquat that exists, one hundred lines from the Drosophila Genetic Reference Panel (DGRP) were assayed for climbing ability under control conditions and after exposure to paraquat. Paraquat reduces climbing ability in both males and females but males show much greater variation in susceptibility. A GWAS was used to map 31 and 15 genes in males and females respectively, which contribute to susceptibility to paraquat. A subset of these are being verified using RNAi. In addition, these results will also be compared via correlations and genetic network analyses to other measures of susceptibility that have been determined in the DGRP lines. This comparison may perhaps reveal correlated responses or tradeoffs in response to different xenobiotics, and also compare genes involved in susceptibility to paraquat at different concentrations.

D1356B *Effects of altered expression of apoptotic and autophagic gene products in novel Drosophila models of Parkinson disease*. *P. M'ANGALE*, B. STAVELEY. MEMORIAL UNIVERSITY OF NFLD, ST. JOHN'S, NL, CA.

The directed expression of genes associated with apoptosis and autophagy, two very important cellular processes, under the direction of the *Ddc-Gal4* transgene in dopaminergic (DA) neurons has distinct effects upon behavioural attributes of *Drosophila melanogaster*.

Buffy, the single pro-cell survival member of the *Bcl-2* family, extends lifespan and improves locomotor ability when overexpressed in the dopaminergic neurons. Furthermore, we showed that *Buffy* overexpression was sufficient to restore lifespan and age-dependent loss in locomotor ability in the robust *α-synuclein*-induced Parkinson disease model in Drosophila. In addition, the overexpression of the pro-apoptotic *Bcl-2* homologue *debcl* leads to a greatly shortened lifespan and severely reduced climbing ability, phenotypes that are strongly associated with Parkinson disease (PD), and may as well provide a novel model for PD.

Subsequently, we have been able to demonstrate that the directed inhibition of two autophagic genes; *Atg6* and *Pi3K59F*; apoptotic genes; *Bax inhibitor-1 (BI-1)*, *Lifeguard (LFG)*, *Growth hormone-inducible transmembrane protein (GHITM)*, *High temperature requirement A2 (HtrA2)*; and multifunctional genes; *pyridoxal kinase (Pdxk)* and *voltage-dependent anion channel (VDAC/porin)* results in shortened lifespans and defective climbing abilities. We show that the overexpression of the anti-apoptotic *Bcl-2* homologue *Buffy* in DA neurons when co-expressed with the apoptotic and autophagic genes suppresses the PD-like phenotypes described above.

The loss in function of a number of autophagic and apoptotic genes in the dopaminergic neurons of Drosophila leads to shortened lifespans and the progressive loss of locomotor abilities and *Buffy* is able to improve the "healthspan" of these flies.

Funded by a School of Graduate Studies Fellowship and by an NSERC Discovery Grant.

D1357C Polyglutamine tract expanded Androgen Receptor and SBMA: humans and flies. *S. MokHTAR*, M. Paliouras, M. Trifiro. McGill Univ., Montreal, Canada.

Introduction: The role of the androgen receptor (AR) in disease pathology is determined by its mutational status and androgen hormone-dependent activity. There is a class of mutations in the AR gene that results in an expansion of a polymorphic trinucleotide-CAG repeat, coding for a polyglutamine tract in exon 1 of the AR gene. Normally, the CAG tract is 20 repeats, whereas in the disease known as spinal and bulbar muscular atrophy (SBMA) the repeat is expanded to >37 repeats. SBMA is a motor neuron disease directly linked to an androgen-dependent toxic gain of function caused by the polyglutamine expansion in the AR. The characteristics of SBMA are weakness, atrophy, and fasciculations of bulbar, facial and limb muscles that are attributable to degeneration of lower motor neurons in the spinal cord and brainstem. Understanding the molecular pathological mechanisms the AR polyglutamine polymorphism disorder could yield potential candidate therapeutic targets.

Aims of the Study: The aim of this project is to reveal mechanistic pathways involved SBMA pathogenesis. We employed an AR transgenic *Drosophila* genetic interaction screen of homologues of protein interactors of the expanded polyglutamine AR (polyQ-AR).

Results: A proteomics-coupled systems biology approach was used to characterize the proteome of wild-type (WT)-AR vs. polyQ-AR complexes. Through this process we have identified a number of androgen-dependent protein interactors known to be involved in RNA splicing. To investigate the role of polyQ-AR in RNA splicing, we undertook a genetic interaction screen using an *UAS-polyQ-AR* and *UAS-WT-AR* humanized-Drosophila transgenic lines and crossing them to *UAS-RNAi* or enhancer trap lines of fly homologues to our polyQ-AR protein interactors, in the presence or absences of androgen hormone ligand. The genetic interaction screen has identified a number of suppressors of the androgen-dependent polyQ-AR phenotype.Most interestingly, we identified *HNRNPA2B1*gene to be the strongest genetic interactor. No observable phenotypes have been noticed in WT-AR files, supporting the novel gain-of-function properties of the polyQ-AR in RNA splicing.

Conclusions: The proteomics and systems biology approach has allowed us understand AR functionality beyond its classical role as a transcription factor. Using Drosophila as a genetic interaction screening tool, we have robustly explored a novel mechanistic property of polyQ-AR. Through these investigations, it is now clear that the polyQ-AR is involved in RNA splicing pathways and has provided new dimensions to our understanding of SBMA disease and potential therapeutic avenues.

D1358A Investigate the Effects of Cyclopamine on *Drosophila melanogaster* Model of Alzheimer's Disease. *P. T. Nguyen*[1], M. Sharoni[1], N. Latcheva[2], M. Krach[1], K. Innamorati[2], A. Saunders[1], D. Marenda[1,2,3]. 1) Drexel University Department of Biology, Philadelphia, PA; 2) Molecular Cell Biology and Genetics Program, Drexel University College of Medicine, Philadelphia, PA; 3) Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA.

Alzheimer's disease (AD), a progressive neurodegenerative disorder, is the most common cause of dementia in older adults. AD pathology includes formation of amyloid plaques, a major component of which is the Amyloid β peptide (Aβ) generated from sequential proteolytic cleavage of Amyloid precursor protein (APP) by β- and γ-secretases. Aβ accumulation is neurotoxic and leads to synaptic dysfunction, neuronal death, and learning and memory defects. Our lab identified the Smoothened receptor of the Sonic Hedgehog signaling pathway in a genetic screen as a regulator of APP metabolism. In a later study, Cyclopamine (an antagonist of the Smoothened receptor) was shown to alter the subcellular trafficking of APP C-terminal fragments (substrates for γ-secretase cleavage) in HeLa cells and primary rat cortical neurons. Thus, we hypothesized that Cyclopamine would have a similar mechanism of action in an *in vivo* AD model. To test this, we used transgenic *Drosophila melanogaster* which overexpress human APP and β-secretase in all postmitotic neurons under the control of the Gal4-UAS system. We showed that Cyclopamine rescues a number of defective phenotypes observed in our AD *Drosophila* model including external morphology, larval locomotion, adult motor reflex, and neuroanatomy. These data suggest Cyclopamine can be investigated further for AD therapeutics.

D1359B Altered metabolism in a TDP-43 model of ALS in Drosophila. *Abigail O'Conner*, Ernesto Manzo, Sylvia Zarnescu, Mary-Beth Roberts, Daniela Zarnescu. University of Arizona, Tucson, AZ.

ALS, or amyotrophic lateral sclerosis, is a fatal progressive neurodegenerative disease for which there is currently no cure. Several genes have been linked to ALS, including TDP-43, which encodes an RNA binding protein and has been associated with the vast majority of ALS cases. While only 2-3% of ALS cases harbor mutations in TDP-43, more than 95% of patients exhibit TDP-43 positive cytoplasmic inclusions. A *Drosophila* model of ALS using both wild-type and mutant TDP-43 expression has been shown to recapitulate several pathogenic hallmarks of the disease, including motor dysfunction and decreased survival.

Because metabolic changes have been seen in ALS patients, we have investigated whether similar defects are also present in our fly model. Indeed, metabolomics studies show that, as in ALS patients, flies expressing TDP-43 in motor neurons exhibit a significant increase in pyruvate, the end product of glycolysis, and alterations in the TCA cycle. Using a larval locomotion assay that quantifies differences in locomotor function, we have tested the effects of dietary changes on larvae expressing TDP-43 in motor neurons or glia. Increased dietary glucose appears to alleviate motor dysfunction in wild-type and mutant larvae (both in motor neuronal and glial expression), while also increasing survival time in adult flies. Consistent with these findings, expression of the glucose transporter GLUT4 in diseased larvae shows a similar improvement in motor function. Additionally, the introduction of different fats into the diet of larvae expressing TDP-43 in motor neurons appears to improve motor function at specific dosages. Taken together, these data suggest that the TDP-43 based fly model of ALS experiences specific alterations in metabolism that parallel the changes seen in patients and offer novel strategies for therapeutic and dietary intervention for ALS.

D1360C Assaying tissue-specific functions of SMN using *Drosophila* models of Spinal Muscular Atrophy. *A. Raimer*, A. G. Matera. University of North Carolina Chapel Hill, Chapel Hill, NC.

Survival Motor Neuron (SMN) protein is essential for development across metazoans, and in humans a reduction of SMN leads to Spinal Muscular Atrophy (SMA). SMA is the leading genetic cause of death in infants, characterized by degeneration of α-motor neurons and atrophy of proximal muscles. In severe cases, these children do not live past two years old. The reduction of SMN in these patients is due to mutations in the *Survival Motor Neuron 1 (SMN1)* gene, but how this disruption of SMN protein levels leads to SMA phenotypes is not well understood.

SMN is known to be involved in small nuclear ribonucleoprotein (snRNP) biogenesis, where it helps load Sm proteins onto snRNAs during snRNP maturation. At the same time, SMN has been implicated in alternative functions, such as mRNA transport and RNP assembly. It is unclear which SMN function is disrupted in SMA or what tissue it is disrupted in. Motor neurons and muscles are the tissues that show abnormal phenotypes in SMA patients, so it is possible that there is a cell autonomous function of SMN that is disrupted in these tissues. Another possibility is that the effect of SMN reduction is cell nonautonomous and a different cell type is losing an important function of SMN, indirectly affecting the motor neurons and muscles.

To test spatiotemporal functions of SMN, we are using *Drosophila* lines that contain mutations orthologous to SMA patient mutations. This set of lines has mutations in each of SMN's functional domains: Gemin2 binding domain, Tudor domain, and self-oligomerization domain.

Previously our lab has shown that these lines display a spectrum of viability phenotypes. We are using the GAL4-UAS system to express wild-type Smn in specific sets of tissues in the mutant backgrounds, then observe any changes in viability. Through these studies we will determine the minimal set of tissues that require functional Smn for proper development. We are also further characterizing these mutant lines by conducting locomotor assays and snRNP assembly assays. These assays will examine the effect of each mutated domain/residue on motor function and Smn's canonical role in snRNP biogenesis. Through these studies we hope to determine the tissues that are severely affected by SMN reduction, and learn more about SMN's function in these tissues.

D1361A Role of steroid-responsive Ecdysone (Ecd) pathway in Aβ42-mediated neurodegeneration. *Matthew Riccetti*[1], Ankita Sarkar[1], Kenneth Moberg[2], Madhuri Kango-Singh[1], Amit Singh[1]. 1) University of Dayton, Dayton, OH, United States; 2) Department of Cell Biology, Emory University School of Medicine, Atlanta, GA, United States.

Alzheimer's disease (AD) is a chronic neurodegenerative condition that affects nearly 44 million people worldwide. The hallmark of Alzheimer's pathology is the accumulation of extracellular Amyloid Beta 42 (Aβ42) protein plaques generated by defective endoproteolysis of amyloid precursor protein (APP) by α– and γ-secretase. We misexpressed human Aβ42 in the third instar larval eye imaginal disc of Drosophila. This stable transgenic line results in GMR-GAL4 driven Aβ42-mediated cell death in the eyes of nearly 100% of adult flies. In a forward genetic screen, we identified the Ecdysone signaling pathway as a modifier of neurodegeneration caused by Aβ42 accumulation in the eye. It has been shown that Ecdysone (Ecd) signaling pathway modulates Hippo transcriptional activity in imaginal disc cells. Our preliminary data suggests that upregulation of the involved Yki-Tai transcription complex rescues Aβ42-mediated neurodegeneration in our Drosophila eye model. We propose to understand the underlying molecular genetic mechanism responsible for Ecd/Hippo-mediated rescue of Aβ42 mediated neurodegeneration.

D1362B Investigating the role of glycogen accumulation in neurodegeneration within brain. *A. Rostami*[1,2], G. L. Boulianne[1,2]. 1) University of Toronto, Toronto, ON, CA; 2) The Hospital for Sick Children, Toronto, ON, CA.

Background: Aging is a complex process involving both genetic and environmental factors. Aging and neurodegeneration share some of these influential risk factors, with aging itself being the most robust risk factor for neurodegeneration. Several recent studies have demonstrated that aberrant accumulation of glycogen in neurons can lead to premature aging and neurodegeneration in both flies and mice. Moreover, granular glycogen bodies have also been observed in postmortem cerebral cortex sections of Alzheimer's disease (AD) and dementia patients. Whether glycogen accumulation in the brain occurs in all neurodegenerative diseases and contributes to disease pathogenesis remains unclear. Thus, my main objective is to determine whether accumulation of glycogen within the brain leads to premature brain aging and neurodegeneration.

Methods: To study the impact of increased levels of glycogen in brain aging and neurodegeneration two approaches are used. First, transgenes encoding constitutively active and catalytically dead versions of human muscle glycogen synthase (hMGS) were expressed within neurons of *Drosophila melanogaster* and an amyloglucosidase-based assay was used to measure glycogen levels from fly brains. The effect of glycogen accumulation was determined by measuring sensitivity to oxidative stress. Alternatively, the glycogen concentration in the heads of AD model flies will be manipulated using pharmacological approaches and its effects will be determined by measuring sensitivity to oxidative stress, lifespan and cognition using an olfactory learning assay.

Results: Flies overexpressing a constitutively active hMGS showed higher sensitivity to oxidative stress compared to control flies.

Conclusion: This study will show us whether glycogen accumulation within the brain is a pathogenic feature of neurodegeneration in AD and whether neurodegenerative phenotypes of AD can be ameliorated by reducing glycogen levels.

D1363C Understanding the role of Wingless (Wg) signaling pathway in Amyloid-beta 42 (Aβ42) mediated neurodegeneration in Alzheimer's Disease. *A. Sarkar*[1], M. Kango-Singh[1,2,3], A. Singh[1,2,3]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Program, University of Dayton, Dayton,OH; 3) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH.

Alzheimer's disease (AD), an age related progressive neurodegenerative disorder manifests as memory loss and reduced cognitive ability. One of the hallmarks of AD is formation of the Amyloid-beta (hereafter Aβ42) plaques, which initiates oxidative stress due to impaired signaling and finally leads to the death of neurons by unknown mechanism. However the exact mechanism causing cell death is still not well understood. We misexpressed high levels of human Aβ42 protein in the developing fly retina, which mimics AD like neuropathology. In a forward genetic screen we have identified members of highly conserved Wingless (Wg) signaling pathway as modifiers of the Aβ42 mediated neurodegeneration. Furthermore, Wg protein levels are upregulated in the dying cells marked by TUNEL staining. We have demonstrated that blocking Wg signaling pathway, by misexpressing negative regulator of Wg like Shaggy kinase (*sgg*) or a dominant negative form of *Drosophila* T-cell factor ($dTCF^{DN5}$) or blocking Wg transport specifically by downregulating Porcupine (using $porcupine^{RNAi}$) can rescue Aβ42 mediated neurodegeneration by reducing the number of dying cells and restoring the axonal targeting from the retina to the brain. We have developed a drug feeding regimen for flies and will test if we can use chemical inhibitors to block Wg signaling in neurons expressing high levels of Aβ42 and thereby prevent neurodegeneration in the *Drosophila* eye. We will test antagonists and agonist of Wg signaling to determine if they can work as chemical inhibitor/modifier of Aβ42 mediated neurodegeneration. The results from our studies will be presented.

D1364A A Drosophila model for neurodegeneration based on gain- and loss-of function of the Cdk5 activator, p35. *Arvind K. Shukla*, Joshua Spurrier, Edward Giniger. National Institute of Health, Bethesda, MD.

The incidence of neurodegenerative diseases rises in parallel to the aging of human being, but the intricate relationship between aging and neurodegeneration (ND) remains extremely murky. We are exploring this relationship in *Drosophila* that lack or overexpress *p35, a* neuronal specific activator of the *Cdk5* protein kinase. Deregulation of Cdk5 activity in humans has been associated with different neurological disorder such as Alzheimer's and Parkinson's, and either gain or loss of Cdk5 activity causes neurodegeneration in mouse models and neuronal loss in cultured mammalian neurons. We now describe a variety of clear neurodegeneration phenotypes in flies with altered expression of p35. Loss of dopaminergic (DA) neurons is the hallmark of Parkinson's disease, and we find significant DA neuron loss in the brain of *p35* deletion and over-expressing flies. DA neuron counting was done in the brains of 3, 10, 30 and 45 days old flies in control, a *p35* deletion mutant and strains that overexpress p35 modestly (2.5-3x). No significant DA neuron loss was observed in control flies till the age of 30 days. However, *p35* null and OE strains showed significant loss of neurons as early as 10days, with substantial loss by 30days, compared to the control. We also observed that the *p35* mutant and OE strains were sensitive towards oxidative stress generator (H_2O_2 and paraquat). In case of H_2O_2 challenge median survival of p35 mutant and OE were 36 and 48 hrs respectively, which was 60 hrs for age matched (30 days) control. *p35* mutant and OE strains also had higher level of reactive oxygen species in comparison to age- matched control (~1.5 and ~2.5x, respectively). Analysis of autophagy markers in the mutant brains revealed ~2.0x higher accumulation of Ref(2)P (homologue of mammalian p62) and similar increase in LC3 cleavage.The alteration in autophagy, is presumably linked to the ND, which we observed in our *p35* gain- and loss-of-function models. Finally, we observed an early onset of impaired locomotor performance in the mutant and OE strains as compared to control flies. All the phenotypes described above in the p35 mutant were significantly rescued by inserting transposon carrying the genomic *p35* locus, demonstrating the specificity of the phenotypes. These data suggest that analysis of the p35 model should be informative for unravelling the complex cellular endophenotypes associated with neurodegenerative disorders.

D1365B Illegally Parked: Investigating the role of Hip1 in a model of Parkinson Disease. *F. A. Slade*, B. E. Staveley. Memorial University of Newfoundland, St. John's, Newfoundland, CA.

Parkinson Disease (PD) is a progressive neurodegenerative disorder characterized by the gradual loss of motor control and cognitive functions. These symptoms, resulting from the breakdown of dopaminergic neurons, often present in mid-late life and may lead to premature death. A number of molecular pathways have been implicated in the progression of Parkinson Disease to provide a variety of treatment options that offer temporary relief from disease symptoms. The search for preventative therapies and cures for the sporadic and inherited forms of the disease is ongoing.

A polymorphism in the *Huntingtin Interacting Protein 1 related* (*Hip1r*) gene in mammals has recently been associated with PD but its role in disease progression remains uncharacterized. Directed manipulation of the single *Drosophila Hip1r* homologue *Hip1* in the dopaminergic neurons was carried out to provide an *in vivo* model of disease progression and symptom development. Investigation of motor ability and longevity of *D. melanogaster* upon overexpression and loss of function of *Hip1* was completed. Motor analysis was examined by ranking the climbing ability of flies weekly in a glass vessel of regulated size and conditions. Longevity was analyzed by scoring experimental genotypes every two days for the presence of deceased individuals, beginning at day two post-eclosion and continuing until the death of all experimental samples. Directed expression of *Hip1-RNAi* in the dopaminergic neurons decreased the locomotor ability of the flies and increased the average lifespan. Overexpression of *Hip1* in the dopaminergic neurons revealed the opposite effect, improving locomotor ability and slightly decreasing average lifespan. This suggests that a delicate balance of *Hip1* exists in the dopaminergic neurons, and alteration of expression influences the motor ability and life expectancy of *D. melanogaster*. Maintaining a healthy balance of *Hip1* in the dopaminergic neurons may offer a new therapeutic option for loss of locomotor ability and pre-mature death. Further investigation of *Hip1r* and its role in human disease progression is needed, and may be crucial to our understanding of PD, and perhaps Huntington Disease, in order to provide new therapeutic targets.

Funded by a School of Graduate Studies Fellowship to FAS and an NSERC Discovery Grant to BES.

D1366C Yorkie Regulates PolyQ Mediated Neurodegeneration in *Drosophila melanogaster*. *Madhu G. Tapadia*, Sandeep Dubey. Banaras Hindu University, Varanasi, 221005 Uttar Pradesh, INDIA.

Expansion of CAG trinucleotide repeats within open reading frame causes the protein to gain toxic properties resulting in dysfunction and degeneration of specific subpopulation of neurons in the brain. Hippo pathway regulates cell number by cell proliferation or cell death by regulating Yorkie, which is the major downstream target of Hippo pathway. Yorkie in turn, when activated leads to activation of *cyclin E*, microRNA *bantam* and apoptosis inhibitor *diap1* which are involved in cell proliferation and apoptosis. Here, we show that Yorkie is robust genetic modifier of polyQ mediated neurodegeneration. Overexpression of Yorkie in polyQ background reduces the severity of pathogenecity caused by inclusion bodies while downregulation has the opposite effect. Yorkie suppresses cytotoxic effect of polyQ by Cyclin E and bantam but does not show significant rescue by Diap1, suggesting that apoptotic pathway is not involved. PolyQ inclusion bodies causes overexpression of Antimicrobial peptides in neurons by activating the Nuclear Factor-κB (NF-κB) transcription factor Relish, a key activator of IMD pathway. We show upregulation of Yorkie downregulates expression of Relish and other components of Toll and IMD pathway in eye imaginal disc. Downregulation of antimicrobial peptides significantly rescues polyQ phenotypes. Finally we show that polyQ interacts with Yorkie to modulate its functions making it a strong genetic modifier of polyQ mediated neurodegeneration by regulating cell cycle genes and innate immune response. .

D1367A Restoration of mitochondrial morphology rescues impaired axonal distribution caused by loss of Opa1 and Mitofusin in Drosophila melanogaster. *T. Trevisan*, A. Daga. IRCSS E. Medea, Ass. La Nostra Famiglia, Conegliano, Italy.

Mitochondrial dynamics play a critical role in the control of organelle shape, size, number, function and quality control of mitochondria. It is regulated by several GTPases that play an important role in fusion and fission processes. In mammals, mitochondrial fusion is controlled by Mitofusin 1 (Mfn1), Mitofusin 2 (Mfn2) and Optic atrophy protein 1 (Opa1), while mitochondrial fission is regulated by Dynamin related protein 1 (Drp1). A significant imbalance of mitochondrial fusion and fission results in iperfused or fragmented mitochondria.

We use *Drosophila melanogaster* as model to study how loss of fusion and fission protein modify the axonal distribution and motility of mitochondria.

We demonstrate that loss of Marf (Mitochondrial associated regulatory factor) or Opa1 cause accumulation of mitochondria in the soma, a severe depletion of mitochondria in neuromuscular junctions (NMJs) and reduced mitochondrial motility. Loss of Drp1 rescue Marf and Opa1 phenotype. This suggests that restoring mitochondrial dimension and morphology is necessary to maintain normal mitochondrial trafficking in axons. .

D1368B The influence of the altered regulation of histone acetyltransferase Tip60 on degeneration in a *Drosophila* model of Machado-Joseph Disease. *A. Watterson*, J. Warrick. University of Richmond, Richmond, VA.

Machado-Joseph Disease (MJD) is a human spinocerebellar ataxia caused by a polyglutamine repeat expansion in the Ataxin-3 protein that leads to motor neuron degeneration. The mechanisms by which the toxic ATX3 protein causes degeneration are not yet known. In MJD, the ATX3 protein misfolds, leading to the formation of aggregates. Histone acetyltransferases (HATs) may get trapped in the aggregates, therefore preventing proper regulation of protein acetylation and gene transcription. As a result, neurons may become dysfunctional and die due to transcriptional dysregulation or other dysregulation. The histone acetyltransferase Tip60 functions in neuronal gene control and apoptosis, and elevated levels have been found to rescue axonal transport defects, characterized by locomotive phenotypes, in a *Drosophila melanogaster* model of Alzheimer's disease. However, HAT Tip60 has not yet been studied in MJD. Polyglutamine expansions have been associated with axon transport defects. This research investigates the effect of up-regulating and down-regulating HAT Tip60 in a MJD *Drosophila melanogaster* model to determine how levels of Tip60 expression affect the progression of the disease. This research may lead to an effective target for MJD, Alzheimer Disease, and other neurodegenerative diseases.

D1369C *In vivo* evidence for a moving HTT-Rab4 vesicle complex in *Drosophila* larval axons. *J. A. White*, K. Zimmerman, H. G. Hoffmar-Glennon, S. Gunawardena. University at Buffalo, Buffalo, NY.

Huntingtin (HTT), the protein responsible for Huntington's disease (HD), is ubiquitously expressed and enriched in neurons. HTT associates with microtubule motors, kinesin-1 and dynein, and is involved in the movement of vesicles within axons. However the type of vesicle or cargo complex that HTT transports within axons is unknown. We previously found that reduction of *Drosophila* HTT perturbed the movement of Rab4 GTPase- containing vesicles in larval axons. Simultaneous dual-view imaging revealed that HTT and Rab4 likely move together within *Drosophila* larval axons. Sub-pixel co-localization analysis revealed that Rab4, HTT, and motor proteins co-localize suggesting that a HTT-motor complex likely exists *in vivo*. Reduction of the huntingtin interacting protein 1 (HIP1) and a known Rab effector, Rip11, perturbed the movement of both HTT and Rab4 *in vivo*. However, reduction of Milton (a protein with some sequence homology to HAP1) and Nemo (a Rab8 effector) had no effect. Taken together, our observations suggest that HIP1 and Rip11 may aid in linking HTT-Rab4-containing complexes to motor proteins. Since expansion of poly-glutamine repeats in the context of human HTT perturbed the motility of Rab4, perhaps HTT normally functions to facilitate Rab4 motility within axons for functions at the synapse.

D1370A Identification and Characterization of the ΔExon7 *PINK1* Mutation Associated with Parkinson's Disease in *Drosophila* and Mammalian Cells. *Huan Yang*[1,2*], Ting Zhang[1,2], Susan Perlman[1], Brent Fogel[1], Ming Guo[1,2,3,4,5]. 1) Department of Neurology; 2) Molecular, Cellular and Integrative Physiology; 3) Department of Molecular and Medical Pharmacology; 4) Brain Research Institute, David Geffen School of Medicine; 5) Molecular Biology Institute, UCLA, Los Angeles, CA.

Mutations in *PTEN-induced Kinase 1* (*PINK1)* and *parkin* lead to autosomal recessive forms of Parkinson's disease (PD). *PINK1* encodes a putative Ser/Thr kinase with a mitochondrial targeting sequence, and *parkin* encodes an E3 ubiquitin ligase. We and others have previously shown that *PINK1* and *parkin* function in the same genetic pathway to regulate mitochondrial integrity and quality in *Drosophila*. *PINK1/parkin* null mutant flies show motility defects and apoptotic muscle death, and mitochondria in neurons and muscles exhibit abnormal morphology and compromised function. Loss of *PINK1* in human cells block Parkin-mediated autophagy of damaged mitochondria (mitophagy), an important mechanism of mitochondrial quality control. Here we identified a new familiar form of *PINK1* mutation in a PD patient family. We found that two PD patients in this family carried two different *PINK1* mutant alleles: one was a previously characterized *PINK1* L347P allele; the other was an uncharacterized splicing mutant resulting in skipping of *PINK1* Exon7. As *PINK1* Exon7 encodes the C-terminus of the kinase domain, we investigated whether ΔExon7 *PINK1* is a loss-of-function allele. We showed that overexpression of human ΔExon7 *PINK1* failed to rescue mitophagy defects in *PINK1* knockout HeLa cells, in contrast to the human wildtype *PINK1*; overexpression of the *Drosophila* homolog of ΔExon7 *PINK1* also failed to rescue mitochondrial morphology defects and apoptotic muscle death in *PINK1* null mutant flies, in contrast to the wildtype *Drosophila PINK1*. In this study, we validated two model systems (*Drosophila* muscle *in vivo* and human cell-based mitophagy assay) for future characterization of newly discovered PD-linked mutations.

D1371B Exploring the molecular basis of the PCH-associated RNA Exosome phenotypes in *Drosophila* . *Xue Yang*[1,2], Vafa Bayat[1], Zhihao Wu[1], Yen-Chi Wu[1], Hannes Vogel[1], Bingwei Lu[1]. 1) Stanford School of Medicine, Stanford, CA; 2) Peking University, Beijing, China.

Pontocerebellar Hypoplasia (PCH) is a rare congenital disorder displaying autosomal recessive inheritance characterized by hypoplasia and atrophy of the cerebellar cortex, dentate nuclei, pontine nuclei and inferior olives. Two of the RNA exosome subunits, EXOSC3 and EXOSC8, have been identified as mutated in PCH type 1 patients, who also exhibit a spinal muscular atrophy phenotype indicating possible motor neuron involvement too. *Drosophila* has a predicted single copy of all of the known RNA Exosome subunits, and is therefore an excellent model organism to investigate potential molecular mechanisms. We have obtained and are generating mutants using imprecise excision and CRISPR in as many of the RNA exosome subunits as possible with the purpose of comparing their null phenotypes. We have already discovered in rrp4 mutants that the expression of key neurodegenerative disease-related proteins is altered, and are further investigating this. Using this data, we are targeting specific pathways to determine whether their RNAi phenotypes can be suppressed using genetic interactions and determining whether the molecular mechanisms are similar to those we are in parallel studying in the PCH-associated TSEN complex. We are in parallel determining whether the cellular and molecular phenotypes we observe are also seen in human cell lines. We hope that our data will give clues to potential treatments, which are presently only supportive in nature.

D1372C Translation Dysregulation in ALS. *S. Yao*, A. Coyne, D. Zarnescu. University of Arizona, Tucson, AZ.

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that affects the lives of at least 30,000 people in the United States, annually. It is often categorized by the progressive neurodegeneration of motor neurons. TAR DNA Binding Protein (TDP-43) is normally found within the nucleus having known roles in RNA splicing as well as DNA-binding. Mislocalization of TDP-43 to the cytoplasm, either due to mutations or to environmental stressors leads to accumulation of TDP-43 in cytoplasmic inclusions. Notably, in the cytoplasm, TDP-43 associates with RNA stress granules and also affects mRNA translation, both of which are thought to contribute to disease pathology. In order to study the effects that cytoplasmic TDP-43 mutations have on translation, a technique called polysome fractionation is utilized. This technique uses sucrose gradients to effectively separate different ribosomal populations. The mRNAs that are bound to multiple ribosomes, or polysomes, are separated from the mRNAs bound to single ribosomes. This allows the pinpointing of specific defects in protein production as well as potential explanations to how it may affect cellular metabolism. Furthermore, this technique is able to provide information about the halting of protein production in response to stress which is a potential factor that contributes to ALS. Western blotting and quantitative PCR (qPCR) allow us to observe the protein and transcript distribution within the polyribosome fractions, respectively. Using these approaches we found that TDP-43 associates with both translating polyribosomes as well and untranslated fractions (RNP and the ribosomal subunits). qPCR of *futsch*, an mRNA target of TDP-43 showed a shift in transcript levels from actively translated to untranslated fractions in the context of TDP-43, indicating that *futsch* mRNA is being repressed by TDP-43 in motor neurons. Taken together, these findings indicate that TDP-43 regulates the translation of specific mRNAs and defects in translation may contribute to ALS. Experiments will focus on additional candidate mRNA targets that will be tested for their distribution within polysome fractionations to determine their translational status in disease. Candidate mRNAs that are identified by Translating Ribosome Affinity Purification (TRAP) that also associate with TDP-43 in complex will be tested. Specifically, qPCR will used to distinguish shifts in translational targets in polysomes. Targets that are downtranslated should exhibit a shift into the RNP fractions while uptranslated targets will exhibit a shift into the polysomes.

D1373A Rescue of neurotoxicity in a TDP-43-based Drosophila model of ALS by a 4-aminoquinoline analog. *Ben Zaepfel*[1], Alyssa Coyne[1], Joel Cassel[2], Allen Reitz[2], Daniela Zarnescu[1]. 1) University of Arizona, Tucson, AZ; 2) ALS Biopharma, LLC, Doylestown, PA.

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons. TAR DNA-binding protein (TDP-43) is an RNA and DNA binding protein that has been implicated in ALS, both as a causative factor and at the level of pathology. Although TDP-43 has been shown to play a key role in RNA metabolism, its mechanism is not fully understood. Specifically, evidence has shown that TDP-43 binds TG-rich sequences within RNA targets. In the context of disease, this binding can lead to alterations in splicing and/or proper regulation of its targets, and is partially responsible for the neurotoxicity that is associated with ALS. A 4-aminoquinoline analog (i.e. AAQ-2) has been shown to inhibit the binding of TDP-43 to TG oligonucleotides. To evaluate the effect of this small molecule in vivo, we administered AAQ probes to larvae expressing wild-type or mutant TDP-43 in motor neurons (D42 GAL4>TDP-43). Our experiments show that feeding of AAQ-2, but not a structurally similar negative control (AAQ-9), rescues the lethality caused by overexpression of TDP-43 in motor neurons. Additionally, AAQ-2 feeding also leads to improved locomotor function of larvae, as well as increased lifespan of flies overexpressing both wild-type and mutant TDP-43. In exploring the role of TDP-43-associated proteins in mediating the protective effect of AAQ-2 in motor neurons, we find that rescue of TDP-43-induced neurotoxicity by AAQ-2 is dependent in Fragile X Mental Retardation Protein (FMRP) in a TDP-43 variant-dependent manner. Currently, experiments are being performed to determine the effect of AAQ-2 on the solubility of full-length TDP-43 and its cleaved C-terminal fragment within motor neurons. These results provide insight into the role of TDP-43 in RNA metabolism, as well as suggest a possible therapeutic strategy for TDP-43-based ALS and related neurodegenerative diseases.

D1374B p97/VCP overexpression suppresses the mitochondrial defects in *PINK1*, *parkin* and *parkin mul1* mutants. *T. ZHANG*[1], M. Guo[2]. 1) UCLA,Brain Research Institute, Los Angeles,U.S.A; 2) Neurology and Pharmacology, UCLA ,Los Angeles, U.S.A.

Mutations in *PINK1* and *parkin* lead to autosomal recessive forms of familial Parkinson's disease (PD). Studies in *Drosophila* first showed that *PINK1* and *parkin* function in the same pathway*, with PINK1* positively regulating *parkin* to control mitochondrial integrity and quality control. Mitochondrial morphology is maintained by a dynamic balance of mitochondrial fusion and fission. Mitofusin (Mfn) is a GTPase located on the mitochondrial outer membrane and controls mitochondrial fusion. Importantly, downregulation of Mfn suppresses the mutant phenotypes in

PINK1 and *parkin* mutants. Parkin, an E3 ubiquitin ligase, degrades Mfn. Mul1 is another E3 ligase that functions in parallel to Parkin to degrades Mfn. *parkin mul1* double mutants have more severe mitochondrial phenotypes and much increased Mfn levels than those flies lacking either *parkin* or *mul1* alone. p97/cdc48/Valosin-containing protein (VCP) encodes a highly conserved and abundant AAA+ ATPase which participates in multiple cellular processes. We have found that overexpression of VCP results in a dramatic rescue of defects of mitochondrial integrity, cell death and tissue damage due to lack of *PINK1* and *parkin.* VCP overexpression significantly decreases Mfn levels in wildtype flies. Overexpression of VCP also decreases the accumulated Mfn levels in *PINK1* or *parkin* null mutants. In addition, VCP overexpression suppresses the mitochondrial morphological defects in *parkin mul1* double mutants. These results suggest that VCP-dependent suppression of *PINK1* and *parkin* phenotypes occurs through downregulaton of Mfn protein level.

D1375C ER shaping protein REEP1 regulates neuronal lipid droplets *in vivo*. *N. D'Elia*[1], S. Gumeni[2], M. Fantin[2], A. Forgiarini[1], M. Corrà[2], A. Martinuzzi[2], G. Orso[1,2]. 1) Institute of Pediatric Research, Padova, PD, IT; 2) Scientific Institute IRCCS Eugenio Medea, Conegliano, TV, IT.

Defects in endoplasmic reticulum (ER) membrane shaping and its interactions to other organelles seem to be crucial mechanisms underlying Hereditary Spastic Paraplegia (HSP) neurodegeneration.

Here we report the analysis of a *Drosophila* model for HSP caused by mutations in the *SPG31* gene, which are linked to autosomal dominant HSP. SPG31 codifies for REEP1, which is a transmembrane protein belonging to the TB2/Dp1/HVA22 family. REEP1 interaction with atlastin-1 (*SPG3A*) and spastin (*SPG4*), the other two major HSP linked proteins, has been demonstrated to modify ER architecture. The last evidence that both atlastin-1 and REEP1 play a role in lipid droplets (LDs) biology prompted us to investigate the *in vivo* role of REEP1 in ER membrane shaping and LDs biogenesis using *Drosophila melanogaster* as a model organism. We show that *Drosophila* REEP1 mutant flies present altered ER membrane morphology in neurons and reduced LDs number in both neuronal and non neuronal tissues. In addition, overexpression of wild type DREEP1 affects LDs number and size. Furthermore, we generated and *in vivo* expressed the mutated pathological form of DREEP1 p.P19R. The p.P19R missense mutation increases LDs size, reduces their number and modifies DREEP1 subcellular localization relocating it from ER to LDs membrane. Modification of LDs biogenesis is also associated to triglyceride levels perturbation *in vivo*, suggesting an altered lipid metabolism.

Our data demonstrate that REEP1 is involved in ER morphogenesis and LDs biogenesis/turnover in neuronal tissues. Our findings support and highlight the importance of lipid metabolism in neural functionality and the neurodegeneration mechanism of HSP disorder. Therefore, lipid metabolism can be considered as a novel HSP target towards which pharmacological efficient treatments could be developed.

D1376A Analysis of glucose metabolism during pathogenesis of Spinocerebellar Ataxia Type 1. *J. R. Diaz*[1,2], A. Perez[1,2], T. Gallego-Flores[1,2], Y. Wan[1,2], T. Inoue[1], A. Chai[1,2], C. Karakas[1,2], I. Al-Ramahi[1,2], M. Maletić-Savatić[1,2], M. Gaber[1], R. Samaco[1,2], Z. Liu[1,2], H. Orr[3], J. Botas[1,2]. 1) Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan NRI, Texas Children Hospital. Houston, TX; 3) University of Minnesota, Institute for Translational Neuroscience, Minnesota.

Spinocerebellar ataxia type 1 (SCA1) is one of nine Polyglutamine Diseases caused by CAG expansion in the coding region of the corresponding gene. In SCA1, CAG expanded repeats cause an abnormally long glutamine tract in ATXN1 protein and trigger a gain of function pathogenic mechanism that leads to a progressive neurodegenerative disorder. The brain regions primarily affected are the cerebellum and the brainstem. Previous study reported that insulin sensitivity and insulin secretion are abnormal in SCA1 patients. We hypothesize that expression of mutant ATXN1 impairs glucose metabolism and use multiple approaches to test this hypothesis using mice and Drosophila disease models. Gene expression analyses in SCA1 mice and flies suggest glucose uptake deficiency in neuronal cells. Confirming these results, positron emission tomography imaging suggests lower glucose level in the cerebellum of SCA1 transgenic mice. Metabolomic analyses in SCA1 fly neurons reveal a decrease in glucose metabolism metabolites. We carried out a genetic screen in Drosophila of all genes encoding glycolytic enzymes for potential modifier genes of SCA1 pathogenesis. We found that knocking down glycolytic genes ameliorates neurodegeneration by reducing steady-state levels of mutant ATXN1 protein. Together these data suggest that glucose metabolism impairments contribute to SCA1 pathogenesis and reveal new therapeutic approaches to decrease ATXN1 neurotoxicity.

D1377B *Drosophila* spastin regulates lipid droplets and lipid metabolism *in vivo*. *A. Forgiarini*[1], S. Gumeni[2], N. D'Elia[1], A. Daga[2], G. Orso[1,2]. 1) Institute of Pediatric Research, Padova, PD, IT; 2) Scientific Institute IRCCS Eugenio Medea, Conegliano, TV, IT.

Hereditary spastic paraplegias (HSPs) are a group of neurodegenerative diseases characterized by progressive weakness and spasticity of the lower limbs, owing to progressive retrograde degeneration of the long corticospinal axons. Mutations in the gene *spastin* (*SPG4*) are responsible for the prevailing form of pure HSP. Spastin is an ATPase that contains a microtubule-interacting domain and has been implicated in several processes involving remodeling of membrane structures. Moreover, spastin harbors a lipid droplet (LD) targeting sequence that allows its targeting to LDs surface. Our group has previously shown that the highly conserved *Drosophila spastin* homolog (*Dspastin*) regulates microtubule stability and synaptic function of the *Drosophila* larval neuromuscular junction.

Considering the lipid droplets formation and maintenance as a possible common pathway in HSP neurodegeneration, we wanted to further investigate a possible role of spastin in lipid metabolism.

We observed that ubiquitous overexpression of Dspastin in *Drosophila* increased triacylglycerol levels and led to bigger and less numerous LDs in fat bodies. In contrast, Dspastin specific overexpression in skeletal muscles or nerves increased LDs number. Downregulation of Dspastin and expression of a dominant-negative variant (Dspastin K467R) decreased LDs number in *Drosophila* nerves, skeletal muscles and fat bodies, and reduced triacylglycerol levels in the whole larva.

Our results suggest the role of spastin as a regulator of lipid metabolism, enforces and strongly support the recent discoveries that dysfunction of LDs in axons may contribute to the pathogenesis of HSP.

D1378C Uncovering cellular energetics at the neuromuscular junction in a Drosophila model of ALS. *E. Manzo*[1], I. Lorenzini[2], A. Joardar[1], A. O'Conner[1], J. Barrows[1], R. Sattler[2], D. Zarnescu[1]. 1) University of Arizona, Tucson, AZ; 2) Barrow Neurological Institute, Phoenix, AZ.

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's Disease, is a fatal neurodegenerative disorder affecting upper and lower motor neurons. TAR DNA-binding protein 43 (TDP-43) is found in cytoplasmic inclusions in almost all non-SOD1 mediated ALS cases and is thought to play a major role in pathogenesis of the disease. Our lab has previously shown that overexpression of either wild type or mutant human TDP-43 in motor neurons of *Drosophila* melanogaster induces motor deficits and reduces lifespan. Using this model we have performed global metabolomics profiling and identified several significant changes consistent with alterations in glucose and lipid metabolism. Specifically, increased pyruvate in both TDP^{WT} and disease associated TDP^{G298S} models is suggestive of altered glucose metabolism. We also found increased tricarboxylic (TCA) cycle intermediates and pyruvate, which are also upregulated in plasma from ALS patients. Based on these preliminary results we hypothesize that improving glucose and lipid metabolism through genetic and dietary intervention can provide protection against neurodegeneration. We employed molecular and genetic techniques to determine the basis of altered glucose metabolism. Our preliminary data indicate that a high sugar diet, or the genetic expression of either the human glucose transporters 3 or 4 (Glut3 or Glut4) in motor neurons, suppresses toxic effects caused by of TDP-43. Additionally, Glut3 expression is altered in both fly and human iPS motor neurons (MNs) . To further test whether the expression of TDP-43 affects glucose transporter dynamics, we have used total internal reflection fluorescence (TIRF) microscopy, and found that in primary MNs expressing TDP^{WT}, there are comparable levels of Glut4-GFP at the plasma membrane immediately after insulin stimulation. In contrast, 14 min after stimulation, Glut4-GFP persists at the surface in TDP-43 expressing cells but not in controls. These data suggest Glut3/Glut4 alterations in expression and dynamics, in both both fly and iPS MNs, and are consistent with defects in glycolysis identified through metabolomics. Indeed, pfk mRNA, a key indicator of glycolytic activity is significantly upregulated in TDP-43 expressing flies and iPS MNs with TDP-43 pathology. Taken together, our findings indicate specific metabolic alterations in ALS and highlight the predictive power of *Drosophila* as a model organism for human disease.

D1379A A Ketogenic Dietary Supplement Eliminates Seizure-Like Activity and Paralysis in the Bang-sensitive (BS) Paralytic Mutants. *M. Nelson*, C. Radlicz, J. Rubera, T. Taylor, A. Chambers, C. Bittner, D. Kuebler. Franciscan University of Steubenville, Steubenville, OH.

The *Drosophila* Bang-sensitive (BS) paralytic mutants undergo seizure-like activity (SLA) and paralysis following a variety of insults such as mechanical shock. While the etiology underlying this defect is poorly understood, a number of recent studies in our lab have demonstrated that metabolic and dietary alterations can suppress, but not complete eliminate, SLA and paralysis in these mutants. Given that a ketogenic diet is known to reduce seizures in many children with refractory epilepsy, we examined the effect this diet had on the BS mutants *easily-shocked (eas), bang-senseless (bss),* and *technical knockout (tko)*. These mutants were fed a standard cornmeal/yeast/sugar diet supplemented with 10% KetoCal 4:1, a commercially available ketogenic formula consisting of a 4:1 ratio of fats to carbohydrates. Newly eclosed BS flies were fed this diet for 3, 5 or 7 days and all three genotypes displayed significant reductions in SLA and paralysis following mechanical shock. After only 3 days on the diet, over 90% of *tko* flies no longer exhibited SLA or paralysis, and complete suppression of the BS phenotype was seen by day 7. In the case of *eas*, only 30% still displayed SLA after 3 days on the diet and SLA was completely suppressed by day 7. The *bss* flies showed a similar but less robust reduction of SLA on the diet as 35% of flies still exhibited SLA and paralysis following 7 days on the diet. In addition to reducing SLA, the diet partially rescued the reduction in basal activity that is normally seen in the BS mutants. Taken together, the data indicates that the BS mutants represent an attractive model for investigating the underlying mechanism by which the ketogenic diet can suppress seizures.

D1380B Tissue specific overexpression of *c-myc* mitigates human poly(Q) induced neurodegeneration in *Drosophila* disease model. *K. Raj*, S. Sarkar. University of Delhi, New Delhi, INDIA.

Polyglutamine or poly(Q) disorders such as Spinocerebellar ataxia(s) (SCAs), Huntington's disease (HD), Spinal and Bulbar Muscular Atrophy (SBMA), Dentatorubral pallidoluysian syndrome (DRPLA) etc. represent a class of dominantly inherited neurodegenerative disorders which develop due to expansion of glutamine (Q) repeats in the coding region of target gene. The key factors for poly(Q) disease pathogenesis involve mis-folding of the mutated poly(Q) bearing protein and their subsequent accumulation in the cell in the form of inclusion bodies, which in turn leads to cellular toxicity and neurodegeneration. However, in spite of several pointers, the precise mechanism of poly(Q) disease pathogenesis and their suitable therapeutic approaches remains elusive. While finding a suitable drug target, we have demonstrated earlier that tissue specific upregulation of *Drosophila myc* (a homologue of human *c-myc* proto-oncogene) dominantly suppresses poly(Q) mediated cellular toxicity *via* histone acetylation and global transcriptional upregulation. Further, the rescue potential of human *myc (c-myc)* in alleviation of poly(Q) mediated neurotoxicity was examined utilizing multiple *Drosophila* transgenic lines expressing three different isoforms of transcripts encoded by human *c-myc gene* under the control of UAS promoter. Extensive genetic crosses, several immunostaining assays and appropriate microscopy techniques revealed that targeted over-expression of human *myc* (*c-myc*) also mitigates poly(Q)-induced cellular toxicity and neurodegeneration in SCA3 and HD models of *Drosophila.* Subsequent investigations suggest that transactivation domain of c-Myc protein which helps in restoring the poly(Q) induced cellular transcriptional impairments might be essential to achieve the rescue events.

We, therefore, propose that enhanced level of *c-myc* make a significant impact on the pathogenesis of human poly(Q) disorders and could be explored as a potential therapeutic target.

D1381C Defining the transcriptional defects of KDM5 mutations associated with Intellectual Disability. *s. zamurrad*, X. Liu, J. Secombe. Albert Einstein College of Medicine, bronx, NY.

KDM5 proteins are multi-domain transcriptional co-factors that function by recognizing and enzymatically altering specific histone modifications. There are four broadly expressed KDM5 orthologs in mammalian cells (KDM5A-D) and a single KDM5 in Drosophila. Emphasizing the importance of KDM5 proteins, KDM5A and KDM5B are over expressed in a number of metastatic cancer types, and KDM5A, B and C are mutated in patients with intellectual disability (ID). To-date, 30 mutations in KDM5C, 7 in KDM5B and 1 in KDM5A have been identified in patients with either syndromic or non-syndromic ID. How mutations in KDM5 family genes result in cognitive phenotypes remains elusive.

As a first step in establishing Drosophila as a model system to understand KDM5-induced ID, we defined direct KDM5 target genes in adults, as this is the stage used to model cognitive disorders. By combining RNA-seq from kdm5 hypomorphic adults with anti-KDM5 ChIP-seq, we identified numerous direct targets. Among these were several genes previously implicated in neurological disorders. Using *Drosophila,* which encodes a single KDM5 protein, our lab carried out RNA-sequencing experiments using *kdm5* hypomorphic adult flies. This revealed that KDM5 is required for the activation of several genes previously implicated in intellectual disability disorders such as fragile-X syndrome, Down syndrome, Autism and Alzheimer's disease. Anti-KDM5 ChIP-seq experiments from wildtype flies demonstrate that many of these genes are direct KDM5 targets. Because patients with mutations in KDM5 genes show cognitive impairment, we used a behavioral assay in flies and found that the *kdm5*^{hypomorph} have impaired appetitive-olfactory associative learning. To complement our analyses of kdm5 hypmorphic mutant adults, we have generated seven fly strains harboring mutations in KDM5 that are analogous to disease-associated missense mutations in KDM5 proteins. 6/7 of the ID mutants generated thus far are expressed at wild type levels re-enforcing that these mutations affect function and not protein stability. To-date, we have examined one ID mutant fly strain in more detail and found it to have a severe learning and memory defect. We are currently testing other ID allele fly strains for similar cognitive impairment, and defining the transcriptional defects in these strains. These analyses will allow us to define for the first time whether similar or distinct target genes are affected by disease-associated alleles. These studies will allow us to define the mechanistic link between KDM5 dysfunction and intellectual disability.

D1382A Use of *Drosophila* wing discs as a model to study the function of miRNAs in epithelial cell invasion. *C. Chang*, F. Chang, S. Cheng, Y. Su, Y. Lee, G. Khoo, Y. Huang, Y. Tsai. Department of Life Science, Tunghai University, Taichung, Taiwan.

Most solid tumors are derived from epithelial tissues. The epithelial cells are highly polarized and hold together by junction proteins. During tumorigenesis, multiple steps are required to transform epithelia into tumor cells. The molecular mechanism of cell migration/ invasion is extensive studied in the culture cells. However, it is difficult to trace the process of tumorigenesis *in vivo*. *Drosophila* wing disc is a monolayer epithelium and pattern formation of wing discs is well studied. miRNAs are endogenous noncoding RNA. Mature miRNAs are 21-22 nucleotides and negatively regulate gene expression at posttranscriptional level. Several miRNAs function as oncogenes. In this study, we use *Drosophila* wing epithelia as a model to establish a system to study whether miRNAs induce tumorigenesis and promote the epithelial cells into invasive cells. To explore whether expression of miRNAs promotes epithelial cells into invasive cells, we use the GAL4/UAS system to express miRNAs in developing wing cells. *dpp-GAL4* is expressed in the anterior/posterior boundary. We expressed UAS-miRNA and UAS-GFP by the *dpp-GAL4* driver. The GFP reporter marks the miRNA expression cells. If the miRNAs promote epithelial cell invasion, the GFP expression cells will move from anterior/posterior boundary to the lateral regions. One hundred and fifty UAS-miRNA lines were screened. Preliminary, we isolated several microRNAs, *mir-8*, *mir-274, mir-310, mir-318* and *mir-1000* which can induce cell migration/invasion in wing epithelial cells. We further examine the epithelial and mesenchymal markers and possible downstream signalings induced by microRNAs. In this study, we established a novel system to study the function of microRNAs on cell invasion in *Drosophila* wing epithelial cells. This system will be useful to study the roles of *microRNAs* and their human *homolog* on tumor metastasis.

D1383B *Bicaudal C* mutation causes *myc* and TOR pathway up-regulation and Polycystic Kidney Disease-like phenotypes. *C. Gamberi*[1], D. Hipfner[2], M. Trudel[2], W. D. Lubell[3]. 1) Concordia University, Montreal, PQ, CA; 2) Institut de recherches cliniques de Montréal, 110 Pine Avenue West, Montréal, QC H2W 1R7, Canada; 3) Département de Chimie, Université de Montréal, P.O. Box 6128, Station Centre-ville, Montréal, QC H3C 3J7, Canada.

Progressive cystic kidney degeneration underlies diverse renal diseases, including the most common cause of kidney failure, autosomal dominant Polycystic Kidney Disease (PKD). Genetic analyses of patients and animal models have identified several key drivers of this disease. The precise molecular and cellular changes underlying cystogenesis remain, however, elusive. *Drosophila* mutants lacking the translational regulator Bicaudal C (BicC, the fly ortholog of vertebrate *BICC1* implicated in renal cystogenesis) exhibited progressive cystic degeneration of the renal tubules (so called "Malpighian" tubules) and reduced renal function. BicC bound to *myc* mRNA in tubules. Elevation of Myc protein levels caused tubular degeneration in *BicC* mutants. Activation of the Target of Rapamycin (TOR) pathway, another common feature of PKD, was found in *BicC* mutant flies. In flies rapamycin administration reversed substantially the cystic phenotype and improved survival. New mechanistic insight on *BicC* function leads us to propose *Drosophila* as a genetically tractable model for dissecting the evolutionarily-conserved molecular mechanisms of renal cystogenesis and for chemical and genetic screens to identify modifiers of the phenotype towards novel therapeutic strategies.

D1384C Short amyloid-β peptides attenuate amyloid-β42 toxcitiy *in vivo*. *B. D. Moore*, J. Martin, L. deMena, J. Sanchez, P. Cruz, C. Ceballos, A. Rosario, C. Janus, D. Rincon-Limas, P. Fernandez-Funez, T. E. Golde. University of Florida, Gainesville, FL.

An attractive therapeutic strategy to treat Alzheimer's Disease is to halt the accumulation of amyloid plaques by decreasing the production of Aβ1-42. A class of compounds called g-secretase modulators (GSMs) shift the g-secretase cleavage site of amyloid precursor protein resulting in

a selective decreased production of amyloid beta (Aβ) peptide 1-42 and a concomitant increased production of the shorter Aβ peptides, Aβ1-37, Aβ1-38 and Aβ1-39. However, the biological significance of these peptides is still unclear. Our initial studies with transgenic mouse models suggest that one such short peptide, Aβ1-40, is a robust inhibitor of Aβ1-42 deposition *in vivo*. The last two hydrophobic residues at the C-terminal of Aβ1-42 are proposed to be critical for its enhanced rate of nucleation. Therefore we **hypothesize** that shorter Aβ peptides are anti-amyloidogenic and modify the toxicity of Aβ42 *in vivo*. In this study we examined the role of shorter Aβ peptides, Aβ1-36, Aβ1-37, Aβ1-38, Aβ1-39, Aβ1-40, Aβ1-42 and Aβ1-43, by generating transgenic *Drosophila melanogaster.* The Aβ peptides were expressed independently or co-expressed with Aβ1-42 specifically in the eye to assess phenotype and the neurons to measure behavioral function. Furthermore, we examined the effect of Aβ38, expressed using our BRI2 fusion strategy, in an APP mouse model. Overexpression of the shorter Aβ peptides, Aβ1-36, Aβ1-37, Aβ1-38 and Aβ1-39, was not toxic in the eye. Overexpression of Aβ1-42 resulted in a degenerative eye phenotype while expression of Aβ1-40 or Aβ1-43 had a slight effect on eye phenotype. Importantly, in flies co-expressing Aβ1-42 and the shorter Aβ peptides, Aβ1-36, Aβ1-37, Aβ1-38 and Aβ1-39, the degenerative phenotype and behavioral function was improved. These studies validate g-secretase modulation as a clinical strategy by characterizing the attenuating effect of shorter Aβ peptides.

D1385A Drug discovery in *Drosophila*. *Tamy Portillo Rodriguez*, Tom Hartl, Ethan Perlstein. Perlstein Lab, PBC San Francisco, CA.

Perlstein Lab is a public benefit corporation that discovers small molecule therapies for orphan diseases. Since our conception in 2014, we have focused on the rare diseases Niemann Pick type C (NPC) and N-glycanase 1 (NGLY1) deficiency. *Drosophila* with mutations in these disorders' causal genes, *npc1* and *ngly1*, are developmentally delayed during larval stages. We will present our *Drosophila* high throughput drug screening platform and our progress on discovering small molecule modifiers of NPC and NGLY1 larval developmental delay.

D1386B The anti-migration/anti-metastatic compound Dihydromotuporamine C signals through RhoI1 and the non-muscle myosin heavy chain and is antagonized by Rac1. *C. Seavey*[1], M. Wang[1], A. Muth[2], O. Phanstiel IV[3], L. von Kalm[1]. 1) University of Central Florida, Orlando, FL; 2) Department of Chemistry, University of Central Florida, Orlando, FL; 3) Department of Medical Education, College of Medicine, University of Central Florida, Orlando FL.

Cancer chemotherapeutics with good anti-migration/anti-metastatic activities are significantly underrepresented in the arsenal of anti-cancer agents. One compound with excellent anti-migration properties in mammalian cell culture is dihydromotuporamine C (Motu 3,3) that was isolated from a sea sponge near Motupore Island, New Guinea. Motu 3,3 has been reported to activate RhoA and to influence sphingolipid metabolism in mammalian cells however the mechanism by which Motu 3,3 inhibits migration in cell culture is not currently understood. Using a leg imaginal disc eversion assay, we show that Motu 3,3 acts through Rho1 in a pathway leading to the activation of the myosin non-muscle heavy chain (Zipper) and the formin actin nucleation (dia) pathway. Additionally, Motu 3,3 activity is antagonized by Rac1. Motu 3,3 exhibits toxicity in cell culture so we tested the mechanism of action of chemical derivatives of Motu 3,3 that have reduced cell culture toxicity but increased ability to inhibit migration. Our data show that the derivatives have the same mode of action as Motu 3,3.

D1387C Effects of Antimalarial Drugs on Motor and Behavioural Programs in *Drosophila melanogaster*. *A. A. Adedeji*, E. Kwikiriza, M. Vicente-Crespo. Kampala International University- Western Campus, Ishaka, Bushenyi, UG.

Background: Antimalarial drugs, used for the control and prevention of malaria infection, affect other parts of the human body to alter certain physiological functions. The neurons form discrete circuits that mediate complex behaviours including circadian rhythms, sleep, learning and memory, courtship, feeding, aggression, grooming, and flight navigation. However, little is known of how the use of antimalarial drugs affects these physiological processes that defines wellness. In the present study, we evaluate the effects of antimalarial drugs on the motor and behavior program activities using *Drosophila melanogaster* fly model.

Materials and Methods: Anti-malarial drugs (fansidar, chloroquine, artesunate, mefloquine and quinine) were administered to Drosophila melanogaster (wildtype) flies on filter paper. The locomotion (climbing), feeding and grooming-aggression assays were performed according to standard methods. The climbing performance indices, feeding events and aggressive presentations were determined. The findings were analyzed and $P< 0.05$ was taken as significant.

Results: The mean performance indices for locomotion were high (>0.6) in all flies treated with the antimalarial drugs but highest (1.0) in female *Drosophila* flies treated with Artesunate and lowest (0.31) in male flies treated with mefloquine. Quinine and mefloquine treated flies had suppressed aggression and grooming behavior compared to untreated flies. The artesunate treated flies had highest indices for aggression and grooming performance ($P=0.021$), In all drugs treated groups, female flies had increased feeding rate ($P = 0.0001$) than male flies. The mean feeding times was 47.25 ± 26.08 minutes and significantly shorter in artesunate treated group (46.07 ± 26.78 min).

Conclusion: The findings from this study provide preliminary information that antimalarial drugs do not only act on parasite but may alter locomotion, feeding, aggression and grooming behaviours in treated individuals. More studies are required to elucidate on molecular basis for these findings using available *Drosophila melanogatser* genetic tools.

D1388A A Drosophila Model of Chronic Traumatic Encephalopathy. *Mingkuan Sun*, Sheng Wang, Liam Chen. Neuropathology Division, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 USA.

Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with repeated concussive and subconcussive brain injury. It has been found most often in contact sports athletes, less frequently in non-athletes including military service members. There is characteristic gross and microscopic changes in the brain, including frontal and temporal atrophy, hyperphosphorylated tau deposition in neurons and astrocytes in a pattern that is distinct from that of other tauopathies, including Alzheimer's disease and frontotemporal lobar

degeneration. In order to understand the mechanisms responsible for its complex spectrum of pathologies, we exploited the versatile molecular tools available for *Drosophila melanogaster* and developed a fruit fly CTE model. Repetitive head trauma inflicted flies demonstrated persistent neuroinflammation, abnormal accumulation of phosphorylated tau protein, and neurodegeneration in the absence of macroscopic tissue damage. Our model offers a new platform to investigate the pathogenetic mechanisms of CTE and provide a rapid means to develop efficacious interventions for prevention and treatment of CTE.

D1389B Human Disease Model Reports in FlyBase. *Sian Gramates*, Madeline Crosby, Kathleen Falls, Beverly Matthews, The FlyBase Consortium. Harvard Univ, Cambridge, MA.

The use of Drosophila melanogaster as a model for studying human disease is well established, reflected by the steady increase in both the number and proportion of fly papers describing human disease models in recent years. In order to improve both the visibility and accessibility of human disease model research, FlyBase has recently begun producing Human Disease Model Reports.

These reports provide an integrated informational resource regarding specific diseases and fly disease models and their potential impacts on translational research. Each report presents background information on a specific disease, including links to outside resources, most notably Online Mendelian Inheritance in Man (OMIM®); a tabulation of related disease subtypes; information concerning relationships between human disease-linked genes and their fly orthologs; and a summary of experimental data and results using fruit flies. We have also incorporated relevant data described elsewhere in FlyBase, including physical interactions, Disease Ontology annotations of alleles, and genetic reagents. These reports are specifically designed to be accessible to non-fly researchers in order to promote collaboration across model organism communities working in translational science, while also providing a convenient entry point for Drosophila researchers interested in disease model systems.

D1390C Genetic screen for Wnt signaling factors that regulate *Drosophila* nociception. *P. R. Freeman*, Andew Bellemer. Appalachian State University, Boone, NC.

The mechanisms that regulate the transduction of noxious stimuli and generation of appropriate behavioral responses in *Drosophila melanogaster* are not fully understood. In larvae, Class IV multidendritic neurons are highly branched sensory neurons that are responsible for detecting noxious chemical, thermal, or mechanical stimuli and generating appropriate behavioral responses. Recent studies have demonstrated involvement of Wnt signaling in regulating nociception and the development of chronic pain in vertebrate models, but the underlying cellular and molecular mechanisms are still not understood. In order to better understand the roles of Wnt signaling in *Drosophila* nociception, I have selected 77 Wnt signaling-related genes and obtained UAS-RNAi lines for each from the Drosophila TRiP collection for an RNAi screen for nociception defects. I have crossed these RNAi lines with flies carrying the Class IV-specific *ppk-GAL4* driver and tested the larvae progeny with a well-established thermal nociception assay. We found the Wnt RNAi behavior phenotypes can be divided into three classes: 1) hypersensitivity to noxious stimuli; 2) insensitivity to noxious stimuli; 3) no change in response to noxious stimuli. These phenotypic categories are established by comparing the response latencies of RNAi animals to the latency of a transgenic control strain. Once candidate genes that produce significant hypersensitivity or insensitivity to thermal stimuli have been identified based on their behavioral phenotypes, I can then further analyze these genes to identify the cellular and molecular mechanisms of how they control nociceptor development and function maintain appropriate sensitivity to noxious stimuli. I have identified the protein tyrosine kinase 7 homolog, *off track2*, as a promising candidate for further analysis based on its reduced sensitivity to noxious thermal stimuli and previously established role in Wnt2 signaling. This further analysis consists of 1) genetic conformation with additional RNAi lines and genetic mutants; 2) gain and loss of function studies and epistasis analysis; 3) functional studies to determine the role of off track2 in Class IV neuron morphological development and electrical activity.

D1391A The Effects of various heavy metals on Drosophila melanogaster third Instar Larvae Polytene Chromosomes. *Osamah Batiha*, Christina Canavati, Ahmed Elbetieha, Rami Alkhatib. Jordan university of science and technology, Irbid, JO.

Heavy metals are naturally present on earth; nevertheless, their bioaccumulation in the environment triggers severe ecological issues, jeopardizing the ecological balance and ultimately producing detrimental effects on human beings and different living systems. To assess the genotoxic effect of various heavy metals, we used *Drosophila* third instar larvae polytene chromosomes as a model. Many chromosomal aberrations (breaks, semibreaks, constrictions and asynapsis) were observed. The frequency of chromosomal aberrations was significantly increased (P-value= 0.001) in groups treated with heavy metals at a concentration of 1000ppm compared to the control group. Understanding the genotoxicity of heavy metals is of great importance. Lack of such information could lead to difficulty in evaluating the danger of such environmental pollutants. For this reason, studying the various effects of these environmental contaminants on *Drosophila melanogaster* polytene chromosomes could aid in measuring their impact at the chromosomal level rather than going to the chemical analysis.

D1392B Polyamines: simple molecule, complex transport system. *D. Brown*, Elena Coronado, Nicole Barnette, Laurence von Kalm. University of Central Florida, Orlando, FL.

Polyamines are small cationic molecules found in all organisms and are essential in growth and proliferation. Intracellular polyamine concentrations are maintained by a combination of biosynthesis, transport, and degradation. Biosynthesis and degradation have been well studied, but even a basic understanding of the transport system remains elusive. Previously our lab identified a membrane-associated P-type ATPase with six protein isoforms necessary for polyamine transport using an in vivo RNAi approach. Current work looks to build on prior work by identifying proteins in complex with the P-type ATPase through Tandem Affinity Purification (TAP) and Tandem Mass Spectroscopy (MS/MS).

The description of components involved in transport will allow us to characterize a poorly understood biological system relevant to all organisms.

D1393C Flies and human disease: Resources at the Bloomington Drosophila Stock Center for human disease-related research. *A. L. Parks*, K. Matthews, K. R. Cook. Bloomington Drosophila Stock Center, Indiana University, Bloomington, IN.

An increasing number of researchers are utilizing the powerful genetic and molecular tools offered by Drosophila to develop and study fly lines which faithfully recapitulate aspects of human disease or human health-related processes. Drosophila models are being used in all aspects of human disease research, including basic research into the underlying mechanisms of disease, screens for disease modifiers for use in drug target discovery, and direct drug screening. To facilitate research into human disease, we are assembling lists of human disease genes, identifying their fly counterparts, and generating a catalog of stocks at the BDSC that can be used to model a disease or study pathways associated with the disease (http://flystocks.bio.indiana.edu/Browse/HD/HDintro.htm). We will present these efforts as well as give an overview of the many types of collections at the BDSC that can be screened for modifers of your favorite Drosophila human disease model.

D1394A Outfast, Outyield, Outlast: Modified foxo increases Drosophila survivorship during amino-acid starvation. *J. D. Slade*, B. E. Staveley. Memorial Univ, St John's, NL, CA.

Disordered eating may lead to diagnosable medical conditions such as anorexia and bulimia, to extreme weight loss or to obesity. While no animal model is able to mimic the entirety of any complex human disease or behaviour, *Drosophila melanogaster* can serve as an uncomplicated model to study the biological basis of abnormal eating patterns. The conserved insulin receptor pathway and its endpoint effector the foxo transcription factor are pivotal for survival during nutritional stress. The loss of *foxo* function results in a defective survival response to amino-acid starvation. Two modifiers of foxo activity, the Akt1 kinase and the Sir2 deacetylase, may adjust the activity of foxo to enhance survival. Novel *Akt1* mutant lines exhibit a moderate decrease in lifespan and growth when aged upon standard media, yet they show a significant increase in survival on amino-acid deprived media. Replacement of Akt1 activity is sufficient to suppress these phenotypes. Combination of the novel *Akt1* hypomorphs and the null *foxo* mutant reveal an epistatic relationship. Biometric analysis and longevity evaluation of these double mutants indicate a phenotype similar to the original *foxo* mutant signifying its necessity in the *Akt1* phenotype. Unlike the *Akt1* mutants, *Sir2* mutant heterozygotes do not have altered growth when raised upon standard conditions. However, the *Sir2* heterozygotes exhibit a greatly extended lifespan when reared on both a standard diet and when starved of amino-acids. These results indicate that the subtle manipulation of foxo, by either *Akt1* or *Sir2* mutants, can enhance survival during adverse nutrient conditions to model the survival of individuals undergoing nutrient deprivation. Ultimately, we believe that a Drosophila model of disordered eating could generate new avenues of potential therapies for related human conditions. Funded by an NSERC PGSD and a School of Graduate Studies Fellowship to JDS and an NSERC Discovery Grant to BES.

D1395B Annotation of the *Drosophila ficusphila* Contig 53 on Chromosome 4 Using Comparative Genomics. *Ahmad W. Al-Abduljabar*, James E. J. Bedard. University of the Fraser Valley, Abbotsford, BC, Canada.

Evidence-based comparative genomics was used to annotate contig 53 genomic sequence spanning 30,500 bp on the dot chromosome of *Drosophila ficusphila*. The project was completed with the help of the Genomics Education Partnership undergraduate student research initiative. In Drosophila the distal portion of the dot chromosome is of interest due to having heterochromatic characteristics but maintains a gene density similar to euchromatic regions. The databases and web-based tools of FlyBase, GEP UCSC Genome Browser Mirror, NCBI BLAST, Gene Record Finder, and Small Exon Finder were used for gene annotation of this region. The genomic DNA sequence was compared with the *Drosophila melanogaster* genome and five putative orthologs were annotated: CG33521, PIP4k, Mitf, Arf102F, and Dyrk3. These genes showed conservation with *D. melanogaster* genes, ranging between 64.1%-98.3% identity (%ID) conservation. Genes known to have many biologically crucial functions, such as Arf102F (%ID=98.3) and PIP4K (%ID=97.5), showed high amino acid sequence similarity. Mitf (%ID=74) and Dyrk (%ID=75.9) have less biologically important roles within the organism and showed lower similarity. Genes identified as not having biologically crucial functions, such as CG33521 (%ID=64.1), showed very low sequence similarity between the two species. CG33521 gene codes for a Zinc ion binding protein. PIP4K gene codes for a protein kinase involved in many pathways including cell growth, cell adhesion, and actin filament organization. Mitf gene codes for a transcription factor involved in the regulation of protein dimerization. Dyrk gene codes for a protein kinase involved in phosphorylating ATP-binding proteins. These findings will help contribute a better understanding of the characteristics and evolution of the dot chromosome within Drosophila.

D1396C The use of comparative genomics in the evidence-based annotation of contig10 on the 3L chromosome of *Drosophila elegans*. *Vivienne K. Beard*, James E. J. Bedard. University of the Fraser Valley, Abbotsford, British Columbia, Canada.

Genomic annotation is the process of locating functionally relevant regions within a stretch of DNA. Specifically, the annotation of genes includes locating gene start sites, stop sites and determining the coordinates of coding exons in addition to describing the function of these genes. The genome of *Drosophila melanogaster* has been entirely annotated, and is therefore an excellent reference species for the annotation of genes in related Drosophila species. The goal of the present study was to use comparative genomics to construct evidence based gene models for multiple isoforms of 8 orthologous genes present on contig10, a 50,000 base pair DNA sequence, of the 3L chromosome of *Drosophila elegans*. The bioinformatics tools utilized included FlyBase, Gene Record Finder, NCBI BLAST, Gene Model Checker, Small Exon Finder, and the UCSC Genome Browser Mirror. Together, these bioinformatics tools were used in conjunction with biological evidence to successfully construct high quality gene models for multiple isoforms of 8 putative orthologs present on contig10: ArfGAP3, CG32452, mael,

CG14450, CG11367, CG32454, CG11241, and l(3)04053. These genes showed variable conservation relative to the putative *D. melanogaster* orthologs, ranging between 38.6-90.4% protein identities. Genes with known crucial ontology, such as ArfGAP3 (ADP-ribosylation factor GTPase activating protein 3) showed high conservation with *D. melanogaster* (%ID = 90.0%), while lesser studied genes such as CG32452 showed much lower conservation (%ID = 38.6%). The construction of high quality evidence-based gene models will contribute to the understanding of the evolution of the heterochromatic dot chromosome compared to euchromatic regions in different Drosophila species.

D1397A Annotation and transcription start site discovery on the dot chromosome of *Drosophila ficusphila* and *Drosophila biarmipes*. *R. E. Boody*, C. M. Brown, S. A. Liber, J. L. Sanford. Ohio Northern University, Ada, OH.

The Muller F element, or chromosome 4 in *Drosophila,* is commonly referred to as the dot chromosome. This chromosome is unique among other *Drosophila* autosomes as it is highly heterochromatic and contains a high density of repetitive sequence. Remarkably, comparative genomic analyses of the dot chromosome show similar proportions of active genes in comparison to the euchromatic control regions. The Genomics Education Partnership (GEP) at Washington University, St. Louis, aims to elucidate the evolutionary mechanisms by which this uncommon gene expression occurs. Undergraduate students across the country work with the GEP to manually annotate genes, develop gene models, and search for transcription start sites in newly sequenced *Drosophila* genomes. The present work focuses on the annotation of contigs 32, 34, 36, 37, and 42 of the *Drosophila ficusphila* dot chromosome assembly and the discovery of transcription start sites (TSS) in contig 62 of the *Drosophila biarmipes* dot chromosome assembly. Annotation of genes relies on standard bioinformatic tools, including NCBI BLAST, *in silico* gene prediction programs, and a mirror to the UCSC Genome Browser to manually curate the gene models. These models include the determination of start and stop coordinates for all the exons of all the genes present on the contig. The process of TSS discovery depends on similar bioinformatic tools, using the UCSC Genome Browser in conjunction with Celniker data to determine the presence, location, and type of the ortholog in *D. melanogaster.* Ultimately, this data is used to determine the presence, location, and type of promoter in the target species. Data obtained from the annotation of contig 32 in the *D.ficusphila* dot assembly revealed 6 genes: Eph, Gat, mav, Ekar, CG11155, and Slip1. Genes gw, CG11360, and myo were found on contig 34. Contig 36 contains the genes ey, toy, and bt, which continues onto contig 37. The only remaining gene on contig 37 is MED26. Lastly, annotation of contig 42 showed the presence of 2 genes, unc and mGluR. TSS discovery in contig 62 of the *D. biarmipes* dot assembly included the elucidation of the transcription start site of the sv gene. Future work will further develop understanding of the TSS discovery via motif hunting for common promoter elements to determine potential differences in motif distribution along the dot chromosome in comparison to the heterochromatic control regions. Analysis of gene structure and TSS annotation in the dot and 3L control chromosomes across multiple *Drosophila* species, including the genes in this study, will aid in highlighting the mechanisms driving gene expression in the highly heterochromatic dot chromosome.

D1398B Messenger RNA-associated processes and their influence on exon-intron structure in *Drosophila*. *F. Catania*, G. Lepennetier. Institute for Evolution and Biodiversity, Muenster, DE.

It is well established that extensively interacting mRNA-associated processes in eukaryotes are crucial for the accurate processing of nascent transcripts. What is less clear—and almost entirely unexplored—however, is whether these interactions affect gene structure.

Drawing from a wealth of knowledge on *Drosophila melanogaster* genetics, molecular and cell biology, and biochemistry, we generated and tested three hypotheses as to how mRNA-associated processes might influence gene architecture. Our findings suggest that the interactions between capping, splicing, cleavage/polyadenylation, and telescripting may impose significant constraints on gene structure. Our study and its results offer an expandable framework for future cross-disciplinary investigations.

D1399C *De novo* evolved genes are essential for spermatogenesis in *D. melanogaster*. *Geoffrey Findlay*, Anna Gubala, Tery Vinh, Michael Kearns, Purva Rumde. College of the Holy Cross, Worcester, MA.

Gene duplication has long been considered the major evolutionary process that gives rise to new, lineage-specific genes. However, recent research in *Drosophila* and other taxa suggests that *de novo* evolved genes are also an important source of evolutionary novelty. *De novo* genes arise from non-protein-coding DNA at a surprisingly high rate, and their expression is often restricted to the male germline. Previous studies have attempted to use organism-wide RNA interference to infer the function of these genes, but interpretation of these results has been impaired by technical issues with the RNAi library that was used. By using tissue-specific knockdown in the testes, we have identified two *de novo* evolved genes, *saturn* and *goddard*, that have become essential for spermatogenesis and sperm function in *D. melanogaster*. RNAi depletion of *saturn* leads to a 60 percent reduction in sperm production and transfer and a near-complete inability of sperm to be stored in the female reproductive tract. Depletion of goddard prevents production of mature sperm by the testes. Molecular evolutionary analyses showed that *saturn* arose near the base of the *Drosophila* phylogeny and has a dynamic evolutionary history that includes gene duplication, gene loss, and positive selection in *D. melanogaster* and its closest relatives. In contrast, *goddard* is present only in the melanogaster group of species and has evolved under purifying selection. While males of all *Drosophila* species produce sperm, there is extensive interspecific variation in the type, size and number of sperm produced in males and in the patterns of sperm storage in females. Our data suggest that lineage-specific *de novo* genes may play a previously unappreciated role in boosting male fitness by shaping the critical process of spermatogenesis.

D1400A New genes play a key role in the reproductive fitness of *Drosophila melanogaster*. *A. R. Gschwend*, N. VanKuren, J. Mihaljevic, S. Allesina, M. Long. University of Chicago, Chicago, IL.

The ability to pass genetic material on to the next generation is a key driving factor of evolution, but the determinants of the evolution of reproductive fitness are relatively unknown. New genes have been shown to be essential for life in *Drosophila melanogaster*, but the extent to

which new genes are important for reproductive fitness has not been thoroughly explored. We assessed the reproductive fitness effects of 91 newly evolved genes, with ages ranging from <1-25 million years, by carrying out constitutive knockdowns using RNAi for each gene in male and female *Drosophila melanogaster*. We quantified male fertilization success and female fecundity by counting and comparing the resulting offspring number from each set of knockdown reciprocal crosses to the reproductive output of non-knockdown parallel control crosses. Over half of the newly evolved genes (53%) we tested had significant beneficial effects on offspring number, revealing important roles of newly evolved genes in the reproductive fitness of *Drosophila melanogaster.* In addition, 7 new genes showed extreme effects on offspring number, resulting in near sterility when knocked down, suggesting a handful of new genes are essential for normal *Drosophila melanogaster* fertility.

Overall, 47% of the new genes tested had a significant beneficial effect on male reproductive fitness compared to the controls, while only 5 new genes had a significant beneficial effects on female offspring number (one gene had a significant beneficial effect for both sexes), revealing differential fitness effects of new genes in males and females. 20% of the new genes had significant differential effects of the same gene on offspring number between males and females; in the majority of these cases (83%), the new gene was significantly beneficial to male reproductive success, whereas it did not significantly affect the female offspring number. Expression data revealed that the majority of new genes were highly expressed in the testis and male accessory gland, providing additional evidence for the role of new genes in male reproductive fitness. Male and female adaptive needs are often different, due to their unique reproductive roles. Our results support the idea that new genes are a driving force in the evolution of reproductive fitness and male-driven evolution is an important component contributing to the retention of new genes in the *Drosophila melanogaster* genome.

D1401B The recombination landscape of *Drosphila virilis* under hybrid dysgenesis. *L. Hemmer*, J. Blumenstiel. University of Kansas, Lawrence, KS.

DNA damage in the germline is a double-edged sword. On one hand, induced double-strand breaks establish the foundation for meiotic recombination, which is essential for proper chromosome segregation. On the other hand, double-strand breaks can also pose a significant challenge for genome stability. Within the germline, transposable elements are powerful agents of double-strand break formation. How different types of DNA damage are resolved within the germline is poorly understood. For example, little is known about the relationship between the frequency of double-stranded breaks, both endogenous and exogenous, and the decision to repair DNA through one of many pathways, including crossing over and gene conversion. We aim to use the *Drosophila virilis* hybrid dysgenesis model to determine how recombination landscapes change under transposable element activation. In this system, a cross between two strains of *D. virilis* with divergent transposable element loads results in the hybrid dysgenesis phenotype, which includes the germline activation of diverse transposable elements, reduced fertility and male recombination. However, only one direction of the cross results in hybrid dysgenesis. This allows us to examine recombination in genetically identical F1 females; those with baseline levels of programmed DNA damage and those with an increased level of DNA damage resulting from transposable element proliferation. We are using multiplexed shotgun genotyping to map crossover events to compare the recombination landscapes of hybrid dysgenic and non-hybrid dysgenic individuals. Finally, we are examining whether the additional crossovers are mitotic or meiotic in origin by characterizing the degree to which increased levels of recombination arising from transposable element activation are clustered among siblings.

D1402C How does replication level contribute to genome size evolution in *Drosophila* species? *C. E. Hjelmen*, J. S. Johnston. Texas A&M University, College Station, TX.

Underreplication (replication of a portion of the total complement of DNA in each chromosome) is well documented in the highly polytene chromosomes of *Drosophila* ovary nurse cells, follicle cells surrounding oocytes, abdominal histoblasts, fat body cells, gut cells, and cells of the late prepupal salivary glands. Recently we found that replication in a majority of thoracic cells in *D. melanogaster* stall between G1 and G2, which means these cells too are underreplicated. Since variation in genome size has been shown to be linked to the amount of repeat and noncoding sequence, we ask, "Is the percentage of replication in DNA of thoracic cells directly related to the genome size of a species, and Is this consistent among species*?"* We also ask, "How much does the late replicating portion of the genome contribute to overall genome size evolution?" To address these questions, we estimate, using flow cytometry, the underreplication level and genome size for more than 100 Drosophilidae from the UC San Diego Species Stock Center. These data are then analyzed using traditional statistic methods as well as newer comparative phylogenetic methods in an effort to measure phylogenetic signal and rate of evolution of genome size of replicated and underreplicated DNA. The *Drosophila* phylogeny used for this analysis was reconstructed with the super-matrix method using Bayesian reconstruction. Preliminary results have shown that with a few striking exceptions, larger genomes of *Drosophila* have lower replication percentages; whereas small genomes have complete or almost complete levels of replication. These data support the conclusion that genome size change is largely, but not always, an increase or decrease in noncoding sequence. Moreover, the rate and mode of change is different for the replicated and unreplicated portions of the genome.

D1403A A high frequency of transposable element tandems is a potential source of new satellite arrays. *M. P. McGurk*, D. A. Barbash. Cornell University, Ithaca, NY.

Eukaryotic genomes are replete with repeated sequences, either dispersed across the genome by transposition events (transposable elements, TEs) or in large tandemly arrayed blocs (satellites). Satellite arrays commonly comprise the centromeres and telomeres, forming structures essential to cell division and replication, evolve by expansions and contractions that are probably largely neutral in nature, and have a high turnover rate. This high turnover must arise through processes of rapid gain and loss. While it is not fully clear how new complex satellite arrays arise, some known satellites clearly originated from TEs, suggesting that TEs can provide source material for the emergence of new

arrays.

With this in mind, we sought to leverage the abundance of available Next-Generation Sequencing (NGS) datasets to assess the extent to which transposable elements form tandem arrays, and how these tandems vary across populations of *Drosophila*. To circumvent the difficulties of mapping repeat-derived reads, we employ an alignment strategy that maps paired-end reads to the consensus sequences of known repeats and implement an Expectation-Maximization algorithm to discover tandem structures in the aligned data.

We applied this method to the hundreds of available *D. melanogaster* NGS datasets. With the exception of a few previously known examples, we rarely detect large tandem arrays of TEs in *Drosophila melanogaster*, however we find that small tandems are surprisingly common. An exception is a rare instance where ~20 copies of the Hobo transposon are arrayed in tandem found in a single line. Further, P-element forms small tandem arrays (<8 copies in tandem) in over 50% of lines; because P-element invaded D. melanogaster in the last century, these tandems must be recently formed structures, suggesting that tandem transposable elements can form and expand into larger arrays over short time scales. These observations suggest that some TE families readily generate the small tandems, which are substrates necessary for the expansion of a larger array. These emerging tandems have the potential to modify chromatin state, concentrate regulatory elements at particular loci, and potentially regulate the expression of other TEs. Going forward, the identification of these strains with novel and low frequency tandems provides us the opportunity to assess the phenotypic impacts of tandem TEs and ask questions about the evolution of young satellite arrays.

D1404B Rapid acquisition of novel immune system genes via duplication and *de novo* origination in dipterans. *Tim Sackton*[1], Brian Lazzaro[2], Andrew Clark[2]. 1) Harvard Univ, Cambridge, MA; 2) Cornell Univ, Ithaca, NY.

Genes encoding immune system proteins are often among the most rapidly evolving genes in the genome due to host-pathogen arms race dynamics. Using new annotations and transcriptome sequencing of infected and control (naive) flies across 10 species of Drosophila and the house fly *Musca domestica*, we show that high rates of novel gene acquisition (by gene duplication and by *de novo* gene origination) also characterize dipteran immune systems. In the house fly, which occupies an unusually pathogen-rich niche among sequenced dipterans, we use new models of gene family evolution to investigate duplication and loss rates across several different classes of immune-related genes. We demonstrate that genes encoding proteins involved in either pathogen recognition or pathogen killing have been duplicating at a significantly accelerated rate along the *M. domestica* lineage compared to other dipterans, perhaps as a consequence of its septic habitat. Some of these same proteins also display marked patterns of nonneutral sequence divergence. Comparative post-infection RNA-seq analysis also indicates considerable functional evolutionary divergence among dipteran species. Taken together, our results suggest that changes in gene content, sequence and regulation may play a critical role in host adaptation to pathogen pressure.

D1405C The Hawaiian *Drosophila* genome project: Transcriptomes. *Haiwang Yang*[1], Terence Murphy[2], Kenneth Kaneshiro[3], Durrell Kapan[4], Brian Oliver[1]. 1) National Institutes of Health, Bethesda, MD; 2) National Center for Biotechnology Information, Bethesda, MD; 3) University of Hawaii at Manoa, Honolulu, HI; 4) California Academy of Sciences, San Francisco, CA.

Understanding how transcriptomes differ within and between species is key to understanding everything from personalized medicine to phenotypic changes on evolutionary timescales. Hawaiian flies provide an exceptional model for understanding population structure as well as sympatric and allopatric speciation. >500 species of *Drosophila* unique to the Hawaiian archipelago have been described. These islands are located thousands of kilometers from continents and it is believed that all the species in this extensive radiation developed from a single founder female approximately 25 million years ago. The individual volcanic islands arise in series as the pacific plate passes over a mantle hotspot, such that the geological age of each is well established, placing firm limits on when new founder populations arrived. In addition to reproductive isolation events due to initial founder effects, there are subsequent isolation events because of subsiding volcanoes and sea level rise that fragment old islands, and lava flows that fragment ecosystems on young islands. There are major microclimate differences due to island features such as altitude and rain shadows that promote adaptive radiation. Mating behavior is particularly striking as males perform elaborate displays and bouts in leks. There are also morphological specializations for male-male competitions (such as battering ram heads in *D. heteroneura*). At abstract submission, we have performed mRNA sequencing for a total of 149 samples (over 1.5 billion RNA-seq reads) to explore expression variance due to sex (N=2), tissue (N=8), strain or species (N=10), and island (N=4). We are assembling transcripts *de novo* and have mapped reads to the *D. grimshawi* genome (the only currently assembled hawaiian genome). Fortunately, >84% of our reads from *D. silvestris, D. hemipeza, D. heteroneura,* and *D. hawaiiensis* uniquely align to the *D. grimshawi* genome. We are also reannotating the *D. grimshawi* genome and evaluating the SNPs and Indels in transcriptome for within- and between-species variance in addition to expression level variance. Variance in male- and particularly testis-biased (sampled separately from male reproductive tract) gene expression is marked. These observations highlight the importance of sexual selection in speciation of these flies (the Kaneshiro hypothesis) at the genome deployment level, despite the many island niche features that one would expect to drive selection for individual fitness. These results indicate that this genomic model will be a powerful tool for exploring population and evolutionary questions.

D1406A The changing biodiversity of Alabama *Drosophila*: important impacts of climate variation, urbanization, and invasive species. *A. Bombin*, L. Reed. The University of Alabama, Tuscaloosa, AL.

Global warming and anthropogenic disturbances significantly influence the biosphere, tremendously increasing species extinction rates. In central Alabama we analyzed Drosophilidae species composition change 94 years after the previous survey. We found ten Drosophilid species that were not reported during the last major biodiversity survey, two of which are invasive pests. In addition, we analyzed the influence of environmental variables characteristic of the subtropical climate zone on *Drosophila* abundance and biodiversity. We found a significant

correlation between temperature and abundance of total *Drosophila* as well as for six out of the seven most represented species individually, with a maximum abundance at intermediate temperatures (18-26°C). In addition, temperature was positively correlated with biodiversity of *Drosophila*. Precipitation produced a significant effect on the abundance of five species of Drosophila, with different optima for each species, but did not affect overall biodiversity. We concluded that in the subtropical climate zone of central Alabama, temperature and precipitation produce a significant effect on *Drosophila* abundance and biodiversity, while local land use also impacts fly abundance, contributing to a substantial shift in species composition over the last century. We expect global climate change and other anthropogenic factors to further impact *Drosophila* species composition in the subtropical climate zone into the future.

D1407B The molecular difference between grey and black genotypes collected in Plopsoru village. *G. A. Butnaru*[1,2], I. Sarac[1], S. Popescu[1]. 1) Banat Univ Agricultural Sci, Timisoara, Banat, RO; 2) Academy of Romanian Scientists.

In 2010, from a sample of fruit flies collected from Plopsoru village a few individuals with a dark color and unusual behavior were separated. Plopsoru village represents a hilly-agro-ecosystem situated in miners' area. After phenotypic checking the sample was split into two: a large grey group – Grey Plopsoru (GP) and few black individuals that generated a new "population" named Black Plopsoru (BP). The new genotype showed different morphological features: very dark black body and eyes, the size of wing imaginal discs at L3 significantly lower, eggs with 6-8 appendices, larval motility during the night, the pupae located in the cotton stopper and strong reproductive barrier for other *Drosophila* lines even for GP ecotype. In comparison to standard genotypes Oregon and white (w1118) and GP the life cycle of BP was longer with 99.2%, 120% and 108% respectively and emphasized a nocturnal behavior. For each ecotype successive generations were produced (six for BP and seven for GP). To establish the similarity vs. evolutionary distance among the analyzed *Drosophila* genotypes the ISSR (*Inter Simple Sequence Repeat*) and RAPD (*Random Amplified Polymorphic DNA*) markers were used. Therefore the polymorphism was emphasized randomly in genome and also for the segments located between microsatellites. It was pointed out that all of the selected markers (eight ISSR and four RAPD) generated a number of fragments ranged between 7 and 23 per primer. For all of the RAPD primers the similarity was 100%. All of the primers amplified 163 fragments of which 159 (97.5%) were polymorphic. The UPGMA (*unweighted pair-group method*) clustering method was used to establish the similarity coefficients and the dendrogram.

According to their genetic background the accessions of BP and GP were grouped in two main clusters, with a coefficient of similarity of 40.20±4.38%. In the first cluster all of the BP generations were grouped, with a very high similarity 96.96±1.13%. All of the GP generations formed the second cluster emphasizing a higher variability compared with BP, the similarity coefficient inside the group being 72.58±10.69. The highest and the lowest coefficients were 87.25% between first and fourth generations for BP, respectively 55.03% between second and sixth GP generations. Therefore, two subclusters were separated, the first being made up from the first five generations and the second one from six and seven generations, pointing out a large distribution of the similarity coefficients. The molecular evaluation revealed the big difference between BP and GP even if they are the "product" of the same evolutionary conditions; BP pointed out a high stability in the offspring six generations.

D1408C Male genotype-specific transcriptional responses to mating in female *Drosophila melanogaster*. *S. Y. N. Delbare*, C. Y. Chow, M. F. Wolfner, A. G. Clark. Cornell University, Ithaca, NY.

Reproduction is a process that involves interactions between males and females at the level of the organism, at a cellular and molecular level, and at the level of the genotype. For *D. melanogaster*, several studies have demonstrated that natural variation in male genes involved in male-female genotype interactions affects female post-mating behavior and reproductive success. How natural variation in male genes affects female gene expression after mating is less well known.

To learn how male-female genotype interactions affect female gene expression after mating, we performed RNA-seq on female flies to detect transcriptome changes before mating and six hours after they had mated with genotypically different males. We used males and females from *melanogaster* inbred lines derived from five geographically dispersed populations. Females from each line were singly mated to a male from each of the same five inbred lines.

Post-mating mRNA levels were compared to those of unmated females from the same population. If a gene's expression changed after mating, we determined if its fold change differed depending on the genotype of the female or the male with which she mated. Linear models were fitted to assess the magnitude of the effect of male genotype, female genotype, and their interaction. Among the genes significant for this interaction effect, we found a highly significant enrichment of immune response genes (EASE score = 2.2×10^{-6}).

Additionally, over 20 transcripts that derive from genes exclusively expressed in male reproductive tissue were found in females after mating. This indicates that *D. melanogaster* males transfer RNAs, along with sperm and seminal fluid proteins during mating, as was reported previously in *D. mojavensis* and *D. arizonae* and in *Aedes* mosquitos. Our dataset expands the suite of male derived transcripts found in female flies after mating and opens the door to apply the technologies available to *D. melanogaster* to investigate potential functional roles of these transcripts.

This work was supported by NIH R01-HD059060.

D1409A Functional networks of locally adapted reproductive proteins in two Drosophila populations. *C. E. Stanley*, R. J. Kulathinal. Temple University, Philadelphia, PA.

Genomic patterns of variation offer powerful insights into the selective conditions that existed in the past. Previous studies highlight the role of rapidly evolving sex and reproductive genes in population divergence and subsequent isolation. Yet whether these highly diverged genes

represent population-specific (i.e., local) adapted functional networks is not well understood. Here, we compare the functional landscape of adaptive change across protein-coding genes involved in various aspects of male and female reproduction between two populations of *D. melanogaster*, an ancestral African population (Gambia) and a more recently derived North American population (Raleigh NC). .

D1410B Conservation and evolution of maternal RNA deposition and early zygotic transcription in *Drosophila* . *Joel Atallah*[1], Susan E. Lott[2]. 1) University of New Orleans, New Orleans, LA; 2) University of California - Davis, Davis, CA.

Transcripts corresponding to thousands of genes are deposited by the mother in the egg. Throughout the earliest developmental stages following fertilization, the embryo is transcriptionally silent, and these mRNA transcripts set the trajectory for subsequent development. During the maternal-zygotic transition (MZT), a substantial fraction of maternal mRNAs are targeted for degradation, and the first zygotic genes are transcribed. While some maternal and early zygotic genes have been exhaustively analyzed, the early embryonic functions of many others are unknown. The precise function of maternal mRNA degradation, and why only specific transcripts are degraded, has been debated. We created a transcriptomic dataset for 14 *Drosophila* species at stages before and after the maternal-zygotic transition, from single embryos, and compared our results with a previously published *Aedes Aegypti* developmental time course. Transcripts that that are represented at both stages are remarkably stable over evolutionary time, while the levels of transcripts that are present at only one stage vary widely between relatively close relatives. Maternally deposited genes that are degraded at the MZT vary dramatically across species, suggesting that these genes may either have an early function that is highly species-specific, or may represent developmental noise that is attenuated through degradation. While purely zygotic transcripts also vary considerably across evolutionary time, we found a small group of purely zygotic genes that are extremely conserved across hundreds of millions of years of evolution. This select group is highly enriched in transcription factors that play critical roles in early development. Our results suggest that the combined contribution of transcripts from the maternal and zygotic genomes meet the developmental constraints of the embryo, while genes expressed only from the maternal genome or zygotic genome evolve more freely.

D1411C Insect Egg Evolution: Diversity of Size and Shape at the Single-Cell Stage. *S. Church*, S. Donoughe, C. Extavour. Harvard University, Cambridge, MA.

Insects eggs come in all shapes and sizes. The morphological diversity at the single-cell stage has implications for the development and adult morphology of the insect. In *Drosophila*, we know of mutations which control egg size, and across flies in Drosophilidae a diversity of egg sizes has been recorded. However far less is understood about how insect eggs have evolved, and few studies compare the diversity of insect eggs across orders or by using methods that take into account the insect phylogeny. To address this problem, we assembled a database of thousands of insect egg size measurements which we used to analyze egg morphological evolution across the insect tree, including within Diptera and within Drosophilidae. Here we show the results of using this database to analyze how egg width and length vary across the insect tree, including repeated convergent evolution of very large and very small eggs. We also analyze the coevolution of egg size and shape with other traits, including body size, ecological habitat (ie aquatic vs terrestrial), and development type (ie syncytial vs holoblastic). The diversity at the single cell stage rivals the diversity of adult forms, and this study reveals rich and complex patterns in egg evolution.

D1412A Functional analyses of the transposable element-derived genes *DPLG1* and *DPLG4* in *Drosophila melanogaster*. *D. Jangam*[1], C. Feschotte[2], E. Betrán[1]. 1) University of Texas at Arlington, Arlington, TX; 2) University of Utah, Salt Lake City, UT.

Our laboratory has described several domesticated transposases from PIF/Harbinger DNA transposable elements (TEs) in *Drosophila* (Casola et al. 2007). Although, the exact functions of these genes are still unknown, these transposase-derived genes appear to have their DNA binding domain conserved but not their catalytic domain and we hypothesize that they might be regulatory proteins. Their regulatory function might include the regulation of TE activity. Here, we present work aimed to elucidate the function of two of the PIF/Harbinger derived transposases named *Drosophila PIF like gene 1* (*DPLG1*) and *DPLG4*. Taking advantage of the UAS/GAL4 system, these two *DPLG*s were knocked down ubiquitously and the effects on the viability and fertility were studied. Using light microscopy and fluorescent microscopy, defects in the testis of knock-down (KD) flies were identified. *In situ* hybridization was used to ascertain the level of transcript reduction in KD testis, and RNA-seq analyses were performed for KD testis and controls to quantify differentially expressed genes and TEs. KD of *DPLG1* does not affect viability in *D. melanogaster*; however, it resulted in male sterility. We observe that the elongated spermatids do not individualize into mature sperms and the seminal vesicles are empty. The testis of these KD males show increased activity of LINE TEs including all three telomeric elements. In addition, *DPLG4* came out in a screen designed to detect genes involved in TE control (Handler et. al 2013). KD of *DPLG4* reduces viability of *D. melanogaster*. This result has been independently validated using a *DPLG4* mutant line. *DPLG4* KD males showed a phenotype analogous to *DPLG1* KD males. Taken together, these results support that *DPLG4* might play a role in the viability of D. melanogaster, and *DPLG1* and *DPLG4* might be needed during spermatogenesis and could potentially have been recruited from PIF/Harbinger TEs to defend against the detrimental activities of other parasitic elements. We are in the process of generating resilient transgenes for *DPLG1* and *DPLG4* which will be used to rescue the defects resulting from the KD and KO experiments. To further advance our understanding of the functional importance of these genes, we are generating null mutants of *DPLG1* and *DPLG4* using CRISPR-Cas9 which will be used to further investigate the role of these genes. This will help us validate the results from our experiments. Additionally, the resilient constructs are HA tagged and will be used to test the nuclear localization of these genes and their interactions with chromatin remodeling proteins. .

D1413B Evolutionary cooperativity between mating position and rotation of male genitalia in Diptera. *I. Momoko*[1], C. Sakuma[2], H. Kanuka[2], K. Matsuno[1]. 1) Osaka Univ., Osaka , JP; 2) The Jikei Univ. Sch. of Med., Tokyo, JP.

In many cases, animal behavior depends on morphology. Thus, during evolution, morphology and behavior should change cooperatively. However, the mechanisms of such cooperative evolution are hardly understood. For example, dipteran insects, *Drosophila melanogaster* and *Aedes aegypti* (mosquitoes) have two typical mating positions, male-above and end-to-end positions, respectively. In these two mating positions, dorsal-ventral direction of the male genitalia is upside-down with respect to female genitalia. However, the genitalia of *Drosophila* and *Aedes* rotate 360 and 180 degree, respectively, which compromises the upside-down direction of male genitalia associated with the evolution of mating position.

In this study, we attempted to analyze the cooperative evolution between the genitalia rotation and mating position during the evolution of dipteran insects. In *Drosophila*, *Myosin31DF* mutation affects the rotation of genitalia. Using *Myosin31DF* mutant males with abnormally rotated genitalia, we found the proper direction of male genitalia is a prerequisite for mating in *Drosophila*, suggesting potential cooperative evolution of them.

Currently, we are making *Myosin31DF* mutant mosquitoes by CRISPR/Cas system to obtain male mosquitos with abnormally rotated genitalia. The effects of abnormally rotated genitalia on the end-to-end mating position will be analyzed. Comparative analyses involving *Drosophila*, *Aedes*, and other dipteran insects should provide us important insights into the cooperative evolution of animal morphology and behavior.

D1414C Step-by-step evolution of Bicoid's anterior patterning functions. *P. Onal*[1], Q. Liu[2], R. Datta[1], J. Cao[1], J. Thornton[2], S. Small[1]. 1) New York University, New York, NY; 2) University of Chicago, Chicago, IL.

How new molecular functions emerge is a fundamental question in evolutionary biology. Here we focus on the homeodomain (HD) transcription factor Bicoid (Bcd), which emerged from a gene duplication that also gave rise to the paralogous protein Zen. After the duplication, Bcd diverged rapidly from Zen throughout its protein sequence, and acquired a pivotal role in anterior embryo patterning. We have used an *in vivo* rescue assay to test chimeric proteins that swap the HDs between Bcd and Zen. These experiments show that the Bcd HD is essential for anterior patterning, and that it can provide many of Bcd's molecular functions when inserted into Zen. To understand how the Bcd HD gained novel functions during evolution, we took an ancestral protein resurrection approach. Using a maximum likelihood-based method, we predicted HD sequences of the ancestors of extant Zen and Bcd proteins. The "ancestral HD" of Zen and Bcd (AncZen-Bcd HD) prefers a consensus Zen DNA-binding motif (ZM), and chimeric Bcd proteins containing this HD sequences cannot replace any of the patterning functions missing in embryos lacking Bcd. Further conservation analysis identified eleven amino acids in the HD as critical for Bcd evolution. Only two of these amino acids significantly change the *in vitro* binding activities of the HD. Substitutions of one (K50) is sufficient to convert the *in vitro* DNA binding specificity of the AncZen-Bcd HD to that of present-day Bcd. *In vivo* analyses showed that this amino acid is also required for Bcd's patterning functions. However, when introduced into the AncZen-Bcd HD, there is only a partial rescue of the *bcd* mutant, and activation of only a subset of Bcd target genes. We also tested R54, a second amino acid previously shown to be required for Bcd's RNA-binding activity. We find that insertion of R54 into the AncZen-Bcd HD does not change its *in vitro* DNA-binding specificity or activate Bcd target genes on its own. However, when both K50 and R54 are introduced into the AncZen-Bcd HD, its DNA-binding activity changes, and it turns on a larger subset of Bcd target genes compared to the K50 substitution alone. Our results show an interdependence between K50 and R54 in DNA-binding activity, and suggest that the evolution of Bcd involved step-by-step single amino acid substitutions that enabled the evolving protein to activate different subsets of target genes.

D1415A Species-specific transcriptional variation underlying *Drosophila* olfactory system structure and development. *J. Pan*[1], P. Volkan[1], C. Jones[2]. 1) Duke University, Durham, NC; 2) University of North Carolina at Chapel Hill, Chapel Hill, NC.

In fast-evolving neural circuits like the olfactory system, some neuronal lineages, and their developmental programs may be more variable, for better adaptability to the environment, while others may be more stable to conserve important functions. To test this, we conducted a comparative analysis of the olfactory system transcriptome of four different *Drosophila* species at multiple points during development. Our analysis revealed that the olfactory receptor neurons (ORNs) expressing Or22a show the highest variation in developmental and transcriptional programs. Interestingly, Or22a ORNs play an important role in the chemosensory adaptations of specialist feeder species to their host plants. Or22a receptor detects host plant odors, and the number of Or22a neurons and their target antennal lobe glomeruli in the brain are increased in both species. To determine whether the species-specific variation in Or22a ORN lineage, number and gene expression is also represented in their developmental program, we analyzed three classes of transcription factors acting at three stages of ORN development: 1) prepatterning transcription factors, which we recently found to partition the antennal disc into multiple rings each with a restricted developmental potential; 2) proneural genes, whose expression selects specific cells from these rings to become the precursors to form specific sensilla and the ORNs they house; and 3) terminal selector transcription factors that regulate olfactory receptor gene expression. Among these three regulatory classes of transcription factors in ORN fate determination, we found the pre-patterning transcription factors to be the most dynamic, both individually and in combinations, across species during development, especially for the combinations that generate the ab3 fate. In contrast, other ORN lineages are very stable both in number and gene expression profiles regulating their identity and developmental programs. Interestingly, pairwise comparison of the developmental gene expression profiles of olfactory system in four species showed that the specialists species are also the more similar in each other then they are to the generalist species. Together, these studies suggest that not only is variation in structural properties of Or22a ORNs associated with behavioral variation, but that this variation is also reflected in the developmental programs culminating in Or22a ORNs fates. Together, these findings may shed light on variation and plasticity in development of specific ORN lineages that may facilitate the adaptation of *Drosophila* chemosensory behaviors to specific ecological niches.

D1416B Butterfly color vision: stochastic patterning mechanisms and expanded sensory receptor diversity. *M. W. Perry*[1], M. Kinoshita[2], G. Saldi[3], L. Huo[1], K. Arikawa[2], C. Desplan[1,3]. 1) New York University, New York, NY; 2) SOKENDAI, The Graduate University for Advanced Studies, Hayama, Japan; 3) New York University Abu Dhabi, Abu Dhabi, UAE.

Butterflies use color vision extensively to navigate the natural world. Their retinas are more complex than those found in *Drosophila*, where development and patterning has been heavily studied. Instead of the eight photoreceptors found in flies, butterflies have an additional ninth photoreceptor per ommatidium ("unit eye"). They also have three main types of ommatidia instead of the two distributed stochastically in the fly retina. We set out to determine how butterflies generate increased sensory receptor diversity to provide improved color vision, and more specifically, to determine how much of the retinal patterning network from *Drosophila* is reused or modified.

Using genome sequencing, gene expression, and CRISPR gene knock out we show that the regulatory network that defines photoreceptor subtypes in *Drosophila* is redeployed in butterflies (*Papilio xuthus* and *Vanessa cardui*) to generate additional subtypes. In *Drosophila,* a complex regulatory network combined with cell-cell signaling specifies photoreceptor subtypes within each ommatidium. Then, a stochastic decision of whether to express the transcription factor Spineless in R7 photoreceptors determines which of two types of ommatidia is specified. We find that the R7 marker Prospero is expressed in two photoreceptors per ommatidium in butterflies. CRISPR knock-out of *Spineless* shows that this gene also controls stochastic choice in each of the two R7s in butterflies, suggesting there is deep evolutionary conservation of stochastic patterning mechanisms. Having two stochastically distributed types of R7s allows for the specification of three ommatidial types instead of two, which in turn allowed for the evolution and deployment of additional opsins, tetrachromacy, and improved color vision. These efforts provide evidence that our extensive knowledge of patterning in the *Drosophila* visual system applies to other groups, and that adaptation for specific visual requirements can occur through modification of this network.

D1417C Next-Generation Approaches to Understanding Evolution of the Insect Germline. *H. Quan*, J. Lynch. University of Illinois at Chicago, Chicago, IL.

Germline cells are unique as they can produce gametes and regenerate themselves, and can be specified by either maternally inherited determinants or by zygotic inductive signals. In the maternal inheritance mode, the germ cells are specified very early by the germ plasm synthesized during oogenesis. This mode is found in most model organisms (fruit fly, zebrafish, frog and nematode), whereas the zygotic induction mode, may be the ancestral model of germline determination .

Among the invertebrates, the only arthropod in which the germ line has been studied in detail is *Drosophila melanogaster* which uses the maternal inheritance mode. However, this mechanism of germ cell specification is a derived feature in insects and seems to be limited Holometabola. The wasp *Nasonia*, like *Drosophila,* uses the maternal inheritance mode, and represents the most distantly branching holometabolous lineage relative to *Drosophila*. The assembly of *Nasonia's* germ plasm is dependent on a regulatory network that is very similar to that of *Drosophila*, and occurs in the same context of polytrophic ovaries, indicating that a regulatory network similar to both fly and wasp was present ancestrally in the Holometabola. Despite the overall similarity *Nasonia* and *Drosophila* germline determination, some aspects are quite distinct, such as the morphology of the germ plasm, the formation of the pole cells, and the migration of the pole cells into the embryo interior.

To characterization the ancestral and novel features of *Nasonia* germplasm relative to *Drosophila* at the molecular the mRNA composition of the *Nasonia* oosome, was characterized by RNAseq. The results confirmed that certain genes, such as *oskar, nasos*, etc., are conserved between the two species, and also revealed previously unknown genes, which could be crucial for the unique properties of *Nasonia* germ plasm and germline.

D1418A Changes in the regulation of *doublesex* led to the diversification of two novel traits. *Gavin Rice*, Kevin Hu, Artyom Kopp. University of California at Davis, Davis, CA.

Whether it is the brightly colored feathers of the male peacock or the horns of male dung beetles, scientists have been intrigued by sexually dimorphic traits. However, the molecular mechanisms responsible for the origin of these sex-specific traits are not well known. Comparative studies investigating the genetic basis of convergent, male-limited phenotypes, will lead to a better understanding of the constraints in the genes and pathways used in the generation of new traits.

We have studied two novel but phenotypically similar traits that arose independently: the sex combs bristles found in forelegs of *D. melanogaster,* and the brush bristles found in the forelegs of the *D. immigrans* species group. The sex combs and foreleg brush are both modifications of the transverse bristle rows of the first tarsal segment in the forelegs and are male-specific . In *Drosophila,* the transcription factor *doublesex* establishes sex-specific transcription in males and females, but is expressed in only a subset of cells. Therefore, for a new cell-type to become sexually dimorphic or for a trait to expand beyond the current set of *doublesex* expressing cells, expression of *doublesex* must be modified.

We have previously shown that changes in the expression of *doublesex* explains a large portion of phenotypic divergence in sex combs. In parallel, we have found that the gene *doublesex* shows a similar pattern in the origin and diversification of the foreleg brush. Species that have secondarily lost the foreleg brush have lost *doublesex* expression, while species that have reduced brush sizes also have corresponding reduced *doublesex* expression. These results indicate that changes to the regulation of *doublesex* may be vital in the origin and secondary modification of sexually dimorphic traits.

D1419B Effect of aging on the Responder satellite in Drosophila melanogaster. *Linhe Xu*, Emerson Khost, Danna Eichbush, Amanda Larracuente. University of Rochester, Rochester, NY.

Eukaryotes have a tight regulation on the expression of satellite DNA, but satellite DNA misexpression is associated with chromosome segregation problems and cancer. Satellite DNA also interests genomic and evolution researchers with its contributions to inter-species genetic incompatibilities. In the fruit fly *Drosophila melanogaster*, the *Responder* (*Rsp*) locus of satellite DNA is particularly interesting because *Segregation Distorter*, a notorious selfish genetic complex, targets it. While the *Rsp* satellite has intrigued researchers for decades, its repetitive and pericentromeric nature make it difficult to study. We took advantage of recent developments in long read single molecule real time sequencing (SMRT) technology from PacBio RS II to assemble the *Rsp* satellite and use this assembly as a platform to study the regulation of *Rsp* satellite expression. Our preliminary Northern blot demonstrates that *Rsp* is expressed and the *Rsp* transcripts differ between the ovary and the female carcass, indicating differences in regulation of the satellite between the germline and the soma. By aligning RNA expression profile data from the modENCODE project to our complete *Rsp* assembly, we show that the *Rsp* satellite expression increases from newly eclosed flies to 30-days-old flies. Our interpretation of these data is that the *Rsp* satellite is misregulated as the flies age, perhaps as a result of more global effects of age-related heterochromatin misregulation. We are studying detailed changes in expression level, transcript size and transcript composition in flies of increasing age using Northern blot analysis and RT-PCR. We aim to understand the molecular details behind this age-related misregulation of satDNA by studying the abundance and types of transcripts produced as flies age. Knowing how factors like aging can affect the *Rsp* locus' expression will shine light on our understanding of how satellite DNAs are regulated.

D1420C Sex-specific divergence for body size and desiccation-related traits in Drosophila hydei from the western Himalayas. *B. Kalra*[1], R. Parkash[2]. 1) Department of Genetics, Maharshi Dayanand University, Rohtak, IN; 2) Department of Genetics, Maharshi Dayanand University, Rohtak, IN.

Sex-specific-differences are awidespread source of genetic variation in various *Drosophila* species. In the present study, we have examined desiccation survival in males and females of Drosophila hydei from colder and drier montane conditions of the western Himalayas (altitudinal populations; 600–2202 m). In contrast with most other studies in drosophilids, *D. hydei* males exhibited comparatively higher desiccation resistance despite smaller body size compared to females. Accordingly, we tested the physiological basis of such adaptations in both sexes of *D. hydei*. Body size traits (wing length, wet weight and dry weight) were ~1.2 fold higher in females than males. However, desiccation resistance was 10 to 13 h higher in males than females. These differences matched enhanced storage of trehalose content (~1.2 fold), higher hemolymph content (~1.2 fold) and enhanced cuticular lipid mass (~1.5 fold) in males than females. Water loss before succumbing to death (dehydration tolerance) was much higher in males (~81%) than females (~64%). A greater loss of hemolymph water until death under desiccation stress was associated with higher desiccation resistance in males. Further, there were lacks of differences in the rate of water loss, rate of trehalose utilization and rate of hemolymph depletion between the sexes in *D. hydei*. Therefore, sex-specific differences in desiccation resistance of *D. hydei* were independent of body size aswell as the exhaustion of metabolite reserves and rather were caused by the higher dehydration tolerance as well as higher acquisition of hemolymph and trehalose contents.

D1421A Genomic regulation of limited lifespan and reproductive senescence in *Drosophila melanogaster*. *Grace A. Parker*, Trudy F. C. Mackay. North Carolina State University, Raleigh, NC.

Limited lifespan and senescence are near-universal phenomena. These quantitative traits exhibit variation in natural populations due to the segregation of many interacting loci and from environmental effects. Due to the complexity of the genetic control of lifespan and senescence, our understanding of the genetic basis of variation in these traits is incomplete. Our goal is to identify causal genes associated with increased lifespan and postponed reproductive senescence in *Drosophila melanogaster* by functional analyses of genetically divergent genes between five long-lived (O) lines selected for postponed reproductive senescence and five unselected (B) lines. Preliminary data assessing productivity of the reciprocal crosses of the O and B lines suggest that genes influencing reproductive senescence are maternally-controlled. Therefore, all of the candidate genes tested are expressed in the ovaries of females. To determine which of these candidate genes exhibit a quantitative change in lifespan or reproductive productivity, I have assessed lifetime reproduction of candidate genes from the Vienna collection of RNAi lines in which gene expression is knocked down in ovaries. Identifying specific genes affecting increased lifespan and delayed reproductive senescence will increase our knowledge of the evolutionary role of naturally segregating populations on overall fitness and may provide potential targets for therapeutic intervention to delay senescence in populations with increasing lifespans.

D1422B The TreadWheel: A novel apparatus to measure genetic variation in response to low impact exercise for *Drosophila*. *L. K. Reed*[1], S. Mendez[1], L. Watanabe[2], R. Hill[1], M. Owens[1], G. Rowe[2], N. Riddle[2]. 1) University of Alabama, Tuscaloosa, AL; 2) University of Alabama - Birmingham Birmingham, AL.

Obesity is one of the dramatic health issues affecting this country and exercise is a well-established intervention strategy. While exercise-by-genotype interactions have been shown in humans, overall little is known. Using the natural negative geotaxis of *Drosophila melanogaster*, an important model organism for the study of genetic interactions, a novel exercise machine, the TreadWheel, can be used to shed light on this interaction. The mechanism for inducing exercise is inherently low impact thus minimizing confounding effects of other stressors. Using this machine, we are able to assess large cohorts of adult flies from eight genetic lines for their response to exercise over one week of training. We have measured their triglyceride, glycerol, protein, glycogen content, and body weight, as well as their climbing ability and feeding behavior in response to exercise, and generally find that exercised flies are more fit than unexercised flies. We have also assessed expression in a panel of genes known to be associated with respiratory fitness and find that many of these genes show expected changes in expression level with

exercise. In addition to demonstrating and overall effect of exercise on flies, we find significant genotype-by-exercise interaction effects for many of the measured phenotypes, and those differences can be partially linked to innate differences in flies' persistence in maintaining activity during exercise bouts. With this study, we have established the TreadWheel as useful tool to study exercise effects in flies, shown significant genotype-specific impacts of exercise, and have laid the ground work for more extensive studies of how genetics, environment, and aging interact with exercise to influence metabolic fitness in *Drosophila*.

D1423C *Drosophila* Lifespan: Effects of RNA Interference (RNAi)-Suppression. *D. M. B. Unselt*, T. G. Campbell, K. Ward, A. Weitzel, T. F. C. Mackay. North Carolina State University, Raleigh, NC.

Understanding the genetic mechanisms affecting variation in lifespan in natural populations is crucial for understanding the genetic basis of age-related diseases. Lifespan is known to vary in natural populations due to the segregation of multiple genetic factors as well as to exposure to different environmental conditions with a heritability of approximately 10-30%. Further, many pathways associated with lifespan, such as the insulin or insulin-like signaling pathway, are evolutionarily conserved between humans and model organisms. *Drosophila melanogaster* is a powerful model for assessing naturally occurring genetic variation in lifespan because of the ability to perform genomic analyses on a large scale while effectively monitoring genetic backgrounds and controlling environmental conditions. The *D. melanogaster* Genetic Reference Panel (DGRP), which consists of 205 sequenced inbred lines, allows for the investigation of natural genetic variation on phenotypically variable traits. To identify polymorphisms associated with variation in aging, a genome wide association (GWA) study utilizing the DGRP was conducted. This resulted in the identification of 28 genes significantly associated with lifespan. To validate their effects on lifespan, we knocked down the expression of these genes using RNA interference (RNAi) and a ubiquitous driver. This revealed several significant results associated with lifespan in the RNAi knockdown genotypes relative to their controls. Specifically, 13 genes (48%) had an effect in at least one sex, demonstrating sex-specific genetic architecture of lifespan. Additionally, 8 of the most significant genes (p-value < 0.01) increased lifespan when knocked down, providing evidence that lifespan is regulated at the transcriptional level. In the future, we plan to further functionally validate the role of these genes in the genetic control of lifespan using an overexpression assay. These experiments will contribute to our overall goal in establishing novel genetic networks associated with variation in aging. Since basic biological processes, such as aging, are evolutionarily conserved, these studies will also provide candidate genes for investigation in other species, including humans.

D1424A Dissecting the Genetics Basis of Learning, Memory, and Thermal Tolerance in a Multi-parental Population. *P. Williams-Simon*, S. Mitchell, E. King, T. Zars. University of Missouri, Columbia, MO.

Learning and memory are complex traits, which are fundamental for the survival of many species. Understanding the complexity of the genes that control these traits, should be of high importance if we want to better comprehend how an individual either learns from or tolerates temperature changes. There have been multiple genes shown to have an effect on learning and memory, however the majority of these previous studies have been using mutagenesis or other "one by one" gene approaches. Here, we dissect the genetic basis of learning, memory and thermal tolerance, using the *Drosophila* Synthetic Population Resource (DSPR). This multi-parental population consists of approximately 1,800 Recombinant Inbred Lines, which allows for high-resolution genome wide scans, and the identification of loci contributing to naturally occurring genetic variation. Using a behavioral assay known as “place learning”, we are able to train flies with a highly sensitive apparatus, the “heat box”. Whenever a fly crosses the midline of the chamber, the whole chamber either warms or cools, and so we are able to test both how well the fly learns to avoid uncomfortable temperatures and how well the fly retains this memory. We found that there was approximately a 2-fold difference between the performance index of flies for learning (.5 – 1). Both memory and thermal tolerance showed a nearly 10 fold range of variation (Memory: 0.1 – 1; Thermal Tolerance: 60s - 600s). We then performed genome scans using the DSPRqtl R package, which uses a Haley Knott regression to test for an association between phenotypes and genotypes. Our results revealed that there is a genetic basis for variability in these traits, and that there are relatively few loci within the fly genome that are important for these traits. These loci have not been previously been implicated in learning or memory. Future work will aim to fine map these loci, identify candidate genes, and validate the function of these genes in learning, memory, and thermal tolerance.

D1425B Identification of QTLs for male courtship song using a high-resolution genetic map of *Drosophila athabasca*. *R. R. Bracewell*, K. Wong-Miller, D. Bachtrog. UC Berkeley, Berkeley, CA.

To understand the initial genetic changes that result in the formation of new species, we need to study species in the earliest stages of divergence. *Drosophila athabasca* is comprised of three closely related semispecies that differ dramatically in male courtship song and these song differences result in strong reproductive isolation among sympatric semispecies. To identify the genomic regions that are associated with these differences in song, we created a high resolution genetic map of *D. athabasca* using low coverage whole genome sequecing of F5 individuals. Our results provide important insights into the relative roles of the autosomes and sex chromosomes in the evolution of *D. athabasca* male courtship song differences and help identify regions of the genome that have promoted the evolution of behavioral isolation in this group.

D1426C Variable rescue of inviability in male hybrids of *Drosophila melanogaster* and the *Drosophila simulans* clade. *J. C. Cooper*, N. Phadnis. University of Utah, Salt Lake City, UT.

Speciation is poorly understood at the molecular level because of the difficulty in identifying hybrid incompatibility genes. Recently, we identified a new hybrid incompatibility gene, *gfzf*, that is required for hybrid F1 male inviability between *Drosophila melanogaster* and its sister species *Drosophila simulans*. Knockdown of only the *D.simulans* ortholog of *gfzf in* male hybrids is sufficient to rescue their viability. However, it

remains unclear whether the incompatibility driven by *gfzf* is a general isolating mechanism between *D.melanogaster* and the *D.simulans* clade (*D.simulans, D.mauritiana, and D.sechellia*) or evolved along the *D.simulans* lineage specifically. To investigate the role of *gfzf*-meditated incompatibility in the *D.simulans* clade, we constructed *D.melanogaster* transgenic flies that express RNAi constructs to knock-down expression from the *gfzf* ortholog of each *D.simulans* clade species. We crossed *D.melanogaster* females that contain the RNAi construct to males of the *D.simulans* clade and counted male and female progeny. We find that *gfzf* knockdown produces variable hybrid male rescue across the *D.simulans* clade.

Further, it is unknown if the cell cycle defects observed in *D.melanogaster* / *D.simulans* hybrids remain consistent in hybrids between *D.melanogaster* and other species in the *D.simulans* clade. To investigate the defects in cell proliferation in developing hybrid larvae, we used a pair of fluorescent constructs that mark cells in a cell cycle specific pattern. We crossed *D. melanogaster* females that contain these transgenic constructs to males of the *D.simulans* clade. Our analyses using developing brains and imaginal discs via immunofluorescent confocal microscopy suggest that the severity of proliferation defects in hybrids of *D.melanogaster* and the *D.simulans* clade may not be uniform. The variation in both rescue of hybrid males by *gfzf* knockdown and hybrid cell cycle defects suggests either the presence of other genes that contribute to dominant male lethality, or a variable role for *gfzf* in this hybrid incompatibility.

D1427A The genetic basis for mate choice evolution between sibling species. *D. Erezyilmaz*, K. Liu. Stony Brook University, Stony Brook, NY.

Although a great deal is known of how genes shape behavior, far less is known of how genes are modified to produce the meaningful differences in behavior that exist among natural populations. As an initial step towards finding natural genetic variants that influence mating behavior, we have focused on differences between two sibling species of *Drosophila*. *D. sechellia*, and *D. simulans*, diverged ~242,000 years ago. Crosses between the two species produce viable males and fertile females. While *D. sechellia* males and *D. simulans* females mate readily, the reciprocal cross, between *D. sechellia* females and *D. simulans* males, is rarely successful. Mate discrimination at courtship in *Drosophila* is believed to occur through 1) female preference for species-specific courtship songs, and 2) male preference for species-specific female contact pheromones. We tested *D. simulans-D. sechellia* recombinant flies in 2-choice mating assays and used a high-resolution seq-based method, Multiplexed Shotgun Genotyping (MSG) to identify regions of the genome that are associated with species-specific mate preference. In females, two large effect QTL overlie enzymes that produce the pheromone, 7,11 heptacosadiene (7,11-HD). Previous work has shown that both enzymes are expressed in *D. sechellia* females, but not *D. simulans.* Moreover, 7,11-HD is produced in *D. sechellia* females, but not *D. simulans* females, and transfer of 7,11-HD to *D. simulans* females has been shown to reduce courtship by *D. simulans* males. We are currently using tissue specific expression to test this prediction. Two different large effect QTL determine mate preference in males, and we are testing candidate genes from these regions. In contrast to previous work on interspecific mate choice and courtship, our data suggests that the genetic basis of a complex behavior can be fairly simple.

D1428B Genetic basis of X-linked hybrid incompatibility betweenDrosophila melanogaster and D. simulans. *C.-T. Ting*, J.-H. Lin. National Taiwan Univ, Taipei, TW.

Genetic basis of reproductive isolation is an important issue to study speciation. Hybrid incompatibility (HI) in the forms of hybrid lethality or sterility, are commonly found in closely related species causing reproductive isolation and thus lead to speciation. One classical model for studying HI is the hybrids from the cross ofDrosophila melanogaster and D. simulans. The cross between D. melanogaster females and D. simulans males only produces sterile F1 females and hybrid males are larval lethal. The reciprocal cross yields only sterile F1 males while the hybrid females are embryonic lethal. Using attached-X chromosome strain, it is proposed that the hybrid female lethality is caused by the present of D. melanogaster X chromosome (Xm) and maternal product from D. simulans. To identify loci which contribute to the hybrid lethality, we conduct a genetic screen by crossing D. simulans females to males of the D. melanogaster X chromosome duplication lines that contain an extra X fragments on Y chromosomes in Zhr background. We have screened 22 lines covering 97.22 % of the X chromosome, and the results show that 2 of the 22 duplications have significant effect on hybrid male lethality. Our results indicate that there are at least two regions on the Xm chromosome contributing to HI between D. melanogaster and D. simulans.

D1429C A comparative genomic approach reveals the rapid evolutionary changes in two putative accessory gland genes throughout the *Sophophora* subgenus. *M. L. Johnson*, K. Vitalone, J. Borja. Notre Dame College, South Euclid, OH.

Accessory gland proteins (ACPs) of *Drosophila* represent a group of proteins that are transmitted to females during mating; and, therefore, influence reproductive success through changes in female behavior, physiology, and sperm utilization. Additionally, ACPs are known to undergo rapid evolutionary changes that are thought to be due to important roles they play in reproductive success during mating. However, the reproductive advantage of ACPs in one species, do not necessarily convey an advantage in other species. Two such ACPs are Sfp79B (Seminal fluid protein 79B) and msopa (male specific opa containing gene). Both of these genes are highly expressed, specifically in the accessory glands of *D. melanogaster*. Additionally, Sfp79B peptides from males have previously been detected in the lower reproductive tracts of mated female in both *D. melanogaster* and *D. simulans*, but not in *D. yakuba*. In this study the two ACP encoding genes Sfp79B and msopa were annotated on the 3L chromosomal arm of *D. elegans* between the RpLP0 and olf413 homologues as part of the Genomics Education Partnership (GEP). To better understand the evolution of these genes, a survey of 19 species in the subgenus *Sophophora* were analyzed, including 16 from the melanogaster group and 3 from the *obscura* group. Both have not always been detected during previous annotations, which is likely due to their small size. Therefore, the genomic regions between the RpLP0 and olf413 homologues were analyzed for the presence of these genes in different species. Of these 19 species, Sfp79B was present in 13 of the species, but was absent in some members of

the *melanogaster*, *suzukii*, and *takahashii* subgroups. Furthermore, in both *ananassae* and *pseudoobscura* subgroups, duplications of Sfp79B were detected. While msopa is present in both the *melanogaster* and *obscura* group, it was not detected in the *ananassae* subgroup. Additionally, in some of the groups msopa was found to be under positive selection based on Ka/Ks values. Future analysis of RNA expression will be conducted to verify whether these annotated gene products of Sfp79B and msopa are produced in select representative species. Together, this data demonstrates while Sfp79B is absent from some species of *Drosophila*, it has a larger role in the *Sophophora* subgenus than originally recognized. Moreover, analysis of both Spf79B and msopa demonstrate that while ACP encoding genes can be found throughout the *Sophophora* subgenus, rapid evolutionary changes in these genes occur in reproductive pockets.

D1430A Functional evolution of *bag-of-marbles,* the key switch for oogenesis in *Drosophila melanogaster*. *Jaclyn E. Bubnell*, Charles F. Aquadro. Cornell University, Ithaca, NY.

In *D. melanogaster* females, *bag of marbles* (*bam*) acts as the master switch for germline stem cell differentiation during oogenesis and in males, plays a key role in regulating spermatogenesis. Interestingly, *bam* is rapidly evolving across the *Drosophila* genus with a strong burst of nonsynonymous changes in both the *D. melanogaster* and *D. simulans* lineages. We have previously shown that the major functional change associated with interspecific differences in the Bam protein is in oogenesis, and not spermatogenesis. We have also discovered a genetic interaction between *Wolbachia* and a *bam* hypomorphic mutant in *D. melanogaster*, where *Wolbachia* infection rescues the *bam* fertility defect. However, the nature of this rescue remains unclear. Our preliminary data shows that *Wolbachia* does not rescue the tumorous ovary phenotype characteristic of the *bam* hypomorph, implying the rescue is indirect. These results have led us to hypothesize that *Wolbachia* may be a driver of positive selection at *bam* in *D. melanogaster* and *D. simulans.* However, since *bam* shows high levels of amino acid diversification across the *Drosophila* genus, it is also possible that *bam's* function as the master switch in oogenesis has arisen in the lineage leading to *D. melanogaster* and *D. simulans* and the refinement of this function has thus been under positive selection in these species. We are currently characterizing the nature of the interaction between *Wolbachia* and *bam* as well as defining *bam* function in *D. simulans* using CRISPR-Cas9 editing to distinguish between these hypotheses.

D1431B The genetic basis of the energy budget in *Drosophila melanogaster* on different diets. *A. M. Perinchery*, P. Stanley, S. Winingear, H. Kleiboeker, E. G. King. University of Missouri, Columbia, MO.

Organisms need to adapt to dynamic environments over time. An organism consumes and stores a finite amount of resources that are used for all daily tasks. In order to survive and thrive, they must allocate these finite resources to different life history traits like reproduction or somatic growth. We used the genetic mapping population, the *Drosophila* Synthetic Population Resource (DSPR), to map the genetic loci responsible for carbohydrate, lipid and protein storage on different diets in *Drosophila melanogaster*. We crossed ~250 Recombinant Inbred Lines (RILs) to a standard inbred line. The adult offspring of these crosses were placed onto one of three diets for 10 days: a high sugar, low yeast or control diet. We then used biochemical assays to measure the total energy budget: lipid, carbohydrate and protein content on the same set of flies. Lastly, QTL analysis was performed to identify possible genetic loci responsible for storage of these different components on different diets.

D1432C The genetic architecture of thermal plasticity in *Drosophila melanogaster*. *Omid Saleh Ziabari*[1], Shampa M. Ghosh[2], Nicholas D. Testa[3], Alexander W. Shingleton[1]. 1) Department of Biology, Lake Forest College, Lake Forest, IL; 2) School of Biological Sciences, National Institute of Science Education & Research, Bhubaneswar, India; 3) Department of Zoology and Ecology, Michigan State University East Lansing, MI.

Little is known about the molecular, genetic, and physiological mechanisms that regulate phenotypic plasticity, while even less is known about how these mechanisms evolve. For selection to act upon phenotypic plasticity, there must first be genetic variation in a plastic response and the mechanisms that generate it. In this study, we measure the genetic variation in the thermoplasticity of abdominal pigmentation in female Drosophila melanogaster among 110 isogenic lineages from Drosophila Genetic Reference Panel (DGRP). We used a novel approach to quantify various aspects of dorsal pigmentation from digital images. Using these data, we characterize the genetic architecture of the thermoplasticity, correlating variation in the thermoplasticity of pigmentation with variation in the thermoplasticity of other morphological traits. In general, the thermal reaction norms for pigmentation were non-linear, and variation in the thermal plasticity of pigmentation among lineages was not correlated with variation in the thermal plasticity of body and organ size. Nevertheless, there was some overlap among the SNPs associated with the thermoplasticity of both pigmentation and size, suggesting some common mechanisms regulate the thermoplasticity of different traits.

D1433A Sex differences in *Drosophila* somatic gene expression: variation and regulation by *doublesex*. *R. M. Graze*[1], F. N. New[3], J. M. Fear[3], T. S. Howard[1], J. E. Dalton[2], M. N. Arbeitman[2]. 1) Auburn University, Auburn, AL; 2) Florida State University, Tallahassee, FL; 3) University of Florida, Gainesville, FL.

Sex differences in gene expression have been widely studied in *Drosophila melanogaster*. Sexually dimorphic expression varies across strains, but many molecular studies of regulation have focused on only a single strain or on genes that show robust sexually dimorphic expression in many strains. How extensive variability is across strains and whether this variability occurs among genes regulated directly by sex determination genes is unknown. To address these questions, we examine differences between strains in sexually dimorphic gene expression in Drosophila adult head tissues. We also examine gene expression in *doublesex* mutants to determine whether a gene with sex-differential expression is regulated by DSX isoforms and the mode by which DSX regulation produces sex-differential expression. The best known mode of

DSX regulation is the classic mode, in which DSX represses in one sex and activates in the other. However, there are multiple additional ways in which DSX regulation can produce sex-differential expression and we examine all possible modes in this study. Our results show that there is substantial flexibility in somatic regulation of sex-differential expression. The sets of genes with sexually dimorphic expression in each strain show remarkably little overlap. The presence and prevalence of different DSX modes also varies between strains, with large differences in the prevalence of different modes among genes with female biased expression. Despite these differences, the genes identified as regulated by DSX by analysis of *doublesex* mutations are enriched in each strain for known sites of DSX occupancy in the genome. Neither the observation of DSX binding in a gene's regulatory regions nor the specific mode of DSX regulation explain why some genes show robust sex-differential expression, while others differ between strains. Thus, regulation by DSX, does not appear to confer a robust pattern of expression with respect to strain differences. Finally, we find that in adult head tissues sex-biased genes in general are enriched on the X and highly enriched on the fourth chromosome in both strains and that the fourth chromosome is more specifically enriched with regions that are occupied by DSX isoforms. Overall these results provide insights into a more complete pool of potential DSX targets within the genome, as well as the molecular flexibility of DSX regulation.

D1434B Genetics, development and plasticity of metabolic performance in *Drosophila*. *K. L. Montooth*, C. R. Julick, O. B. Matoo, K. R. O'Brien. University of Nebraska-Lincoln, Lincoln, NE.

Development generates a dynamic internal environment that is predicted to impact the fitness effects of mutations that arise in populations. Moreover, the external environment in which development occurs can impact the expression of traits within and across life stages. We and others have shown that even fundamental properties of metabolism (e.g., the scaling laws that are thought the govern the relationship between metabolic rate and mass) can vary across development and as a function of the developmental environment (Greenlee, Montooth, Helm. 2014 ICB 54:307). For example, I will show that the scaling of metabolic rate as a function of mass in *Drosophila* depends on the developmental thermal environment, and that mitochondrial-nuclear genotype can significantly affect larval metabolic rate. This latter genetic effect is itself conditional on the developmental thermal environment, and we have found that interactions between genotype and developmental environment affect metabolic rate plasticity (i.e., the Q_{10} for metabolic rate) (Hoekstra, Siddiq, Montooth. 2013 Genetics 195: 1129). In addition, I will show that genetic variance in metabolic rate varies across larval development. To better understand the underlying mechanisms that govern the development of energetic processes, we have characterized larval metabolic rate and aspects of mitochondrial physiology across development for a number of natural *D. melanogaster* genotypes, as well as for mitochondrial-nuclear genotypes that generate energetic inefficiencies. To better understand how metabolic development and plasticity impacts organismal performance, I will relate these patterns to plasticity in development and survivorship in the presence of ethanol, an important ecological challenge and resource for *Drosophila* larvae.

D1435C Parthenogenomics: Assembly, annotation, and analysis of the facultative parthenogenetic fruit fly, Drosophila mercatorum. *C. E. Stanley*[1], D. E. Miller[2,3], T. A. Markow[4,5], R. S. Hawley[2,3], R. J. Kulathinal[1]. 1) Temple University, Philadelphia, PA; 2) Stowers Institute for Medical Research, Kansas City, MO; 3) University of Kansas Medical Center, Kansas City, KS; 4) 4Laboratorio Nacional de Genomica de la Biodiversidad, CINVESTAV, Irapuato, Guanajuato CP, Mexico; 5) 5Univserity of California San Diego, San Diego California, La Jolla, CA.

Parthenogenesis, or reproduction without fertilization, provides a "last resort" method of reproduction when the availability of males is low, resulting in impaternate offspring. While parthenogenesis is extremely rare among species, Drosophila mercatorum has been long-known to be facultative parthenogenetic. To elucidate the underlying genetic basis of parthenogenesis, we generated a draft genome of both sexual and parthenogenetic strains of D. mercatorum. Here, we characterize their genomes, identify genomic variants and genes exhibiting biased expression differentiating sexual and parthenogenetic strains, and discuss the potential contribution of these variants to the parthenogenetic process.

D1436A Partial loss of function in the *Drosophila melanogaster* septin gene *Sep5*. *R. S. O'Neill*, D. V. Clark. University of New Brunswick, Fredericton, New Brunswick, CA.

Gene duplication is a major evolutionary process. One mechanism of gene duplication, retroduplication, generates a retrogene via retrotransposition of a parent gene's mRNA. Retroduplication is thought to facilitate the evolution of novel functions by separating a parent gene's coding sequence from its untranscribed regulatory region. Septins are a family of cytoskeletal proteins that form heter-oligomeric, rod-like complexes which can further assemble into higher-order structures. The septin family is divided into subgroups. The general model for septin complex assembly is that each position in the complex is occupied by a member of a specific subgroup, and thus subgroup members are thought to be interchangeable within the complex. *Drosophila melanogaster* has five septin genes, including *Sep2* and its retrogene duplicate *Sep5*. *Sep2* and *Sep5* are the only *D. melanogaster* SEPT6 subgroup members. About 15% of amino acid sites in a multiple sequence alignment of Sep2 and Sep5 from 20 *Drosophila* species are conserved across orthologs but different between paralogs, suggesting functional diversification. We explored the functions of *Sep2* and *Sep5* to provide insight into the functional diversification of the complex-forming septin family. *Sep5* mutants have no obvious phenotype. About half of *Sep2* mutants die at the end of pupation when grown at 25°C, and those that emerge are sterile and have short eggs. About one fifth of *Sep2* mutant egg chambers are fusions containing multiple germline cysts, suggesting a role for *Sep2* in follicle cell encapsulation. A *Sep5* transgene fails to rescue the *Sep2* mutant egg chamber fusion phenotype, showing functional diversification of Sep2 and Sep5 proteins. *Sep2 Sep5* double mutants lack imaginal discs and die as prepupae. Mosaic analysis showed that double mutant follicle cells can encapsulate germline cysts but fail to proliferate and die at mid-oogenesis, whereas double mutant germline cysts appear normal. Thus, *Sep2* and *Sep5* are redundant for proliferation of some cell types. Sep2-GFP and Sep5-GFP localize

similarly, including as double flanking rings at the germline ring canals, and at the follicle cell cortex. Pnut localization, determined by immunostaining, is similar to Sep2-GFP and Sep5-GFP, and is maintained in both *Sep2* and *Sep5* single mutants but is lost in double mutants. The redundancy of *Sep2* and *Sep5* for Pnut recruitment suggests that they are interchangeable in and required for the formation of septin complexes. While we cannot rule out a unique function for *Sep5* in an untested context, we suggest that *Sep5* has undergone partial loss of its parent *Sep2*'s function; Sep5 has retained the ability to form part of the *D. melanogaster* septin complex, but has lost an ancestral follicle cell-specific function.

D1437B The effects of insulin signaling on sexually dimorphic gene expression in head tissues. *T. S. Howard*[1], R. Tzeng[2], M. N. Arbeitman[2], R. M. Graze[1]. 1) Auburn University, Auburn, AL; 2) Florida State University, College of Medicine, Tallahassee, Florida.

Insulin signaling is generally involved in nutrient sensing and growth and the pathway, overall, is highly conserved from fruit flies to man. In many organisms insulin signaling frequently plays a role in control and development of sexually dimorphic traits, including both differences in growth and in behavior. In Drosophila, this pathway plays a direct role in regulating body size dimorphism, activity level dimorphism and in female fertility and mating behaviors. This is consistent with direct sex specific regulation of the pathway, but the targets of this regulation and the downstream effects on expression in each sex are unknown. To understand how sex differences shape the regulatory effects of the insulin signaling pathway, we examined similarities and differences in the effect of perturbation of the pathway on gene expression in adult males and females. Expression of a dominant-negative *InR* transgene (InRDN) was driven by actin-5C-GeneSwitch controlled GAL4 in males and females. Expression was assessed by RNA-seq in replicate for each sex expressing InRDN and for genetically matched controls. By integrating perturbation data with genomic signatures of sex specific regulation, we are able to shed light on how the InR pathway is sex specifically regulated and on the indirect effects of that regulation.

D1438C How do polymorphic Y-chromosomes modulate genome-wide epigenetic states: analyses of a whole Y-chromosome dosage series. *B. Lemos*, A. T. Branco, L. DellBem. Harvard T. H. Chan School of Public Health, Boston, MA.

Heterochromatin remains a mysterious component of eukaryotic genomes. The amounts and kinds of repetitive elements within heterochromatin of a focal segment have emerged as potential determinants of gene expression across the genome (trans-regulation). Highly repetitive segments of heterochromatin represent one of the last frontiers of eukaryotic genomes and the mechanisms by which heterochromatin dosage exert their effects across the genome have remained elusive. Here we report two sets of results. First, we describe our efforts constructing a whole Y-chromosome dosage series with polymorphic Y chromosomes (XY, XYY, XX, XXY) in a homogeneous background of a autosomes and X chromosomes. Second, we report novel functional genome analyses across a number of attributes in these genotypes that help illuminate the impact of perturbing the nuclear environment with extra Y chromosomes. We discuss the results in view of several proposed models for heterochromatin action in trans, including a classical hypothesis known as the heterochromatin sink as well as others.

D1439A A Facilitated Diffusion Mechanism Establishes the Drosophila Dorsal Gradient. *G. T. Reeves*, S. N. Carrell, M. D. O'Connell. North Carolina State University, Raleigh, NC.

During the first three hours of Drosophila embryogenesis, the transcription factor Dorsal (homologous to NF- κB) patterns the dorsal-ventral axis. Dorsal is present in a nuclear concentration gradient with the highest concentration at the ventral midline and a steady decay to about 40% of the embryo's circumference. The Dorsal gradient is initialized on the ventral side of the embryo via Toll signaling, which phosphorylates the inhibitor protein Cactus (homologous to IκB), marking it for degradation. In the absence of Cactus, Dorsal can enter the nuclei and activate expression of target genes in a concentration-dependent manner. Recent work measuring the dynamics of the Dorsal gradient has shown that, while Dorsal nuclear levels are initially uniform, Dorsal protein diffuses towards the ventral midline, which causes the overall accumulation of a Dorsal gradient peak. To explain this accumulation phenomenon, which seems to act against standard Fickean diffusion, we hypothesize a facilitated diffusion, or "shuttling" mechanism. The Dorsal/Cactus system has each of the features required for facilitated diffusion: (1) Dorsal binds to a "carrier" molecule (Cactus) that protects it from capture; (2) the Dorsal/Cactus complex is diffusible; and (3) the complex is broken in a spatially-dependent manner.

After using a photoactivatable GFP to show that nucleus-to-nucleus diffusion of Dorsal occurs within the embryo, we extended a previous computational model of Dorsal/Cactus interactions. Our model generically predicts that shuttling occurs in the embryo, and that three experimental perturbations would reveal a shuttling-specific phenotype. First, embryos with a half dose of Dorsal develop gradients that are wider and flatter than normal. Second, slowing Dorsal or Cactus diffusion causes the gradient to widen. And third, expanding the spatial domain where Toll signaling causes Cactus degradation results in a widening or even splitting of the Dorsal gradient. We performed each of these model-guided experiments and observed the predicted phenotypes.

Our results suggest that Cactus plays multiple roles in dorsal-ventral axis specification. Besides its predominant role, which is to maintain Dorsal in the cytoplasm, a secondary, but important role is to shuttle Dorsal towards the ventral midline. Given that this mechanism has been found in other, independent systems, we suggest it may be more prevalent than previously thought.

D1440B Pax6 and the Polycomb group proteins promote eye formation by repressing alternate non-ocular fates. *J. Zhu*, A. Ordway, K. Buddika, J. Kumar. Indiana University Bloomington, Bloomington, IN.

The suppression of alternative tissue fates is important for the normal development of an organ. It has been shown that the Polycomb group (PcG) proteins, which are epigenetic regulators, are necessary for transcriptional repression of developmental regulated genes, such as Hox genes. Reductions in PcG protein levels can result in homeotic transformations of the wing, leg and antenna. Despite the importance of the PcG

complex in regulating Hox gene expression, its relationship to endogenous gene regulatory networks and its function in tissue/organ fate specification are not well understood. To address this question, we removed Polycomb (*Pc*) from the developing eye-antennal imaginal disc and observed ectopic expression of the Hox gene *Antennapedia* (*Antp*) and the wing selector gene *vestigial* (*vg*). These dramatic changes in gene expression accompanied a homeotic transformation of the eye into a wing. The mutant tissue also underwent hyperplastic growth and increased in size by several orders. However, knocking down other PcG proteins was insufficient to induce this phenotype. The Pax6 gene is required for eye formation in all seeing animals from Drosophila to humans. Although previous studies have revealed that *Drosophila* Pax6 homologs, *eyeless* (*ey*) and *twin of eyeless* (*toy*) are required for eye specification and head capsule development, the potential roles of Pax6 in the suppression of non-ocular tissue fates remain elusive. We discovered that simultaneous removal of PcG proteins and Pax6 could also induce the eye to wing transformation, indicating Pax6 and PcG proteins cooperate to control growth of the eye and to prevent the establishment of alternative tissue fates. Using different Gal4 drivers, we found that the tissue transformation only happened in the eye progenitor cells ahead of furrow where *ey* and *toy* are normally expressed. Removal of Pax6 and PcG proteins in the differentiated photoreceptor cells did not cause the tissue transformation. Moreover, *vg* was activated mainly in the dorsal part of the eye tissue, when Pax6 and PcG proteins were knocked down using *ey*-Gal4. This suggests that dorsal section of the eye disc is more capable to transform compared to the ventral domain. We found that the physical transformation of the eye to wing takes place in the third larval instar, when *vg* was activated ectopically in the eye imaginal disc. However, this transformation requires that PcG proteins and Pax6 are removed from the eye disc starting from the first larval instar stage.

D1441C SoxNeuro and Shavenbaby act cooperatively to shape denticles in the embryonic epidermis. *Nicholas P. Rizzo*, Amy Bejsovec. Duke University, Durham, NC.

During development, morphogenetic processes require the integration of numerous signals to properly shape cells and tissues. These signals are interpreted by cells to induce the precise transcriptional circuitry that controls morphogenesis. The cuticle pattern of the developing embryonic epidermis provides a valuable system to study how this process is executed. It is well established that the fly Wnt, *wingless* (*wg*), generates naked cuticle zones that separate the ventral denticle belts by repressing expression of *shavenbaby* (*svb*), which encodes a transcription factor required for denticle formation. What is not known is how Wg and Svb interact to produce the stereotyped diversity of denticle shapes within each belt. One possibility is that graded levels of Svb, responding to graded levels of Wg signaling, may determine denticle shape. Indeed, we found that the *svb* promoter responds differentially to altered Wg activity levels. However, artificially increasing the levels of ectopic *svb* does not produce morphologically distinct denticles, suggesting that additional factors may be involved. We have discovered that a second Wg-responsive transcription factor, encoded by *SoxNeuro* (*SoxN*), cooperates with Svb to shape the denticles. Co-expressing *SoxN* with *svb* in an ectopic location within the naked cuticle zone is sufficient to rescue shaping of the ectopic denticles. Strikingly, the expression of *SoxN* in *svb* null embryos produces rounded, remnant denticles resembling those present in *svb* null embryos, suggesting that SoxN activity in *svb* mutants promotes these remnant denticles. Consistent with this idea, *svb*;*SoxN* double mutant embryos secrete cuticles that completely lack ventral denticles and dorsal hairs. The lack of denticles in *svb;SoxN* embryos is not due to hyperactivity of Wg, because expression of the Wg target gene, *engrailed*, is not altered. We propose that Svb and SoxN together are required for proper denticle shaping, and we discuss target genes that show differential response to these transcription factors.

D1442A *eyeless* Participates in the Establishment and Maintenance of the Retinal Dorsal/Ventral Axis. *Luke R. Baker*, Justin Kumar. Indiana University, Bloomington, IN.

The eye of the adult *Drosophila* comprises 800 unit eyes known as ommatidia. Each ommatidium is composed of 8 photoreceptors and 12 accessory cells. Ommatidia are organized into dorsal and ventral chiral forms that are determined by their position relative to an equator that bifurcates the adult eye. This midline is established in larval development through the juxtaposition of independent dorsal and ventral compartments specified by differentially expressed and antagonistic gene networks. Here, I show that the Pax6 transcription factor, *eyeless* (*ey*), has a role in mediating the establishment and maintenance of the early DV patterning of the *Drosophila* eye. Flies harboring mutations in *ey* have either small or no eyes. I have examined the developing and adult *ey* mutants and have evidence that D/V patterning is disrupted. The developing eye shows an increase in the expression pattern of the dorsal selector gene *mirr* which suggests ventral eye tissue is reduced or lost. Consistently, the adult eye of *ey* mutants is composed solely of ommatidia of dorsal chirality. Additionally, the dorsal selector gene *wg* and the dorsal head capsule selector *otd* also show increased spatial expression in *ey* mutants, supporting the hypothesis that the loss of *ey* disrupts D/V patterning. The midline, which can be visualized by the expression of a *fj-lacZ* reporter, is shifted in accordance with the increased expression pattern of dorsal selector genes. Together, these data suggest that the loss of *ey* affects the formation of dorsal and ventral compartments in the developing eye. This represents a new role for Pax6 in eye development.

D1443B Role of axial patterning genes in growth regulation during eye development. *N. GOGIA*[1], M. KANGO SINGH[1,2,3], A. SINGH[1,2,3]. 1) Department of Biology, University of Dayton, 300 College Park Drive, Dayton, OH; 2) Premedical Program, University of Dayton,OH; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, 300 College Park Drive, Dayton, OH.

An important question in developmental biology is that how growth regulation and cell fate specification genes work together during organogenesis to form a three-dimensional organ. In any multicellular organism, organogenesis requires axial patterning to determine Antero-Posterior (AP), Dorso-Ventral (DV), Proximo-Distal (PD) axes. Any deviation in these axes during development leads to congenital birth defects. We use *Drosophila melanogaster* (a.k.a fruit fly), eye as our model. In *Drosophila,* Dorso-Ventral (DV) patterning marks first lineage restriction event where axial patterning genes, regulates the formation of dorsal, ventral compartments in the eye. We have identified *defective*

proventriculus (*dve*-a Homeobox gene), an ortholog of SATB homeobox 1 (special AT-rich sequence binding protein 1), as a new member of DV patterning gene hierarchy. We have shown that *dve* acts downstream of *pannier* (*pnr*, GATA-1 transcription factor), and upstream of *wingless (wg)* in dorsal gene hierarchy. Loss-of-function of *dve* or *pnr* results in dramatic dorsal eye enlargements, whereas gain-of-function suppresses the eye fate. We have demonstrated that Wg is a downstream target of Hippo growth regulatory pathway (highly conserved) in eye. Furthermore, Wingless (Wg), which acts downstream of *dve*, also exhibits similar eye enlargement and suppression phenotypes and has been shown to play a role in growth. Here, I present that DV patterning genes interact with Hippo signaling to regulate the common downstream target, Wg during growth and patterning of developing *Drosophila* eye. We are pursuing genetic epistatsis studies to determine how the members of these two highly conserved pathways regulate Wg signaling in the developing eye. Our data (using Gain-of-function studies) states that activation of Hippo signaling in *dve*, *pnr* expression domain results in change of fate. We have tested retinal determination fate markers in these backgrounds. This study will address an important question-if the axial patterning genes (*dve, pnr*) and Hippo pathway regulates patterning, growth independently or in-coordination with each other by regulating Wg signaling to form an eye/or any organ. Our study will have significant bearing on developmental mechanisms during organogenesis of the eye.

D1444C Defining the role of Glass, a zinc finger transcription factor, in eye development. *C. A. Morrison*, J. E. Treisman. New York University School of Medicine, New York, NY.

Cell fate specification is achieved through the combinatorial activity of transcriptional regulators, which activate the developmental program necessary to establish a cell's identity. The zinc finger transcription factor Glass (Gl) acts as a key determinant to establish photoreceptor identity in the *Drosophila* eye imaginal disc. In *gl* mutants, eye progenitor cells exhibit certain aspects of early neuronal differentiation and begin to organize into ommatidial clusters, but these cells fail to express photoreceptor-specific genes and display abnormal axon projections into the brain. It is thought that Gl directs the transition from an unspecified neuron to a photoreceptor identity by activating numerous downstream target genes. To determine if Gl is sufficient to induce any or all aspects of photoreceptor development we generated a UAS-*gl* transgenic line and performed misexpression experiments in neuronal and non-neuronal cells. We show that Gl misexpression in neuroblasts is sufficient to induce ectopic expression of the photoreceptor-specific gene *chaoptin* (Chp) in the larval brain. We also show that Gl is sufficient to induce Chp in non-neuronal cells of the larval wing and leg imaginal discs and that the Gl-expressing cells are not converted to a neuronal identity. Furthermore, Gl misexpression in imaginal discs or abdominal cells leads to ectopic eye pigment in the adult. We have performed RNA-seq in order to identify which genes can be ectopically induced by Gl in neuronal and non-neuronal cells. We find that some predicted Gl targets, including *PvuII-PstI homology 13* (*Pph13*) and *Eye-enriched kainate receptor* (*Ekar*), as well as *cardinal* (*cd*), which encodes a pigment biosynthetic enzyme, can be induced in both cell types, while others are induced only in either neuronal cells or non-neuronal cells. Surprisingly, we find that many genes predicted to be Gl targets because of the presence of Gl binding sites in their regulatory regions and the co-variation of their expression with Gl, such as *lozenge* (*lz*) and *kin of irre* (*kirre*), are not induced in either context. We are currently performing a promoter analysis of these genes in order to identify possible repressors of Gl function. Our misexpression data and RNA-seq analysis also suggest that Gl may promote cone cell and pigment cell specification in addition to photoreceptor identity. We are currently investigating this possibility using cell type-specific knockdown and rescue experiments. .

D1445A Zinc finger transcription factors, Teashirt and Tiptop: Their role in promoting early eye-antennal disc development and maintaining segregated eye-antennal fates. *Sneha Palliyil*, Justin Kumar. Dept. of Biology, Indiana University, Bloomington, IN.

How a small set of cells go on to become tissues and organs is one of the fundamental questions in the field of developmental biology. The eye-antennal imaginal discs give rise to the compound eye, ocelli, antennae, maxillary palps and the head capsule. Fourteen genes that form the retinal determination(RD) network are responsible for the proper specification of the compound eye and for many aspects of its development. Among them are the zinc finger proteins, Teashirt(Tsh) and its paralog,Tiptop(Tio). Understanding the specific functions of Tsh/Tio in the early eye-antennal imaginal disc development is the primary focus of my work. Tsh/Tio have redundant expression patterns in the larval third instar imaginal discs and are suggested to be functionally equivalent. Knockdown of each gene individually has very mild,if any, phenotype in the eye, while knocking down both genes simultaneously results in flies with small or missing eyes. We have evidence suggesting that *tsh*, functions with another RD gene *twin of eyeless*(*toy*),to maintain cells in a proliferative state. We determined that Tsh and Toy are required together during the embryonic and first larval instar stages. Simultaneous removal of both *tsh* and *toy* during these stages results in headless flies. We are examining how the loss of Tsh and Toy affects the expression of other RD network genes and whether the headless phenotype is due to increased cell death or a loss of cell proliferation. Also,I have evidence that Tsh/Tio play a part in antagonizing non-ocular fates.My results reveal that Tsh/Tio are capable of repressing *cut*, a key specification gene of the antenna and head epidermis when misexpressed in the antennal disc.Work from several labs has shown that the Sine Oculis(So) and Eyes Absent(Eya) proteins, which are also members of the RD network, function to repress the expression of gene regulatory networks that control surrounding non-ocular fates. My results suggest that the Tsh/Tio repress *cut* independent of the So-Eya complex. Finally,I have evidence that this Tsh/Tio require the co-repressor C-terminal Binding Protein(CtBP).

D1446B Cytoneme-mediated cellular synapsis for Hh signaling. *L. GONZÁLEZ-MÉNDEZ*, I. GUERRERO. CBMSO, MADRID, MADRID, ES.

Hedgehog (Hh) is a morphogen with a central role in development, and also implicated in other processes in the adult organism, including stem cells maintenance, cell migration, axon guidance and cancer. In many systems, Hh morphogen is secreted from a defined group of cells and specifically distributed to create a concentration gradient that drives differential genes expression and subsequent specification in target cells. The mechanism/s responsible for Hh gradient establishment through a tightly controlled dispersion of the morphogen remains

controversial. Increasing evidences support the role of specialized and highly dynamic filopodia (cytonemes) in the transport of morphogens during development, facilitating the concentration and spatial restriction of the signal. In this context, we have recently demonstrated that Hh transport via exosomes along cytonemes emanating from Hh producing cells is an essential mechanism for the restricted distribution of Hh in Drosophila epithelia development. It has recently suggested that signaling between epithelial cells occurs through direct cell-cell contacts even if they are separated by several cell diameters, in a similar manner to a synaptic connection. Currently, we are focusing on the study of the cytoneme-mediated communication between Hh producing and receiving cells during Drosophila wing disc patterning. In order to identify the physical interaction sites along these cytonemes, we use CD4-based GRASP technique combined with LexA/lexO and Gal4/UAS binary expression systems. We observe a strong connection between A and P compartments cytonemes at the basal surface of the epithelium. In addition, we study this interaction between Hh sending and receiving cytonemes during Hh reception by expressing some of the components of the Hh reception complex, such as Ihog/Boi, Patched (Ptc) and the proteoglycans Dally and Dlp. Interestingly, high levels of Dally, Dlp and Ihog accumulate at the contact sites along A and P cytonemes. Also, we have evidences for the presence of both Ptc (Hh receptor) and Hh at cytonemes emanating from Hh receiving cells. We find besides a recycling process of Ptc and Ihog to reach the plasma membrane as exosomes; Ptc and iHog colocalize with exosome markers, and different conditions (UAS-RNAi) that disturb the exosome formation in the Hh receiving cells lead to a block in Ptc recycling, modifying the Hh gradient. Our results agree with a cytoneme-mediated Hh release and reception, and suggest a mechanism of cell-cell signaling mediated by exosomes containing Hh and Ptc, resembling synaptic contacts.

D1447C Steep difference in Dpp signaling triggers JNK-dependent transcriptional activation of *reaper*. *H. Nojima*, JP. Vincent. The FRANCIS CRICK institute, London, GB.

Extensive evidence suggests that Dpp signaling controls patterning and growth in wing imaginal discs. Patches of cells undergoing excessive Dpp signaling trigger overgrowth while at the same time inducing JNK dependent apoptosis at their boundary. To investigate this process, we have developed several reporters of JNK signaling and pro-apoptotic gene expression. Using these tools, which we hope will be widely useful, we confirmed that JNK signaling is activated on both sides of the boundary. We also found that this leads to transcriptional activation of *reaper*, but not *hid*. Suppression of JNK signaling prevented *reaper* expression and led to a reduction in the number of apoptotic cells. Moreover, in the absence of *reaper*, apoptosis was no longer detected at the boundary of clones undergoing excess Dpp signaling. We conclude that morphogenetic apoptosis is mediated by JNK dependent activation of *reaper* transcription.

D1448A Trithorax Group proteins interact with Pax6 to specify proper organ number in the *Drosophila* eye-antennal disc. *A. J. Ordway*, G. M. Teeters, J. P. Kumar. Indiana University, Bloomington, IN.

The control of organ number is vital to the proper development of an organism. For example, the decision to make two lungs but just one heart allows vertebrate body to function correctly. My work is focused on understanding how this type of decision is made in *Drosophila* –in particular; I am interested in understanding how each eye-antennal disc produces a single adult antenna. I have uncovered a role for the Trithorax Group (TrxG) of epigenetic regulators in this decision. Reductions in some members of the TrxG result in an adult fly with four antennae instead of the normal two. In other cases, this antennal duplication requires the simultaneous reduction of a TrxG member and *twin of eyeless* (*toy*), a selector gene for eye fate and the *Drosophila* homolog of vertebrate Pax6. Data from our lab also shows that eliminating *toy* and a second Pax6 gene, *eyeless* (*ey*), during certain developmental windows causes identical antennal duplications. These results demonstrate novel roles for Pax6 in antennal development and for TrxG proteins in regulating organ number. There is precedent for communication between Pax proteins and TrxG members. It has been reported that vertebrate Pax2 physically interacts with PTIP (Pax Transcriptional Activation Domain Interacting Protein), a TrxG protein. To uncover how Pax6 and TrxG cooperate to control antennal numbers we are using candidate gene approach beginning with the Wingless (Wg) pathway. Prior studies have shown that some TrxG members regulate Wg signaling and that reductions in early Wg expression within the antennal field can lead to its duplication. In addition to its role in antennal development, Wg signaling is also necessary for preventing the initiation of ectopic furrows. We have observed ectopic furrows in discs with reduced levels of TrxG. Our model suggests that Pax6 and TrxG cooperate to regulate Wg signaling in the eye and early antennal discs. Disrupting this regulatory circuit results in ectopic furrows and an increase in the number of the antennal segments.

D1449B Computational analysis of spatiotemporally-patterned intercellular Ca^{2+} transients in the *Drosophila* wing imaginal disc. *P. A. Brodskiy*, Q. Wu, C. Narciso, J. J. Zartman. University of Notre Dame, Notre Dame, IN.

The calcium ion (Ca^{2+}) is a second messenger known to have important roles in regulating physiological processes such as proliferation, apoptosis and cell differentiation. However, how the spatiotemporal properties of Ca^{2+} transients reflect the underlying physiological state of tissues is still poorly understood. Here we use an established model system of an epithelial tissue, the *Drosophila* wing imaginal disc, to investigate how tissue properties impact the propagation of Ca^{2+} transients in simple epithelia. We recently observed oscillatory waves in cultured larval wing discs. A computational model of Ca^{2+} signaling was developed to explain these oscillatory Ca^{2+} waves. The computational model predicts that modulating the variance of phospholipase C (PLC) activity leads to multiple signaling regimes that transition from quiescence to coordinated long range waves at the tissue scale to noisy locally uncoordinated Ca^{2+} transients. As validation of this prediction we confirmed that these predicted regimes can be recapitulated experimentally through manipulating levels of serum-based stimulation. Imposing a pattern of Ca^{2+} signaling kinetics also explains relative differences in Ca^{2+} signaling frequencies between the anterior and posterior compartment of the wing disc. This model provides an important basis to computationally test mechanisms of Ca^{2+} regulation and function in organ development and homeostasis.

D1450C Rab11, a multi talented determinant of morphogenesis in *Drosophila*. *Jagat Kumar Roy*, Divya Singh, Praween Choubey, Nabarun Nandy. Banaras Hindu University, Varanasi, Uttar Pradesh, IN.

The small GTPase Rab11, a major regulator of endomembrane traffiking, has been linked to developmental defects. Using loss and gain-of-function approaches in *Drosophila,* new roles for Rab11 in mediating the process of myoblast proliferation, migration and differentiation during *Drosophila* indirect flight muscle development have been identified. Rab11 function in the developing IFMs is a prerequisite for the differentiation and *de-novo* development. It functions in the proliferating adult muscle precursors (AMPs) for normal division and for maintaining cellular contact between the neighbouring myoblasts, failure to which results in pre matured differentiation of myoblast. Genetic interaction studies show that Rab11 partners with Notch in patterning the IFMs, since expression of ligand independent Notch in Rab11 mutant background can rescue the muscle defects and restores the myoblast number. Rab11 is also involved in the morphogenesis of Malpighian tubules which is one of the important organs for excretion and osmoregulation in insects and escapes histolysis during metamorphosis. It has two cell types, the principal cells (PCs) and stellate cells (SCs). Rab11 downregulation in PCs by using Gal4c-42 leads to shortening of MTs due to reduced endoreplication. Also interestingly, the SCs do not show mesenchymal to epithelial transition and do not differentiate into characteristic star shaped cells during pupal stages, show altered physiological functions and individuals die at pharate adult stage. In another set of experiments we found Rab11 playing an instrumental role in epithelial morphogenesis of developing *Drosophila* embryos. We observed that embryos lacking a functional Rab11, fail to undergo Dorsal Closure and show improper cuticle synthesis. The epithelial cells of these embryos, lack proper cell-adhesions and show anomalous expression patterns of activated JNK, which interestingly, are quite similar to the cells which lose their apical-basal polarity, suggesting a putative interaction of Rab11 functions with the JNK pathway. We suggest that Rab11 mediated regulation of inter-cellular membrane trafficking may be an additional mechanism to regulate the intercellular signaling during *Drosophila* development..

D1451A *Macroglobulin complement-related* is required for border cell migration and proper egg shape during *Drosophila melanogaster* oogenesis. *H. Alhadyian*, R. Ward. The University of Kansas, Lawrence, KS.

Macroglobulin complement-related (*Mcr*) encodes an ~200 kDa protein with α-2-Macroglobulin and LDL receptor class A domains. We initially identified *Mcr* in a genetic screen of mutations that dominantly enhanced a malformed leg phenotype in *broad* mutant animals, suggesting a role for *Mcr* in imaginal disc morphogenesis. We subsequently determined that *Mcr* is a core component of epithelial septate junctions (SJs), which are analogous to the vertebrate tight junction in providing an essential occluding function to the epithelium. Interestingly, homozygous mutations in *Mcr* are embryonic lethal with defects in developmental process including head involution and dorsal closure that occur prior to the establishment of the SJ, suggesting a role in morphogenesis that is independent of its role in the occluding junction. To extend these studies we are investigating the role of *Mcr* during morphogenetic events that occur during oogenesis. First, we investigated the expression of Mcr in oogenesis, and determined that it is expressed in the germarium, where it is enriched in the germline stem cells. It is also expressed in follicle cells, with the strongest expression in the polar cells. We next used cell-type and stage specific Gal4 drivers and mosaic analysis to examine the function of *Mcr* during oogenesis. We find that reducing *Mcr* in border cells using *slbo-GAL4* to drive *Mcr-RNAi* results in 45% of the border cell clusters splitting apart. Knocking down *Mcr* in all the follicle cells including the follicle stem cells (*GR1-GAL4>Mcr-RNAi*) resulted in degeneration of middle stages egg chambers, whereas knocking down *Mcr* in all the follicle cells from stage 8 onwards (*c204-GAL4>Mcr-RNAi*) perturbs eggs elongation, resulting in stage 14 egg chambers that are significantly rounder than wild type egg chambers. Mosaic analysis using the strong loss of function allele *Mcr1* revealed that germline clones formed germ cell cysts that fail to separate from the germarium. In addition, *Mcr* clones in the anterior and posterior follicle cells, including stalk cells, results in rupture of the epithelium. Together, these observations suggest that *Mcr* plays an important role in multiple aspects of egg chamber morphogenesis during oogenesis.

D1452B Unstable enhancer activity during early development of Drosophila. *S. Casas-Tinto*, M. Arnés, A. Ferrús. Cajal Institute, Madrid, Madrid, ES.

Gene expression is a tightly regulated process that eventually determines cell fate. The regulation of this process relies on short gene regulatory sequences, enhancers, whose activity fluctuates until the on or off state is fixed. Based on the G-TRACE analysis system, we show here that a set of nervous system specific enhancers (elav, D42, OK6) have ectopic activity in epithelial tissues early in development. This ectopic activity is variable, unstable and mainly influenced by the primary sequence of the enhancer and the insertion site in the chromosome. In addition, the LN2 enhancer from the sex lethal (sxl) gene showed sex dependent features in its ectopic expression. Although variable and unstable, the non-canonical expression of enhancers seems to be regulated in terms of tissue and extent. The factors that determine enhancer activity are relevant for transgenic animal models and the engineering of biotechnology tools. As well as for the interpretation of many current developmental biology studies in which Gal4 lines are used. In addition, since all 12 neural enhancers analyzed here showed unstable expression, we argue that this general phenomenon may be relevant for evolutionary change.

D1453C The *Drosophila melanogaster tfiia-s-2* gene encodes a male germline-expressed homolog of the small subunit of the TFIIA general transcription factor. *M. Hiller*, E. Collins, J. Mohammadi. Goucher College, Baltimore, MD.

Eukaryotic General Transcription Factors (GTF) are protein complexes that help position RNA polymerase at promoters and initiate transcription. The general transcription factors TFIIA and TFIID are multi-subunit protein complexes that physically associate with each other and assemble on promoters early in the process of transcription initiation. TFIIA consists of three protein subunits. In *D. melanogaster*, a single gene, *tfiia-l*, encodes a 48 kD polypeptide that is proteolytically cleaved to form two proteins of 30 kD and 20 kD. *tfiia-s* encodes the small subunit of 14 kD. Both the large and small subunits are widely expressed in many tissues and cell types. A homolog of the small subunit, *tfiia-s-*

2 (CG11639), is expressed only in the male germ-line. We have shown that two transcripts are encoded by *tfiia-s-2*, and that both proteins are able to interact with the generally expressed large subunit when expressed in *E. coli*. Together, this suggests that three different forms of TFIIA might be present in the male germ-line. TFIID is comprised of TATA-binding protein (TBP) and up to fourteen TBP-associated factors (TAFs). Several testis-specific TAFs have been identified. Mutations in the testis-TAFs cause defects in transcription and block spermatid differentiation, however, there is no mutant allele of *tfiia-s-2*. We are investigating the possibility that the testis-specific TFIIA and TFIID homologs work together to during transcription initiation in the male germ-line. We are also characterizing the ability of complexes containing TFIIA-S-2 to physically associate with TFIID subunits and testis-expressed homologs of TFIID subunits.

D1454A Identification of transcriptional regulators and enhancer regions of the *Alk* locus in *Drosophila*. *P. Mendoza-Garcia*[1,2], F. Hugosson[1], K. Hens[3], K. Pfeifer[1], G. Wolfstetter[1], D. Popichenko[2], B. Deplancke[4], R. Palmer[1]. 1) Sahlgrenska Academy, Gothenburg University, Gothenburg, Sweden; 2) Molecular Biology, Umea University, Umea, Sweden; 3) Centre for Neural Circuits and Behaviour, University of Oxford, Oxford, United Kingdom; 4) Laboratory of Systems Biology and Genetics, Lausanne, Switzerland.

Specification of the *Drosophila* visceral trunk mesoderm occurs upon inductive signals from the ectoderm. This induces the transcription of some key regulators for visceral muscle differentiation such as Tinman, Bagpipe and/or Biniou. The **receptor tyrosine kinase (RTK)** Alk is expressed in the developing CNS but predominantly in the embryonic trunk visceral mesoderm (VM), where is activated in response to the secreted ligand Jelly Belly (Jeb). **Jeb/Alk signalling** is responsible for founder cell (FC) specification in the forming VM and for the subsequent fusion to the fusion competent myoblasts (FCMs) to form the functional larval midgut. In this study we have investigated the transcriptional control *Alk* expression in the embryonic VM. The *Alk* locus was examined by several approaches including functional enhancer activity in vivo, deletion analysis, yeast one-hybrid screening and *in silico* analysis of predicted transcription factors binding sites. Furthermore, we have studied *in vivo* relevance of several identified and *in silico*-predicted transcription factors that could modulate *Alk* gene expression by single or combinatorial effect. Here we describe the identification of *cis*-regulatory elements in the *Alk* gene active during early embryogenesis and sufficient for the embryonic expression of *Alk* gene in the VM. This regulatory region is highly conserved among different *Drosophila* species. We also show the role of some of the identified transcription factors and their potential regulation of *Alk* transcription during embryo development through the identified regulatory region.

D1455B Myb and Mip120: an oncogenic dyad that causes tumorigenesis in Drosophila 3rd instar larvae. *P. J. Vorster*, J. S. Lipsick. Stanford University, Stanford, CA.

The *Myb* proto-oncogene family is recurrently mutated in human leukemia, brain cancer, breast cancer, and cancer of the salivary gland. Whereas vertebrate animals have three different *Myb* genes, Drosophila has a single non-redundant gene. The Drosophila Myb protein regulates gene expression in concert with other proteins in the RBF E2F2 and Myb-interacting proteins (dREAM) complex. Apart from Myb, homologs of all proteins contained within the dREAM complex were identified by mutations in the group B synthetic multivulval (synMuvB) genes in *C. elegans*. In humans, the MuvB core associates either with Rb-E2F-Dp proteins or with Myb proteins. In the absence of Myb the MuvB core suppresses the transcription of other genes during G0/G1 of the cell cycle, while B-Myb is bound to the MuvB core during S/G2 of the cell cycle and activates transcription. The Myb-interacting protein of 120 kDa (Mip120, homologous to LIN54 in humans and *C. elegans*), is part of the MuvB core. While Myb and other components of the dREAM complex are misregulated or mutated in many human cancers, a role for Mip120 remains unclear. In the course of studying the epigenetic regulation of Drosophila eye development by the dREAM complex, we have discovered a new role for Mip120 in tumorigenesis. Dual expression of two members of the dREAM complex, Myb and Mip120, caused solid tumors in 3rd instar larvae, while expression of Myb or Mip120 alone did not have this effect. This result suggests that protein stoichiometry and abundance of interactors of the dREAM complex play a vital role in regulation of tumor development. Our work also highlights the role of Mip120 as a critical member of the Myb-MuvB/dREAM complex in oncogenesis.

D1456C Novel function of the class I bHLH protein Daughterless in postmitotic neurons. *E. A. Waddell*[1], E. L. Robinson[1], M. D'Rozario[1,2], D. R. Marenda[1,3]. 1) Drexel University, Philadelphia, PA; 2) Washington University School of Medicine, St. Louis, MO; 3) Drexel University College of Medicine, Philadelphia, PA.

Class I basic Helix Loop Helix (bHLH) proneural proteins are highly conserved transcription factors. Class I bHLH proteins are broadly expressed in multiple tissues and have critical roles in many developmental processes such as neurogenesis. However, little is known about how class I bHLH proteins function in mature, differentiated neurons. Class I bHLH proteins function during development by forming heterodimers with class II bHLH proteins to activate transcription or by forming homodimers to both activate or restrict transcription of target genes. Class I bHLH proteins can also heterodimerize with class V HLH proteins, preventing gene expression. Our laboratory has identified a novel role for the class I bHLH protein Daughterless (Da), the only class I bHLH protein in *Drosophila*. Neuromuscular junction dissections were performed on third instar larvae and mature motor neuron synapses were imaged from this tissue. We have shown that Da functions to restrict synaptic branching and synapse number in these postmitotic neurons. Furthermore, our laboratory has shown that Da accomplishes this in part through restricting the expression of Neurexin, a cell adhesion molecule required for synapse formation. Third instar larvae were dissected and direct immunohistochemistry was performed for α-Nrx1 to show Neurexin levels in Da knockdown and overexpression genotypes. The mammalian ortholog of *Da*, *Transcription Factor 4* (*TCF4*), is a well-identified risk factor for a number of neurodevelopmental disorders including Pitt-Hopkins syndrome and schizophrenia, both of which are characterized by defects in behavior, learning, and memory. It is hypothesized that defects in postmitotic neurons may contribute to the phenotypes associated with these neurodevelopmental disorders. Therefore, identifying

how Da functions to restrict synaptic branching and synapse number in postmitotic neurons will have a significant impact on our understanding of the etiology of these diseases.

D1457A Germline silencing of pUASt depends on the piRNA pathway. *YC. Huang*, H. Moreno, WM. Deng. Florida State Univ, Tallahassee, FL.

One of the widely used techniques in *Drosophila* is the Gal4/UAS system, which allows tissue-specific misexpression or knockdown of interested genes. The original UAS vector, the pUASt, can only be activated in somatic tissues, not in the germline. By replacing the hsp70 promoter and the SV40 3'UTR with the P transposase promoter and the K10 3'UTR, respectively, Rørth (1998) generated a modified UAS vector, called pUASp, which can respond to Gal4 in both somatic and germline tissues. However, the underlying mechanisms for UASt silencing in germline cells remained unclear. Here, we report that the piRNA pathway is involved in suppressing UASt expression in ovarian germline cells. When piRNA pathway components (Piwi, AGO3, Aub, Spn-E, and Vasa) were knocked down individually in germline cells, the UASt-RFP/GFP can be detected in germline cells in the ovary. To determine how piRNAs silence UASt-transgene expression, we performed RNA-seq analyses of small RNAs and found that the hsp70 promoter of pUASt is a potential piRNA target. Additionally, since the UASt vector also contains the SV40 3'UTR, which happens to be targeted by the Nonsense-mediated RNA decay (NMD) pathway. To find out whether NMD is also responsible for UAST silencing in the germline, we knocked down several NMD key components in ovarian germline cells, and observed a low frequency of UASt-RFP expression in germline cells with *Smg5* knockdown. Taken together, our findings suggest an important role of the piRNA pathway, and a potentially minor role of the NMD pathway in germline UASt silencing.

D1458B Transcriptome profile of abnormal testis reveals potential function for ceramidase in *drosophila melanogaster*. *M. Zhang*[1*], Z. Zhu[2]. 1) Zhejiang Univ., Hangzhou, Zhejiang, CN; 2) Zhejiang Univ., Hangzhou, Zhejiang, CN.

Sphingolipids are important components of eukaryotic cell membranes. Some sphingolipid metabolites, such as ceramide, sphingosine and sphingosine-1-phosphate, also act as signaling molecules to mediate various biological processes, like programmed cell death. However, little is known about the mechanisms responsible for the abnormality in testis development in ceramidase knock down strain. Genomic information for the phenotype is currently unavailable, therefore, to facilitate research on it, we present a method for *de novo* assembly of drosophila transcriptome using short read sequencing technology (Illumina) combined with a tag-based digital gene expression (DGE) system. Illumina sequencing generated the drosophila transcriptome from the reproductive organ of two phenotypes with normal and abnormal testes. In total, we obtained 46,498,038 uniquely mapped reads that covered 87.53% of the current annotated transcripts, which represented 16246 mRNA transcripts, across all the samples. Among them, 34 differentially expressed genes ($p < 0.05$, false discovery rate $q < 0.05$) between the normal and abnormal testes were revealed, and we confirmed their altered expression levels by quantitative real-time PCR (qRT-PCR). Gene ontology and KEGG pathway analysis demonstrated that the 34 differently expressed genes were enriched in specific biological processes with regard to actin filament-based process, oxidative phosphorylation, metabolic pathways, phenylalanine metabolism and ECM-receptor interaction ($p < 0.05$). The obtained transcriptome and DGE profiling data provided comprehensive gene expression information at the transcriptional level that could facilitate our understanding of the molecular mechanisms of the abnormal testis development.

D1459C *In vitro* characterization of Zelda zinc fingers – discovery of a new DNA binding activity. *N. C. Kirov*[1], H. Y. Liu[1], S. N. Higa[1], C. Y. Nien[1], D. Ouyang[1], A. Yang[2], T. Hughes[2], C. Rushlow[1]. 1) New York Univ, New York, NY; 2) University of Toronto, Toronto, ON.

The transcription factor Zelda (Zld) is essential for zygotic genome activation in Drosophila embryo. It is maternally deposited and activates groups of pre-blastoderm genes required for sex determination, cellularization and embryonic patterning, either alone or in combination with other transcription factors. Zld is a large DNA binding protein (1596 aa) containing six predicted C2H2 type Zn finger domains. Two of them (ZF1, ZF2) are located in the N-terminal half of the protein, while the others are clustered close to the C-terminal end. Zld cognate binding sites, CAGGTAG and related sites (TAGteam sites), are enriched in the regulatory elements of its target genes. *In vitro* binding experiments have shown that the C-terminal Zn fingers are required for binding to DNA, but it is not known how each of them is involved in the binding. The function of the N terminal Zn fingers is unknown. Here we use *in vitro* mutagenesis and DNA binding assays (gel shifts and protein binding microarrays) to further characterize the functional properties of Zld's Zn fingers. Surprisingly, we discovered a new DNA binding activity to G-rich sequences for ZF2 and adjacent conserved amino acids. We also quantified the contribution of the C-terminal cluster to the affinity and specificity of binding to different TAG team sites. We discuss the implications of these findings for Zld function in Drosophila embryos.

D1460A The circadian clock orchestrates *de novo* rhythmic expression of oxidative stress-response genes in aging *Drosophila*. *Rachael Kuintzle*, Eileen Chow, Tara Bonar, Jaga Giebultowicz, David A. Hendrix. Oregon State University, Corvallis, OR.

Maintenance of robust circadian rhythms is associated with healthy aging and delayed onset of age-related diseases. Disruption of these rhythms in flies increases susceptibility to oxidative stress and neurodegeneration. Loss of core clock gene function in mice similarly accelerates the onset of late life diseases and mental decline, indicating that the links between circadian desynchrony and neuronal aging are conserved from flies to mammals. However, the epigenetic mechanisms underlying this relationship have remained enigmatic. Here, we used RNA sequencing (RNA-seq) to compare gene expression in young and old *Drosophila melanogaster* heads at 4-hour intervals around the clock. Although attenuations of core clock gene expression were minor, we identified diverse age-induced transcriptional changes among oscillatory genes, including phase shifts and weakened or abolished rhythmicity. Remarkably, we also discovered a subset of mRNAs that adopted synchronous, *de novo* rhythmicity during aging. These genes were termed "Late Life Cyclers" (LLCs) and include, among others: small heat shock protein *Hsp22*, cytokine *bnl*, lactate dehydrogenase *ImpL3*, and Hsp40-like *CG7130*. Because the majority of LLCs were known to be

upregulated in oxidative stress, we used qRT-PCR to test their expression in heads of young flies challenged with hyperoxia. Interestingly, hyperoxic insult approximately phenocopied the aging effect by inducing rhythmic upregulation of the LLCs, supporting our hypothesis that increased oxidative stress during aging contributes to the LLC phenomenon. We also show that LLC expression was significantly reduced in the heads of Clk^{out} and cyc^{01} flies compared to controls with functional clocks, implicating the circadian transcription factors CLK and CYC as important regulators of LLC transcription. In addition, novel transcript assembly with our RNA-seq data uncovered many age-induced, unannotated genes, including several that exhibit LLC behavior. Besides providing a genome-wide, round-the-clock expression profile in heads of young and old *Drosophila*, our data more broadly offer a temporally unbiased measure of age-dependent differential expression. We have developed a web-based database to enable convenient viewing of these RNA-seq results for individual genes and isoforms. In summary, this study demonstrates a novel role for the circadian clock during aging and begins to uncover the mechanistic relationship between the senescent organism's timekeeper and its changing cellular environment.

D1461B Two temporal functions of Glass: ommatidium pattarning and photoreceptor differentiation. *X. Liang*, Simpla Mahato, Chris Hemmerich, Andrew Zelhof. Indiana University Bloomington, Bloomington, IN 47405, IN.

Much progress has been made in elucidating the molecular networks required for specifying retinal cells, including photoreceptors, but the downstream mechanisms that maintain identity and regulate differentiation remain poorly understood. Here, we report that the transcription factor Glass has a dual role in establishing a functional *Drosophila* eye. Utilizing conditional rescue approaches, we confirm that persistent defects in ommatidium patterning combined with cell death correlate with the overall disruption of eye morphology in *glass* mutants and reveal that Glass exhibits a separable role in regulating photoreceptor differentiation. In particular, we demonstrate the loss of photoreceptors in adult *glass* mutant retinas is due to a failure of photoreceptors to complete differentiation. Moreover, the late reintroduction of Glass in these developmentally stalled photoreceptors is capable of restoring differentiation in the absence of correct ommatidium patterning. Mechanistically, transcription profiling at the time of differentiation reveals that Glass is necessary for the expression of many genes implicated in differentiation, i.e. rhabdomere morphogenesis, phototransduction, and synaptogenesis. Specifically, we show Glass directly regulates the expression of *Pph13*, which encodes a transcription factor necessary for opsin expression and rhabdomere morphogenesis. Altogether, our work identifies a fundamental regulatory mechanism to generate the full complement of cells required for a functional rhabdomeric visual system and provides a critical framework to investigate the basis of differentiation and maintenance of photoreceptor identity.

D1462C Transcriptional regulation by Drosophila Suppressor of Hairy-wing: Investigating contributions of a newly discovered interacting protein HIPP1. *Steve Glenn*, Pamela Geyer. University of Iowa, Iowa City, IA.

Transcription is regulated by three classes of DNA regulatory elements: enhancers, silencers and insulators. Transcription factors bind these elements to coordinate gene expression across cell types and developmental stages. Most metazoan transcription factors carry Zinc finger (ZF) DNA binding domains, often with five or more ZFs. Many polydactyl transcription factors have distinct regulatory functions at different endogenous binding sites. How these activities are regulated is unclear. To better understand how a single transcription factor establishes enhancer, silencer and insulator functions, we study the Drosophila multi-functional twelve ZF insulator protein Suppressor of Hairy-wing [Su(Hw)]. Su(Hw) functions primarily as a repressor, and this activity is required in the ovary for female fertility. Su(Hw) is also important for male fertility through unknown mechanisms. We hypothesize that the diversity of Su(Hw) regulatory functions depends upon recruitment of partner proteins. Recently a novel Su(Hw) interactor was identified, called HP1 and insulator partner protein 1 (HIPP1). We find that HIPP1 is the major Su(Hw) interacting protein, localizing to more than 80% of Su(Hw) sites. HIPP1 is homologous to the vertebrate Chromodomain on Y (CDY), a transcriptional repressor that is required for spermatogenesis in mouse. These data suggest that HIPP1 may mediate Su(Hw) repressor function. To test this hypothesis, we undertook a genetic approach using genome editing to generate numerous *HIPP1* deletion mutants. We are currently using these mutants to determine the role of HIPP1 in Su(Hw) function, as well as defining its role in heterochromatin stability and function.

D1463A Split-ends is required for Ecdysone production during Larva development. *Sattar Soltani*, Qiuxiang Ou, Kirst King-Jones. Alberta, Edmonton, CA.

Steroid hormones coordinate many biological processes during development, metabolism and reproduction. *Drosophila* provides an excellent platform for studying steroid hormone regulation, since many studies have examined the role of ecdysone, the major insect steroid, and its roles during the development of the fly. Larval ecdysone production occurs in the prothoracic gland (PG) and is carried out by a group of well-examined enzymes that are encoded by the "Halloween" genes. Although the enzymatic reactions carried out by the Halloween enzymes are fairly well characterized, little is known about the molecular and genetic factors regulating this pathway. We report here that the *split-ends* gene is required for ecdysone regulation in the PG. *Split-ends* is a transcriptional co-repressor and that harbors three N-terminal RNA-binding motifs and a highly conserved C-terminal SPOC domain. PG-specific knock down of *split-ends* via RNAi resulted in developmental arrest during late third instar larvae, with no animals progressing to the pupal stage. Our data suggest that the down-regulation of *split-ends* affects ecdysone production due mainly to altering the expression level of Halloween genes. Furthermore, arrested animals can be rescued when fed with ecdysone or precursors thereof. Taken together, we provide new insights into how a co-repressor can modulate ecdysone production during *Drosophila* development.

D1464B Whole genome analysis of the transcriptional corepressor, Atrophin, reveals interactions with Trithorax-like and regulation of Dpp and Notch signaling. *K. Yeung*[1,2], D. Yap[3], P. H. Holmqvist[4], A. Boija[4], M. Moksa[5], S. Aparicio[3], M. Fanto[6], M. Hirst[5], M. Mannervik[4], H. McNeill[1,2]. 1) Lunenfeld-Tananbaum Research Institute, Toronto, CA; 2) Department of Molecular Genetics, University of Toronto, Toronto, CA; 3) Department of Molecular Oncology, BC Cancer Research Centre, Vancouver, CA; 4) Department of Molecular Biosciences, The Wenner Gren Institute, Stockholm University, Stockholm, Sweden; 5) Department of Microbiology & Immunology, University of British Columbia, Vancouver, CA; 6) MRC Centre for Developmental Neurobiology, King's College London, Guy's Campus, London, UK.

Atrophin (Atro, also known as Grunge) is a transcriptional corepressor. Mutation of a mammalian *Atro* homolog, *Atrophin-1*, causes a neurodegenerative disease called dentatorubral-pallidoluysian atrophy in humans, while mutation of the second mammalian *Atro* homolog, *Atrophin-2*, causes severe developmental defects. Although *Drosophila Atro* mutants have a large range of phenotypes including neurodegeneration, segmentation and planar polarity defects, very little is known about Atro's binding partners and downstream targets. We present the first genomic analysis of Atro using ChIP-seq against Atro. Our Atro ChIP-seq identified 1300 potential targets of Atro; these include *engrailed*, and components of the *dpp* and *Notch* signaling pathways. Using *Atro* mutant clones, we show that Atro regulates *dpp* signaling and the expression of Engrailed in imaginal discs. Interestingly, Atro mutant imaginal disc clones have similar phenotypes to the ones caused by loss of Notch signaling. In addition, our bioinformatic analyses, sequential ChIP and coimmunoprecipitation experiments show that Atro and Trithorax-like (Trl, *Drosophila* GAGA factor) physically interact and bind to the same loci. *Atro* and *Trl* also interact genetically. We propose that Atro and Trl function together as a complex to regulate transcription.

D1465C Characterization of a *grainyhead* neuroblast enhancer. *Thomas Brody*, Alexander Kuzin, Svetlana Smith, Ward Odenwald, Neural Cell-Fate Determinants Section. NINDS/NIH, Bethesda, MD.

Analysis of *cis*-regulatory sequences using comparative genomics reveals that enhancers consist of clusters of conserved sequence blocks (CSBs) that are made up of unique and repeat sequence elements; both often consist of identifiable motifs corresponding to binding sites of known transcription factors. Our goal has been to address aspects of enhancer structure, including the basis for spatial and temporal regulation of gene expression and the functions of the large number of CSBs in neural enhancers. We have identified a late temporal network enhancer of *grainyhead*. The enhancer, *grh-15* contains two separable activities, one that drives expression in the brain and another in the ventral cord. We have systematically truncated, rearranged and mutated each of the nine CSBs present in the brain-specific portion of *grh-15*, expressed in a ~12 embryonic brain neuroblasts and in ~15 larval neuroblasts and their lineages. While our results indicate that information regulating enhancer expression is carried in a highly redundant fashion, individual CSBs convey expression in subsets of larval lineages. We also show that the *grh* enhancer is co-expressed within a subset of cells that express the temporal determinants Cas and Pdm, that it marks a subset of both type I and type II neuroblasts and their lineages in the larval brain, and that it is expressed in only some of the Grh^{+} lineages. These studies highlight the advantages of using evolutionary conservation as a guide to the analysis of *cis*-regulatory sequences.

D1466A Fiber-specific Troponin C isoform switching in Drosophila thoracic muscles. *M. Chechenova*[1], S. Maes[2], S. Oas[2], C. Nelson[2], A. Bryantsev[1], R. Cripps[2]. 1) Kennesaw State University, Kennesaw, GA; 2) University of New Mexico, Albuquerque, NM.

During development and differentiation, structural tissue-specific proteins undergo isoform switching. Although instances of multiple isoform expression have been described for all multicellular organisms, particular mechanisms of isoform switching in many cases still remain unclear. Among the five Drosophila Troponin C isoforms, *TpnC4* is predominantly expressed in the fibrillar-type fibers of the indirect flight muscle, whereas *TpnC41C* is the main isoform of the tubular-type fibers of jump muscle. In our study, we show that these two isoforms are essential for the functioning of thoracic muscles, and can mutually replace each other when the expression of each is down-regulated.

To analyze functional importance of the two TpnC isoforms, we created genetic lines with single gene deletions for either *TpnC4* or *TpnC41C*, and a line with a double deletion for both genes. We found that the removal of one of the isoforms resulted in reciprocal activation of expression of the other isoform in both fiber types. Moreover, minimal enhancers of *TpnC4* and *TpnC41C* can be activated in both fiber types, suggesting that normal isoform expression pattern is rather a result of transcriptional repression in non-native muscles. However, functionally these two isoforms were not equivalent. Although *TpnC4* expressed in jump muscles was able to support effective jumping, *TpnC41C* expressed in flight muscles could not sustain flight. Flies with the *TpnC4/TpnC41C* double deletion showed flightless and jumpless phenotypes. Similar data were obtained in experiments with RNAi knockdown of these genes. Analysis of the ultrastructural muscle organization in the flies with the double deletion revealed defects in myofibril alignment and Z-line integrity.

Our study provides insight into isoform switching mechanisms for muscle genes, and defines the importance of TpnC for normal muscle assembly.

D1467B The activity of the *en* imaginal disc enhancers is dependent on chromatin structure. *Y. Cheng*, J. Kassis. Eunice Kennedy Shriver NICHD, NIH, Bethesda, MD.

engrailed (*en*) is a gene important for many different aspects of development including embryonic segmentation and formation of the posterior compartment in imaginal discs. *en* and the co-regulated, redundant gene *inv* reside in a 113 kb domain covered with tri-methylated histone H3 (H3K27me3), the distinctive mark of genes regulated by the Polycomb group proteins. We have been identifying *en* enhancers that drive its complex expression during development. We previously reported that a 79kb genomic *en* construct (*HA-en79*) contains enhancers for both embryonic and wing imaginal disc (WD) expression, and rescues a chromosomal deletion of the *inv/en* domain. Interestingly, even though the WD enhancers are present in the transgene, their ability to drive HA-*en* expression is subject to *en* protein (EN) auto-repression; in the presence of endogenous EN, HA-en is only expressed in subsets of the wild type domain. In contrast, the endogenous *en* gene is not repressed

by the presence of the *HA-en79* transgene. Further, reporter constructs that contain the imaginal disc enhancers driving lacZ show lacZ expression only in the anterior compartment; our data strongly suggest that EN silences *lacZ* expression in the posterior compartment. These data indicate that removing the imaginal disc enhancers from the *inv/en* domain alters their activity, rendering them sensitive to repression by EN. Chromatin-immunoprecipitation experiments suggest that EN binds directly to a DNA fragment that contains one of the imaginal disc enhancers. We suggest that when this imaginal disc enhancer is within the *inv/en* domain, its activity is high, and it cannot be repressed by EN, but when this same enhancer is taken out of the domain, EN is able to repress it. Experiments to examine the chromatin marks present in the endogenous locus and the small and large transgene are in the planning stages.

D1468C Concentration Dependent Activity of the Bicoid Transcriptional Activator. *Colleen Hannon*, Shelby Blythe, Eric Wieschaus. Princeton University/HHMI, Princeton, NJ.

In order for embryonic development to proceed correctly and reproducibly, the expression of genes in individual cells must be coordinated with precision. In *Drosophila*, graded expression of the maternal transcription factor Bicoid (Bcd) provides positional information to pattern the anterior-posterior (AP) axis of the developing embryo. Bcd is known to bind hundreds of sites in the genome, activating target genes at different positions along the AP axis. To measure Bcd binding states at specific concentrations along its gradient, we have developed a series of transgenic lines that express defined uniform concentrations of Bcd. Using chromatin immunoprecipitation for Bcd followed by high throughput sequencing in these transgenic embryos, we group Bcd-bound target regions into several "affinity" classes based on their *in vivo* occupancy by Bcd at different concentrations. We find that the occupancy of a given target sequence for Bcd is dependent not just on the biochemical affinity of its Bcd binding sites, but its genomic context. Further, we find that some low affinity target regions are dependent on Bcd for maintaining an open chromatin state. This suggests a model in which Bcd is able to influence chromatin structure to gain access to low affinity targets at high concentrations. In contrast, high affinity targets are more accessible either through their native chromatin structure or as a result of other chromatin modifying factors.

D1469A Expression of reciprocal antagonists Mirr and Mid is regulated by localized input from EGFR, JAK/STAT, and Dpp signaling pathways. *Scott De Vito*[1], Mariana Fregoso Lomas[1], Jean François Boisclair Lachance[2], Josée Houde[1], Laura Nilson[1]. 1) Department of Biology, McGill University, Montreal, Quebec, CA; 2) Ben May Department for Cancer Research, University of Chicago, Chicago, Illinois, USA.

EGFR signalling is central to tissue patterning. In Drosophila ovarian follicular epithelium, EGFR signalling is activated by Grk, a ligand secreted by the oocyte. Localized secretion of Grk leads to localized EGFR signalling, which in turn specifies distinct cell fates at different stages . In early oogenesis, Grk/EGFR signalling at the posterior induces a posterior fate characterized by expression of the paralogous T-box transcription factors Mid and H15. In later stages, dorsal anterior Grk/EGFR signalling induces dorsal anterior fates characterized by the expression of the transcription factor Mirr. These fates contribute to structure of the eggshell. Patterning of this epithelium thus involves activation of the same signalling pathway leading to different outcomes in different regions of the tissue. Here we show that EGFR signalling outcome is determined by spatially localized input from other signalling pathways. Here we show that at the anterior of the epithelium, Dpp signalling cooperates with Grk to induce expression of Mirr while also repressing Mid and H15. At the posterior of the epithelium, JAK/STAT signalling is required for expression of Mid and H15 while also repressing Mirr. Moreover, we show that Mid and Mirr mutually repress each other, contributing to the complementary expression of Mirr in the dorsal anterior and Mid at the posterior. Input from Dpp and JAK/STAT signalling thus defines whether EGFR activity will induce expression of Mirr or expression of Mid/H15, and this choice is refined by the mutual repression between Mirr and Mid/H15. The components comprise a molecular switch where graded inputs are converted into bistable outcomes.

D1470B Methionine Sulfoxide Reductase expression in response to anoxic stress conditions in *D. melanogaster*. *E. Rakitina*, D. Binninger. Florida Atlantic University, Boca Raton, FL.

Anoxia is the condition of extreme oxygen deprivation. It presents real danger to the viability of tissues, subjected to inadequate oxygen supply. Different organisms have various degrees of susceptibility to anoxic stress conditions. Thousands of human deaths each year are attributed to the events of induced anoxic conditions – ischemic strokes and heart attacks being one the most numerous. But whereas mammals tolerate anoxic stress very poorly, *D. melanogaster* is known to develop a special protective mechanism – spreading depression, allowing it to withstand hours of oxygen deprivation. Period following reintroduction of oxygen is the most dangerous consequence of anoxia and is characterized by the abundance of ROS (Reactive Oxygen Species), which oxidize vital molecules in cells rendering them non-functional. Methionine is exceptionally susceptible to oxidation by ROS, but it can be catalytically restored by the enzyme Methionine Sulfoxide Reductase (Msr). Two forms of Msr are known – MsrA and MsrB, which act upon two different epimers of oxidized methionine. Currently, little is known of the relationship between Msr gene activity and anoxic stress recovery in *Drosophila*. Activity of the Msr-encoding genes in response to anoxia is the subject of the proposed study. We use anoxia chamber to induce comma in flies, followed by recording recovery times. Preliminary results show, that single mutant flies do not take significantly longer than wildtype to recover from spreading depression. However, double mutants take significantly longer to recover and greater number of Msr-deficient flies die as a result of anoxia. Failure to recover becomes more pronounced as animals approach senescence. These studies offer insight into the role of oxidative damage during reperfusion period following cardiac stroke.

D1471C CLAMP: Sex Specific or Global Transcription Factor. *W. Jordan*, J. Johnson, C. Doherty, E. Larschan. Brown University, Providence, RI.

Dosage compensation is a mechanism by which gene dosage of X-linked genes between males and females is equalized. In Drosophila, the Male Specific Lethal Complex (MSL) regulates this process. This complex is responsible for the upregulation of a single X-chromosome in males (XY) so that expression of male X chromosome genes is equal to those on the female (XX). MSL complex also includes two non-coding RNAs called roX (RNA on the X) that are important for targeting of the complex. The protein, Chromatin-Linked Adaptor for MSL Proteins, CLAMP, is directly responsible for mediating the interaction between MSL and DNA elements called MSL Recognition Elements (MRE) that are two-fold enriched on the X chromosome compared with autosomes. While much of what we currently know about CLAMP is in the context of its function during X chromosome dosage compensation, CLAMP is localized to similar MRE-like DNA sequences in all regions of the genome. Therefore, we used CRISPR to knockout the gene encoding CLAMP to determine how it functions in vivo. We determined that in the absence of CLAMP males die earlier than females but there are no viable flies of either sex. Also, CLAMP functions as a repressor of transcription of the roX RNAs in females to assure that aberrant dosage compensation does not occur. Future experiments include retargeting CLAMP and roX RNAs to determine which factors are sufficient for X chromosome identification.

D1472A An integrated causality-based regulatory network for Drosophila S2 cells. *Hangnoh LEE*[1], Yijie Wang[2], Michael Buckner[3], Quentin Gilly[3], Dong-Yeon Cho[2], Stephanie Mohr[3], Norbert Perrimon[3], Teresa Przytycka[2], Brian Oliver[1]. 1) National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892; 2) National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD 20892; 3) Drosophila RNAi Screening Center, Department of Genetics, Harvard Medical School, Boston, MA 02115.

Reconstruction of regulatory networks and prediction of biological interactions are important aims of network biology. Advances in high-throughput genomics technologies have accelerated the building of improved network models. However, delineation of the overall network topology requires further effort because many of current network models are based on correlation, rather than causation. In order to overcome this limitation, and to generate a comprehensive network based on causality, we depleted 483 transcription factors (TFs) expressed in S2 cells, and profiled gene expression changes upon knockdown by sequencing a total of 1,920 RNA-Seq libraries. Our knockdown reduced transcript level in approximately 98% of target genes and significantly induced changes in gene expression in more than 40,000 non-target genes. Based on these observations, we described 25 different modules of genes affected by different combinations of TFs. We then integrated chromatin Immunoprecipitation data for 137 TFs and histone marks from the same cell line as well as 1,264 TF binding motifs and implemented a mathematical modeling approach. Our network model predicted more than 70,000 gene interactions that a few of which were previously known, but primarily these were novel predictions. The known interactions include regulatory pathways that govern sex determination. The novel ones largely describe cell cycle progression of the proliferative cells. Our result modeled a regulatory network based on causality and provided a testable catalog of genetic interactions.

D1473B Competitive binding of transcription factors drives dominance in regulatory genetic pathways. *A. H. Porter*[1], N. A. Johnson[1], A. Y. Tulchinsky[2]. 1) Univ Massachusetts, Amherst, MA; 2) SUNY New Paltz, New Paltz, NY.

We report a new mechanism for allelic dominance in regulatory genetic interactions and its sensitivity to genetic background. We investigated a biophysical model of gene regulation, where the fractional occupancy of a transcription factor (TF) on the *cis*-regulated promoter site it binds to is determined by binding energy (*–ΔG*) and TF concentration. Transcription and gene expression proceed at the regulated *cis* site while the *trans*-acting TF is in the bound state. In diploids, individuals may be heterozygous at the *cis*-site promoter, at the TF's coding region, or at the TF's own promoter, which determines allele-specific TF concentration. We find that when the TF's coding region is heterozygous, TF alleles compete for occupancy at the *cis* sites and the tighter-binding TF is dominant in proportion to the difference in binding strength. When the TF's own promoter is heterozygous, the TF produced at the higher concentration is also dominant. *Cis*-site heterozygotes have additive and therefore codominant phenotypes. While this dominance is inevitable at the molecular level, it may be difficult to detect in the phenotype under some biophysical conditions, more so when TF concentration is high. In three-locus linear pathways of loci A->B->C where A and B are TF's, dominance propagates down the pathway: locus A can show dominance with respect to expression at locus C. This dominance is attenuated at the phenotypic level such that only low-expression A alleles appear recessive, especially when TF concentration is high at the B locus. Two types of genetic background effects occur. In the proximal background of the two-locus interaction, where a TF is heterozygous in both its promoter and coding sites, their effects on competitive binding interact to increase, decrease or even reverse dominance of the other; heterozygosity in the *cis*-site promoter has no effect. In more distant interactions of three-locus pathways, locus A's dominance with respect to C-locus expression can be modified by allelic variation at locus B, especially at its coding site. Many empirical findings of dominance in TF and *cis*-site interactions in the literature can be explained by this simple mechanism of competitive binding.

D1474C Patterns of gene expression variation in a natural population of *Drosophila melanogaster* provide evidence for GRN robustness through compensatory *cis trans* interactions. *J. Fear*[1,2], L. León-Novelo[3], A. Morse[1,2], A. Gerken[1,2], K. Van Lehmann[5], J. Tower[4], S. Nuzhdin[4], L. McIntyre[1,2]. 1) Molecular Genetics and Microbiology University of Florida, Gainesville, FL; 2) Genetics Institute University of Florida, Gainesville, FL; 3) Department of Biostatistics; University of Texas Health Science Center at Houston-School of Public health, Houston, TX; 4) Molecular and Computation Biology Program, Dornsife College of Letters, Arts and Sciences, University of Southern California, Los Angeles, CA; 5) Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY.

Regulatory variation in gene expression can be described by *cis* and *trans* genetic components. Here we use RNA-seq data from a panel of *Drosophila melanogaster* test crosses to compare allelic imbalance (AI) in female head tissue between mated and virgin flies, an environmental

change known to affect transcription. Indeed, 3,048 exons (1,610 genes) are differentially expressed in this study. We use a Bayesian model for the detection of AI and control type I error with an intersection test. There are ~200 genes with AI exclusively in mated or virgin flies and on average 34% of genes within a cross, and 54% of all genes across the entire panel show evidence for genetic regulation of transcription. Nearly all differentially regulated genes are affected in *cis*, with an average of 63% of expression variation explained by the *cis* effects. *Trans* effects explain 8% of the variance in AI on average and the interaction between *cis* and *trans* explains an average of 10% of the total variance in AI. In both environments c*is* and *trans* effects are compensatory in their overall effect, with the opposite signs of effects of the allele coupled 63% of the time. There is a negative association between *cis* and *trans* effects (85%) across lines. We hypothesize that the gene expression level perturbed by *cis*-regulatory mutations is compensated through *trans*-regulatory mechanisms, e.g. *trans* and *cis* by *trans* factors buffering *cis*-mutations. In addition, when AI is detected in both environments, *cis-mated, cis-virgin,* and *trans-mated-trans-virgin* estimates are highly concordant. We conclude that the GRN are robust and that *trans* buffering explains robustness.

D1475A A novel Smad/Su(H)-target enhancer drives *hedgehog* expression in a signaling hole of the posterior wing disc compartment. *Timothy Fuqua*, Elizabeth Stroebele, Christian Noblett, Albert Erives. Department of Biology, University of Iowa, Iowa City, IA, 52242-1324, USA.

Developmental patterning involves dynamic integration of signals from the BMP, Notch, Wnt, Hedgehog and other signaling pathways. These signals are integrated by transcriptional enhancers in a manner that is customized for each target gene. To advance our understanding of this signal integration, we identified regulatory belts that are conserved across the *Drosophila* genus and contain binding sites for the Notch-signaling effector Su(H), the Dpp/BMP effectors Mad:Medea, and the LIM-homeodomain selector Apterous (Ap). We report identifying a novel wing imaginal disc enhancer for *hedgehog* driving an interesting expression pattern. While the *hedgehog* gene is normally expressed only in the posterior compartment of the wing imaginal disc, this novel enhancer, "*hh-E*", drives twin spots of expression on either side of the Dpp expression stripe in the dorsal-proximal region of the columnar epithelial side of the wing disc. Of these twin spots of *hh-E* driven expression, one (ectopic) spot is located in the anterior compartment and the other (normal) spot is in the posterior compartment. While the *hh-E* expression pattern is consistent with a Dpp-readout licensed in part by Ap, it is not consistent with it being a canonical Notch/Su(H) target. To understand the role of the conserved Su(H) site in *hh-E*, we mutated this site and found that it functions as a dedicated repressor in the peripodial membrane of the wing disc. Mutated *hh-E* continues to drive twin spots of expression in the dorsal-proximal regions of both the anterior and posterior wing disc compartments. Thus, *hh-E* likely features a dedicated Su(H) repressor binding site that is refractive to Notch-induced activation. To understand what must be silencing the ectopic spot of anterior expression driven by *hh-E*, we "restored" anterior silencing in an expanded *hh-DEF* fragment. One of these flanking regions,"*hh-F*", is another Su(H) binding site containing enhancer driving posterior-compartment specific expression in the distal regions of the wing disc, including the wing pouch. A combined *hh-DEF* enhancer with a mutated *hh-F* Su(H) site has attenuated wing pouch expression without diminishing the posterior spot of dorsal proximal expression driven by the embedded *hh-E* enhancer. In summary, the modular *hh-DEF* enhancers, which are different from the previously identified *hh-ABC* regulatory modules, are each responsible for various signaling holes in the wing disc and collectively piece together the full *hedgehog* expression throughout the posterior compartment. We conclude that some Su(H)-targeted enhancers may function independently of Notch signaling at unexpected loci and do so via additional unknown contextual cues present in these enhancers.

D1476B Strong interactions between copies of *spineless* drive interchromosomal communication independent of homologous chromosome pairing. *K. C. Viets*, R. J. Johnston. Johns Hopkins University, Baltimore, MD.

Fifty years ago, Nobel laureate Ed Lewis observed that two mutant alleles of a gene, one lacking a functional regulatory region and one lacking a functional protein-coding region, can interact in *trans* to rescue gene expression. This process, which he called transvection, requires homologous chromosome pairing and is disrupted by chromosomal rearrangements. With only a handful of DNA elements linked to transvection and chromosome pairing since Ed Lewis's initial discovery, many questions remain in the field. What role do these phenomena play in regulating gene expression in the wild? Does crosstalk between alleles always require chromosome-wide pairing? What DNA elements are necessary for gene copies to locate each other in the genome, and are these elements similar between genes? Our work seeks to answer these fundamental questions using stochastic gene expression in the *Drosophila* retina as a model.

In the retina, the gene *spineless* (*ss*) is expressed in a random on/off manner in 67% of R7 photoreceptors. A transvection-like mechanism known as Interchromosomal Communication (InterCom) allows cross-regulation of expression states between *ss* alleles within each R7. InterCom is one of the only known examples of transvection in a biological context, acting to average the expression frequencies of functional, naturally derived *ss* alleles. Using a recently described DNA FISH technique called DNA Oligopaints, we have developed a system to study *ss* pairing and InterCom in a quantitative manner. With this system, we have determined that InterCom requires copies of *ss* to pair. We have also found that, unlike classical transvection, pairing and InterCom can occur between copies of *ss* located in heterologous genomic locations, independent of homologous chromosome pairing. Pairing and InterCom of *ss* loci requires surprisingly minimal regions of homology: a *ss* transgene of only 110 kb can drive pairing between non-homologous chromosomes. Additionally, fragments of *ss* as small as 46 kb can pair and perform InterCom with endogenous *ss* when inserted 0.4 or 4.6 Mb away on the same chromosome arm, indicating that even small portions of the gene can mediate interactions. Our data suggest that strongly interacting loci such as *ss* may hold chromosomes together at specific nodes, rather than chromosome-wide homology driving tight pairing along the entire chromosome.

D1477C The developmental effects of germline ablation contribute more strongly to somatic gene expression than the maternal effects of piRNA. *Alexandra A. Erwin*, Justin P. Blumenstiel. University of Kansas, Lawrence, KS.

piRNAs are key regulators of transposable elements (TEs) that have been also shown to have strong effects on gene expression. Since piRNAs are transmitted maternally, they play a key role in maintenance of genome defense across generations. The degree to which they contribute to variation in gene expression across generations is poorly known. Here, using a syndrome of hybrid dysgenesis in *D. virilis*, we evaluate how gene expression profiles differ in progeny whose mothers greatly differ in their piRNA profile. Moreover, because gonadal atrophy occurs in about 50% of progeny of the dysgenic cross, we can contrast the maternal effects of a divergent piRNA pool with the effects on gene expression that arise from loss of the germline. We have evaluated these contrasting effects by performing RNA-seq on head and thorax tissue of reciprocal males and females, with and without atrophied gonads. We find that differences in maternal piRNA contribution have little to no effect on gene expression in the heads and thorax of progeny. However, when we compare expression profiles of flies with ovaries to genetically identical flies that lack ovaries, but share the same maternal piRNA profile, we find that germline ablation results in changes in the expression of metabolic and neuronal genes in tissues distant from the germline. This indicates that the effects of germline ablation contribute more strongly to somatic gene expression than the maternal effects of piRNA. Interestingly, we do not find significant differential gene expression of genetically and epigenetically identical males with and without testes. This indicates that maintenance of male sex organs in *D. virilis* has little consequence on gene expression of somatic tissues outside of the abdomen.

D1478A Development and optimization of light-dependent switches for spatiotemporal control of gene expression. *L. De Mena*, P. Rizk, C. A. Cruz, Y. Zhang, P. Fernandez-Funez, D. E. Rincon-Limas. University of Florida, Gainesville, FL.

Tools that enable manipulation or perturbation of gene function in a spatiotemporal manner are critical to define its contribution to normal development and disease. Unfortunately, current inducible expression systems preclude accurate spatiotemporal control of gene expression and involve steroid hormones, antibiotics, heavy metals, or heat shock, which can induce toxicity or pleiotropic effects. What if transgene expression could be rapidly activated and immediately reversed with a switch triggered by light? Here we present the generation and implementation of a new genetically encoded light-dependent expression system in animal cells based on the fast, reversible photoactivation of Phytochrome B (PhyB) from plants. Phytochromes are light activated biological switches that control plant growth and development. In response to red light, PhyB is activated and moves to the nucleus, triggering a cascade of signal transduction events. Under far-red light, it returns to the inactive state, promptly ending signaling. This system, which we have called PhotoGal4, possesses all the elements required for the formation of active phytochromes in animal cells, including genes essential for chromophore formation as well as important motifs required for transcriptional activity. Our hypothesis is that this system will serve as a high-resolution device to sculpt gene expression in Drosophila with agile on-off control and with unprecedented precision and resolution. Finally, we anticipate that this system will have a variety of applications in many areas of biomedical research, from the development of new therapeutic strategies, to the analysis of complex and multi staged biological processes such as embryogenesis, neurodegeneration and cancer.

D1479B Investigating Effects of TDP-43 on Metabolic Gene Expression in a Drosophila model of Amyotrophic Lateral Sclerosis. *J. Barrows*, E. Manzo, A. Joardar, A. Coyne, D. C. Zarnescu. University of Arizona, Tucson, AZ.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that primarily affects motor neurons, disrupting muscle function, which eventually leads to death by respiratory failure. TDP-43, a DNA/RNA binding protein that plays numerous roles in RNA processing, has been associated with RNA stress granule pathology in over 95% of ALS cases. TDP-43 has also been linked to dysregulation of specific mRNA targets at both the transcriptional and translational levels. Metabolomic analyses demonstrated that several metabolites in glycolysis and the TCA cycle are altered in *Drosophila* larvae expressing human TDP-43 compared to controls, suggesting that enzymes within these metabolic pathways are being affected in ALS. To confirm this hypothesis, we are using the GAL4-UAS system in *Drosophila* to express TDP-43 specifically in the motor neurons or glia. Indeed, transcriptional profiling shows that phosphofructokinase (PFK; Pfk in *Drosophila*) and glucose-6-phosphate dehydrogenase (G6PD; Zw in *Drosophila*) expression levels are altered in the context of TDP^{WT} or disease associated TDP^{G298S}. These findings are consistent with increased glycolysis and high levels of pyruvate identified using metabolomics in TDP-43 expressing flies. Current experiments are aimed at identifying additional transcriptional and translational alterations in metabolic pathways that control cellular energetics. Next, we will use genetic approaches to reduce (in the case of upregulation) or increase (in the case of downregulation) the expression levels of metabolic genes in the context of TDP-43. These experiments will determine whether restoring specific targets can rescue TDP-43 dependent phenotypes. This combination of molecular and genetic analyses will establish physiologically significant targets of TDP-43 that can be used for developing novel therapeutic strategies in the future.

D1480C Sex chromosome-wide transcriptional suppression and compensatory *cis*-regulatory evolution mediate gene expression in the *Drosophila* male germline. *Emily L. Landeen*[1], Christina A. Muirhead[1], Lori Wright[1], Colin D. Meiklejohn[2], Daven C. Presgraves[1]. 1) Univ. of Rochester, Rochester, NY; 2) Univ. of Nebraska Lincoln, Lincoln, NE.

The evolution of heteromorphic sex chromosomes has repeatedly resulted in sex chromosome-specific forms of regulation, however how the X chromosome is regulated in the *Drosophila* male germline has been unclear. In *Drosophila melanogaster* expression of transgenes driven by spermatogenesis-specific promoters show significantly lower expression on the X chromosome compared to the autosomes. The ~3-fold or greater difference in X-autosome expression is established premeiotically, suggesting that this regulatory process is distinct from canonical sex chromosome dosage compensation or meiotic sex chromosome inactivation. We use transgenes and chromosomal transpositions to compare X and autosomal expression across multiple tissues and find that both non-specific and testis-specific endogenous X-linked genes are

transcriptionally suppressed ~2-4-fold specifically in male germline cells. However, in wildtype testes, this sex-chromosome transcriptional suppression is undetectable, as it is effectively compensated by the evolution of strong promoters at X-linked genes. We identify one promoter element sequence motif, in particular, that is enriched immediately upstream of transcription start sites of testis-specific genes, evolutionary conserved across species, associated with increased expression in the testes, and overrepresented on the X. We transgenically validate that this promoter element and show via site-directed mutagenesis that it results in 2-4-fold increased expression in the testes, effectively offsetting X suppression.. Our results help clarify how the X chromosome is regulated in the *Drosophila* male germline and show that global expression of X-linked genes reflects a balance between chromosome-wide epigenetic transcriptional suppression and long-term compensatory evolution. Our results have broad implications for the evolution of gene expression in the *Drosophila* male germline and for genome evolution.

D1481A The insect specific Drosophila gene *banshee* (*bshe*; *CG8878*), a putative protein kinase, has an acid-rich region inserted within the catalytic domain. *J. Locke*, L. Canham. Univ Alberta, Edmonton, AB, CA.

Changes in chromatin structure can occur through histone modification, such as phosphorylation. Changes in chromatin structure can also be detected by mutations that suppress or enhance of the phenotypic expression of easily visible marker loci, such as *white*, as observed in Position Effect Variegation (PEV). Seven mutations in *banshee* (*bshe*, *CG8878*) were recovered in a genetic screen for dominant enhancers of PEV. The predicted amino acid sequence of BSHE suggests a protein kinase within the CK1 protein kinase family. Phylogenetic analysis of *Drosophila melanogaster* BSHE with sequences from other Dipterans, insects, and arthropods shows that BSHE represents a type of protein kinase that is insect specific, unlike the other groups within the CK1 family. The closest protein kinase sub-family to BSHE is *ballchen*, a vaccinia related kinase (VRK), which is known to modify histone proteins. BSHE is unusual in that it has an acid-rich amino acid stretch inserted within the predicted kinase catalytic domain. I-TASSER 3D structure predictions of BSHE put the insert stretch in a position that retains the catalytic site 3D structure and thus its predicted enzyme activity. The length of the insert varies among insect families with Drosophila species having the longest inserts. BSHE appears to play a role in changing chromatin structure, possibly via its kinase activity.

D1482B Investigations into the action of the CHD1 remodeler on chromatin across a transcriptionally active gene in larval salivary glands. *J. A. Armstrong*, M. A. Erb, L. Bugga, C. Kim. Claremont Colleges, Claremont, CA.

CHD1 (chromodomain, helicase, DNA-binding domain) is a conserved member of the SWI2 family of chromatin remodeling factors. While not an essential gene, *Chd1* is important for male and female fertility and wing development. CHD1 stoichiometrically co-localizes with elongating RNA Polymerase II (Pol II) on polytene chromosomes from salivary glands of third instar larvae, suggesting that CHD1 may be important to allow or facilitate the passage of Pol II through chromatin. However, CHD1 is not required for persistence of elongating Pol II on polytenes. Instead, we observed that CHD1 is important for genome-wide deposition of H3.3, a histone variant enriched at transcriptionally active genes, suggesting that CHD1 may be important for the deposition of new or recycled nucleosomes in the wake of Pol II. These findings are consistent with studies of the Chd1 remodeler in mammalian cell lines and yeast. To investigate the function of CHD1 across active genes in a metazoan, we have used both native and cross-linked chromatin immunoprecipitation (ChIP) to investigate changes in nucleosome composition across the transcriptionally active *CrebA* gene in salivary glands of third instar larvae. The use of salivary glands allows us to correlate our ChIP results with our low-resolution immuno-staining of polytene chromosomes. Consistent with prior immuno-staining results, we observe that CHD1 is enriched at *CrebA* relative to the transcriptionally inactive *white* gene, and the remodeler is not required for transcription of *CrebA* in salivary glands. Using both native and cross-linked ChIP, we investigate changes in H3/H3.3, H3K36me3, H3K56Ac and H3K9me2 across the *CrebA* gene in salivary glands lacking CHD1.

D1483C Determining how chromatin structure impacts DNA replication and cell cycle progression. *Robin L. Armstrong*, Robert J. Duronio. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Temporal control of DNA replication is essential for maintenance of cellular proliferation and homeostasis, where loss of control can result in genomic instability, developmental abnormalities, and cancer. Origins of replication act in cis to establish temporal control of DNA replication through cell cycle-controlled recruitment of trans-acting factors. Metazoan studies have yet to find a consensus sequence associated with origins where, instead, origin definition is suggested to be strongly influenced by chromatin architecture, comprised of both nucleosome density and histone tail post-translational modifications (PTMs). PTMs modulate nucleosome density along a DNA molecule, establishing chromatin states that are either “open” or “closed” to protein complexes required for DNA replication.

Chromatin PTMs that contribute to heterochromatin formation and those associated with early origin firing and replication timing are being investigated to address central questions including whether specific histone PTMs are required for origin definition and replication timing, whether PTMs that define heterochromatin are required to create conditions permissive for heterochromatin replication during late S-phase, and whether specific PTMs function in cell cycle regulation. Using a *Drosophila melanogaster* histone replacement platform, we show that specific histone PTMs function in cell cycle control, regulation of DNA replication, and concentration of *trans*-acting factors.

D1484A Characterizing the role of HP1 proteins in aging. *Tandy Dolin Petrov*, Nicole Riddle. University of Alabama at Birmingham, Birmingham, AL.

Aging organisms show declines in both physical and mental abilities. Epigenetic processes, such as those that control chromatin structure, have been associated with these age-related declines. The highly conserved chromosomal proteins of the Heterochromatin Protein 1 (HP1) family have a variety of functions, including in maintenance of chromatin structure, in transcription regulation, and in DNA repair. Disruption of HP1 localization is often associated with aberrant gene transcription, which can contribute to disease. In *Drosophila melanogaster,* there are

three somatic HP1 proteins: HP1a, HP1B, and HP1C. These three proteins share a subset of binding sites despite the fact that initial reports suggest that HP1a localizes mainly to heterochromatin, HP1C localizes mainly to euchromatin, and HP1B localizes to both heterochromatin and euchromatin. Published data suggest that HP1a is redistributed during aging: while young individuals show a clear distinction between heterochromatin and euchromatin, this distinction disappears in older individuals. However, HP1a protein levels do not change with increased age. Moderate overexpression of HP1a in Drosophila increases lifespan (~23%), and under starvation conditions, flies lacking HP1B have a longer median lifespan (~24%) than control flies. These findings suggest that HP1 proteins impact longevity and health span. In order to address the roles of HP1 proteins in aging, we are investigating the HP1 distribution during aging and evaluating cognitive performance. In control, HP1a overexpressing, and Su(var)3-9 overexpressing Drosophila strains, the distribution of HP1a throughout lifespan will be determined via ChIP-seq. Using classical conditioning techniques with y-mazes, aversive and appetitive olfactory memory throughout lifespan will be assessed. HP1a overexpressing lines that show increased maximum lifespan will be tested to determine if memory is improved due to increased HP1a levels. Performance indices will be calculated from the behavior assay results, which will be analyzed via ANOVA and Chi-Square tests.

D1485B An RNA Topoisomerase Complex Interacts with RNAi Machinery to Promote Heterochromatin Formation and Transcriptional Gene-silencing. *Seung Kyu Lee*, Weiping Shen, Yutong Xue, Yongqing Zhang, Muzammil Ahmad, Yuyoung Joo, Supriyo De, Elin Lehrman, Kevin Becker, Sige Zou, Weidong Wang. National Institute on Aging / NIH, Baltimore, MD.

Topoisomerases resolve topological problems generated during DNA metabolism (Wang et al. NRMCB 2002), but the roles of topoisomerases in RNA metabolism remain unclear. Our prior study has identified human Topoisomerase 3b (Top3b) as the first RNA topoisomerase in eukaryotes (Xu et al. 2013), whereas another study has linked *Top3b* gene deletion to schizophrenia and intellectual disability (Stoll et al. 2013). Mechanistically, Top3b forms a stoichiometric complex with TDRD3 (Tudor domain containing 3); and a fraction of this complex associates with FMRP (Xu et al. 2013), an RNA-binding protein known to regulate translation of mRNAs important for synapse development and autism. FMRP is encoded by the *fmr1* gene, which is inappropriately silenced in the Fragile X mental retardation syndrome, a leading cause of autism. Using *Drosophila* as a model, we showed that *Top3b* genetically interacts with *fmr1* to promote synapse formation.

Increasing evidence has shown that *Drosophila* FMRP is a component of the RNAi-induced silencing complex (RISC), which includes AGO2, p68 helicase, and Vig. Mutations in *fmr1*, other components of RISC, as well as *Dicer-2* (*Dcr-2*; a protein essential for siRNA biogenesis), disrupt heterochromatin formation, transcriptional gene silencing, and repression of transposable elements. Here, we show that similar to FMRP, the *Drosophila* Top3b-TDRD3 complex also stably associates with RISC; and mutation of *Top3b* disrupts heterochromatin formation and transcriptional silencing in Position Effect Variegation (PEV) reporter assays. In addition, *Top3b* genetically interacts with *AGO2, p68,* and *Dcr-2* in PEV assays, indicating that Top3b works coordinately with the siRNA machinery to facilitate heterochromatic gene silencing. Moreover, an epigenetic marker of heterochromatin, H3K9me2, displayed abnormal distribution in *Top3b* mutant flies. Furthermore, microarray and RT-qPCR analyses revealed that *Top3b* mutant flies exhibit global derepression of many genes in the pericentric heterochromatin region, as well as in other regions of different chromatin states. Finally, several transposable elements are de-silenced in the *Top3b* mutant flies. Together, our data suggest that Top3b interacts with the siRNA machinery to promote heterochromatin formation and transcriptional silencing.

D1486C Comparative Genomics Analysis of *Drosophila ficusphila* heterochromatic chromosome 4 contig 5. *D. Schiller*, Paula Croonquist. Anoka Ramsey Community College, Coon Rapids, MN.

The Drosophila chromosome 4, known as the “dot chromosome” or Müller F element is predominantly heterochromatic. There is evidence however that 80 genes are actively transcribed in this region. The mechanism by which active transcription is carried out in this environment is under investigation. It is hypothesized that chromosome 4 genes share common elements in their transcriptional start sites that may explain their levels of transcription in this highly compacted environment similar to the other euchromatic autosomes. As part of the Genomics Education Partnership (GEP) undergraduate research initiative, the goal of this study was to annotate all features in Contig 5 of *Drosophila ficusphila* chromosome 4 utilizing a comparative genomics approach and several bioinformatics tools including the Basic Local Alignment Search Tool (BLAST), the UCSC Genome Browser, and gene predictors such as GENSCAN and N-SCAN, as well as RNA-Seq data and TopHat junctions. The *Drosophila melanogaster* genome was used as a reference for gene orthology. It was found that three genes, *pan, ank, and ank2*, with orthologs in *D. melanogaster* are located in contig.5. Gene models were proposed based on all gathered evidence that allowed for annotation of exact coordinates for each intron and exon of all isoforms for each gene homolog. We are currently analyzing the transcriptional start sites that may shed light on how these genes can be expressed in such a highly heterochromatic environment.

D1487A Investigating the molecular basis of dominant male sterility associated with X-autosome translocations in *D. melanogaster* using RNA-seq and cytological analyses. *J. H. Wong*, J. A. Kennison. Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

Dominant male sterility in *D. melanogaster* has been associated with approximately 75% of translocations between the sex chromosomes and autosomes. The current model for male infertility suggests condensation of the sex chromosomes directed by an element in the centric heterochromatin silences X-linked genes earlier than the autosomes during spermatogenesis. By generating a collection of X-ray-induced translocations between the autosomes and X-chromosomes, we will locate breakpoints to map the heterochromatin site responsible for X chromosome regulation and use RNA-seq to determine if there are altered expression levels of either X-linked or autosomal genes during spermatogenesis. This will allow us to test the current models for sterilizing effects involving the X-autosome translocation and to further understand the underlying factors in male infertility.

D1488B Rapidly Evolving Stonewall maintains Germline Stem Cells and regulates Transposons. *Daniel Zinshteyn*, Daniel Barbash. Cornell University, Ithaca, NY.

Germline stem cells (GSCs) are the progenitor cells for the entire population of an organism's germline. In *Drosophila*, these cells reside in a well-defined cellular niche that is required for both their maintenance (self-renewal) and differentiation (asymmetric division resulting in a daughter cell that differs from the GSC). Dozens of genes have been implicated in both maintenance and differentiation and most of them are required for production of viable gametes. The critical function of these genes suggests that they should be highly conserved across *Drosophila* taxa. However, population genetic analyses have shown that many of them have undergone adaptive evolution within the *D. melanogaster* lineage. One possible cause of this evolutionary signature is the critical role that many of these genes play in regulating genomic parasites, particularly transposons. It may be that some of these genes are locked in a dynamic arms-race with rapidly evolving selfish elements.

The stem-cell maintenance factor Stonewall (Stwl) is a particularly intriguing candidate, as it has undergone adaptive evolution and has been implicated in heterochromatin maintenance. We hypothesized that Stwl is required for regulating transposons in *D. melanogaster*. We performed RNA-seq on *stwl* mutant ovaries and testes to assay the transcript abundance of transposable elements in the absence of a functional Stwl. We find that mutant *stwl* ovaries show significant de-repression of many transposon families. Surprisingly, heterochromatic genes are not preferentially misregulated relative to euchromatic genes. These data suggest that Stwl may be required for regulation of transposons via a mechanism other than general heterochromatin maintenance. Our findings support the hypothesis that Stwl is a target of positive selection as a response to rapidly evolving TEs.

D1489C Characterization of essential domains in the BEAF-32B insulator protein. *S. V. Satya Prakash Avva*, Craig M. Hart. Louisiana State University, Baton Rouge, LA.

Like enhancers and promoters, insulators (or boundary elements) are a specialized class of regulatory DNA sequences. Insulators are defined by their ability to function in transgene assays to limit enhancer-promoter communication when placed between a promoter and an enhancer. They are also known to function as barrier elements that protect transgenes from chromosomal position effects. Two of the first insulator elements to be identified are the scs and scs' sequences which bracket two *Hsp70* genes at the *87A* locus of *Drosophila*. BEAF-32 was identified as a Boundary Element-Associated Factor of 32 kDa that binds to the scs' insulator, and was subsequently shown to immunolocalize to hundreds of sites on polytene chromosomes, suggesting that BEAF-dependent insulators are a common feature in the *Drosophila* genome. This is substantiated by genome-wide mapping experiments, which have identified from 1800 to 6000 binding regions. The *BEAF-32* gene encodes two isoforms, 32A and 32B, that differ only in their amino-terminal 80 amino acids. Both of these regions include a different atypical zinc-finger DNA binding domain. The remaining 200 amino acids are identical. BEAF-32 can form homo- and hetero- dimers, trimers and possibly also larger oligomers with varying ratios of 32A to 32B through interactions mediated by the sequences in the carboxy-end of the proteins. Yet transgene rescue experiments utilizing a lethal null *BEAF-32* mutation found that *32B* is essential, while *32A* is not. In order to understand BEAF-32 better, we have used a combination of *in vivo* and *in vitro* experiments to map essential domains in the common portion of the proteins.

D1490A Boundary elements-anchored chromatin loops may facilitate genome rearrangement. *Zhibo Ma*, Matthew Romine, Haini Cai. University of Georgia, Athens, GA.

Genomic architecture lays the physical foundation of gene regulation. Interactions between chromatin boundary elements anchor chromatin loops, which can modulate enhancer-promoter interactions and the extent of active or repressive domains. We have been using the Drosophila ftz gene region as a model to study how chromatin boundaries regulate local gene expression. In Drosophila, the ftz gene is nested within the regulatory sequences of the Scr Hox gene. We have recently shown that SF1 and SF2, two boundary elements flanking the ftz region in D. melanogaster, form a stage-specific chromatin loop. This loop insulates the ftz gene from the interference by the surrounding Hox enhancers and the PcG-mediate repression. SF1 and SF2 cancel their enhancer-blocking activity when arranged in tandem. This is an indication of their pairing in vivo, which is consistant with our 3C data, providing a mechanism for the distal Scr enhancer to bypass their blocking. The ftz gene region is highly conserved among Drosophila species. However, it is found in an inverted orientation in several Drosophila species as compared to D. melanogaster. We found that SF1 and SF2 are located immediately outside the "flipped" ftz gene region in all 12 sequenced Drosophila species. Similar boundary-flanked and inverted intervals are found at multiple locations in the Drosophila Hox complexes. We hypothesize that boundary-anchored chromatin loops facilitate genomic rearrangement and buffer the pressure of misregulation caused by these rearrangement. To test this, we have cloned SF1 and SF2 homologs from D. wilistoni and D. virilis, two species with inverted ftz. We found that all these elements contain strong enhancer-blocking activities, as assayed in transgenic D. melanogaster. Importantly, they cancel each other when arranged in tandem. This suggests that SF1 and SF2 loop with each other in D. wilistoni and D. virilis. New data to extend our preliminary findings will be discussed.

D1491B Characterization of the SCS' insulator. *M. Maharjan*, C. Hart. Louisiana State University, Baton Rouge, LO.

DNA insulator elements are thought to affect gene regulation at least in part by influencing chromatin structure and nuclear organization. Insulator function is determined by sequence-specific DNA binding proteins that bind to them. The insulator protein BEAF-32 usually binds near transcription start sites, suggesting it might play a direct role in promoter function. One example is the scs' insulator, which contains divergently transcribed promoters with a BEAF-32 binding site by each. Identifying protein partners of BEAF-32 through a detailed analysis of the scs' insulator would provide insight into the role of BEAF-32 in the insulator and promoter activity of scs'. Half of scs' retains insulator activity, and this is lost if the BEAF-32 binding site is mutated. Through a linker scanning analysis of this half of scs', we found that mutation of a

20 bp sequence decreases the insulator activity of the BEAF-32 binding site. Additional mutations are being tested to determine if other sequences also play a role. These variations are also being tested for effects on promoter activity to determine whether or not insulator and promoter activity can be separated. For instance, promoter activity is impaired if the BEAF-32 binding site or 20 bp sequence is mutated. In addition, we are using DNA affinity chromatography to purify proteins that bind to the identified sequence with the goal of identifying proteins by mass spectrometry. Characterization of minimal scs' sequences required for insulator and promoter activity, and identification of proteins that bind these sequences, will provide insight into BEAF-32 function.

Progress on this project to identify proteins that work together with BEAF-32 for proper insulator and promoter function will be presented..

D1492C The *Dm*-Myb oncoprotein coordinates higher-order chromatin structure to potentiate expression of target genes as well as stabilize facultative heterochromatin. *Juan Santana*, Mrutyunjaya Parida, Abby Long, Joshua Wankum, John Manak. Univ of Iowa, Iowa City, IA.

Myb is a proto-oncogene that when mutated causes leukemias and lymphomas in birds and mammals. Vertebrates contain three representatives of the *Myb* gene family consisting of A-, B- and c-*Myb*, all of which encode DNA-binding factors that are important for the proper expression of genes. Several studies have described Myb's primary function as a factor that upregulates transcription by binding to promoter regions, thus controlling the expression level of genes adjacent to these sequences. In flies, this regulation has been shown to be accomplished epigenetically such that *Dm*-Myb is only required after initial activation of a gene in order to potentiate this distinct transcriptional state. Here, we further characterize the potentiator role of Myb and show that its absence leads to a reduction in H3K4me3 along promoters, and RNA polymerase occupancy across gene bodies, resulting in downregulation of transcription. However, up to now, no other mechanisms have been proposed that account for the thousands of genes whose expression is altered in the absence of dMyb, including those that appear to be "repressed" by Myb, or lie a distance away from any Myb binding sites. Here we uncover a novel and critical role of Myb in demarcating and maintaining silent chromatin domains, as well as promoting intra-chromosomal interactions which can influence gene expression at sites distal from Myb binding regions. We observe that Myb demarcates and stabilizes H3K27me3 domains associated with silent genomic regions, and in its absence, these domains become reduced in length and less enriched for this chromatin mark, promoting an enrichment of H3K4me3 and subsequent derepression of the genes within these domains (including transcription factors). Notably, the genes whose expression levels change in the absence of Myb (~2,000 genes) are directly influenced or regulated by Myb, with virtually no genes showing altered expression through secondary consequences of aberrant transcription factor upregulation, thus providing strong evidence for a chromatin "buffering" effect in cells that helps prevent misprogramming. .

D1493A Don't Steer Me Wrong: Rustling for Connections Between BEAF Insulator Protein, PBAP Chromatin Remodeler and Gene Expression. *Jamie L. Wood*[1], S. V. Satya Prakash Avva[1], M. Maharjan[1], J. Keller McGowan[1], Fabiana M. Duarte[2], Jacob M. Tome[2], John T. Lis[2], Craig M. Hart[1]. 1) Louisiana State University, Baton Rouge, LA; 2) Cornell University, Ithaca, NY.

Our understanding of gene regulation has grown to include the role of chromatin in managing where and when genes are expressed. Chromatin, the organizational framework that packages DNA into the nucleus, affects the access of specific regulatory proteins to DNA sequences. As part of the regulatory structure, chromatin is divided into domains. Specific sequences of DNA called insulators, which are bound by proteins called insulator binding proteins (IBPs), assist in establishing these domains. We are interested in how the *Drosophila melanogaster* IBP Boundary Element Associated Factor (BEAF) acts within the chromatin landscape to control gene expression. Previous data shows BEAF localizes to hundreds of promoter regions within the genome, often at housekeeping genes. This may indicate a role in promoter function, a non-traditional role for an IBP. We have evidence to suggest a positive interaction of BEAF with the Polybromo-associated Brahma (PBAP) chromatin remodeling complex, specifically the Polybromo subunit. We are probing the nature of this interaction using animal and cell culture experiments to determine the effect of this association on transcription. We have tested for genetic interactions using our rough eye screen and have dissected Polybromo to determine which domains specifically interact with BEAF. We have also used the high through-put techniques PRO-seq and MNase-seq after RNAi mediated knockdowns of BEAF and Polybromo in S2 cells. Progress in analyzing the relationship between BEAF and PBAP will be presented.

D1494B Analysis of *Sex combs reduced* HOX gene cis-regulatory elements. *M. T. Cooper*, J. A. Kennison. Eunice Kennedy Shiver National Institute of Child Health and Human Development, Bethesda, MD.

The Drosophila *Hox* gene, *Sex combs reduced* (*Scr*), is required for patterning the first thoracic segment. The *Scr* transcription unit spans 35 kb, with at least 35kb of upstream cis-regulatory sequences. Transcriptional silencing of *Scr* in the second and third thoracic segments requires the Polycomb group genes. We have identified several *Scr* genomic fragments as Polycomb group response elements (PREs).

D1495C Maintenance of tissue pluripotency by epigenetic factors. *D. Huang*, D. Sadasivam. Academia Sinica, Taipei, Taiwan, TW.

Pluripotent stem cells often adopt a unique developmental program while retaining certain flexibility. The molecular basis of such properties remains unclear. Using differentiation of pluripotent *Drosophila* imaginal tissues as assays, we examined the contribution of epigenetic factors in ectopic activation of Hox genes. We found that over-expression of Trithorax H3K4 methyltransferase can induce ectopic adult appendages by selectively activating the Hox genes Ultrabithorax and Sex comb reduced in wing and leg discs, respectively. This tissue-specific inducibility correlates with the presence of paused RNA polymerase II in the promoter-proximal region of these genes. Although the *Antennapedia* promoter is paused in eye-antenna discs, it cannot be induced by Trx without a reduction in histone variants or their chaperones, suggesting additional control by the nucleosomal architecture. Lineage tracing and pulse-chase experiments revealed that the active state of Hox genes is

maintained substantially longer in mutants deficient for HIRA, a chaperone for the H3.3 variant. In addition, both HIRA and H3.3 appeared to act cooperatively with the Polycomb group of epigenetic repressors. These results support the involvement of H3.3-mediated nucleosome turnover in restoring the repressed state. We propose a regulatory framework integrating transcriptional pausing, histone modification, nucleosome architecture and turnover for cell lineage maintenance.

D1496A A genetic screen for Polycomb group mutants. *J. A. Kennison*, M. T. Cooper. NIH, Bethesda, MD.

Genetic studies first identified the Polycomb group genes by their defects in transcriptional silencing of the homeotic genes. To identify new Polycomb group genes, we have developed a transgene assay using pairing-sensitive silencing of the mini-white reporter gene caused by a Polycomb Group Response Element (PRE) from the Sex combs reduced homeotic gene. Recessive mutations that disrupt silencing are recovered in mitotic clones in heterozygous flies. We have screened about 98% of the genome and isolated mutations in most of the known Polycomb group genes. We have also isolated mutations in several new genes required for silencing. Using a combination of meiotic recombination mapping, deletion mapping, and whole genome sequencing, we have identified the transcription units corresponding to most of these new silencing genes.

D1497B The chromatin remodeling protein Kismet regulates synaptic pruning by controlling steroid hormone receptor expression. *N. K. Latcheva*[1,2], D. Melicharek[4], D. R. Marenda[1,2,3]. 1) Department of Biology, Drexel University, Philadelphia, PA; 2) Molecular Cell Biology and Genetics Department, Drexel University College of Medicine, Philadelphia, PA; 3) Department of Neurobiology and Anatomy, Drexel College of Medicine, Philadelphia, PA; 4) Fox Chace Cancer Center, Philadelphia, PA.

Epigenetics relies in large part on histone modifications and the proteins which make and recognize these modifications. Chromatin readers are a family of proteins which recognize different histone modifications and coordinate the appropriate chromatin rearrangements. A subset of chromatin readers are chromodomain containing proteins that are thought to recognize methylated histone tails, though how they influence gene expression remains largely unknown. One such chromatin reader is encoded by the *kismet* (*kis*) gene in *Drosophila melanogaster*. Decreased *kismet* function leads to defects in axonal guidance and pruning in mature neurons. The Kismet protein shares homology with the mammalian Chromodomain Helicase DNA binding (CHD) subgroup III family of proteins, including CHD7, an ATP-dependent chromatin remodeling protein. Haploinsufficiency of the *CHD7* gene leads to CHARGE syndrome, a congenital neurodevelopmental disorder that affects approximately 1 in 10,000 individuals worldwide. Here we show that axon pruning defects are present in both *kis* mutants and flies expressing RNAi mediated knockdown of *kismet*. Further, we show the pruning defects are due to a decrease in expression of the steroid hormone receptor Ecdysone Receptor isoform B1 (EcR-B1) in the mushroom body Kenyon cells. Supplementing with exogenous EcR-B1 rescues pruning defects in two *kis* mutant backgrounds. Additionally, knocking down *kismet* leads to a global decrease in H3K4me3, a histone modification associated with actively transcribed chromatin. Elucidating the mechanism of Kismet-mediated *EcR-B1* expression will provided greater insight into the epigenetic regulation of neuronal gene expression in general, and will be crucial to further the understanding of neurodevelopmental events that lead to CHARGE syndrome. .

D1498C The recognition of target gene transcriptional state by epigenetic regulators and establishment of Polycomb-group-mediated repression. *P. Ye*, E. Ghotbi Ravandi, J. AlHaj Abed, J. Benes, R. Jones. Southern Methodist University, Dallas, TX.

Polycomb group (PcG) proteins are conserved epigenetic transcriptional regulators that maintain the transcriptional repression of silenced genes. *giant* (*gt*), a PcG target gene, is a zygotic gap gene involved in the development of the head and abdominal regions in *Drosophila*. We have generated a genetic system, triple mutant embryos (*bcd osk tsl*), in which maternal *hunchback* (Hb) is expressed ubiquitously but zygotic Hb expression is absent, resulting in the ubiquitous repression of *gt* by PcG proteins. Chromatin immunoprecipitation (ChIP) experiments on these embryos is being carried out at multiple developmental stages to determine the timing and locations of binding by transcription factors, PcG proteins, and deposition of histone modifications. We have initiated studies to (a) identify the roles of individual proteins in recruitment of PcG complexes and (b) determine the mechanisms by which PcG proteins distinguish between repressed and active target genes. Progress on these studies will be presented.

D1499A Stuxnet Facilitates the Degradation of Polycomb Protein during Development. *Alan J. Zhu*, Juan Du, Junzheng Zhang, Tao He. Peking University, Beijing, CN.

Polycomb-group (PcG) proteins function to ensure correct deployment of developmental programs by epigenetically repressing target gene expression. Despite the importance, few studies have been focused on the regulation of PcG activity itself. Here, we report a novel *Drosophila* gene *stuxnet* (*stx*) that controls Pc protein stability. We find that heightened *stx* expression reduces *Pc* activity, leading to de-repression of PcG targets and homeotic transformation. Conversely, *stx* mutants display developmental defects associated with hyper-activation of *Pc*. Apart from its role on classical PcG targets, this Stx-controlled Pc activity is required for Notch signaling. Mechanistically, Stx facilitates Pc degradation in the proteasome independent of ubiquitin modification. Furthermore, this mode of regulation is conserved in vertebrates. Mouse *stx* promotes degradation of Cbx4, an orthologous Pc protein, in vertebrate cells, rescues *stx*-associated Notch defects and induces homeotic transformation in *Drosophila*. Our results highlight an evolutionarily conserved mechanism of regulated protein degradation on PcG homeostasis and epigenetic activity.

D1500B Mechanisms of epigenetic gene regulation by the Drosophila COMPASS-like complex. *C. B. Zraly*[1], M. G. Slattery[2], A. K. Dingwall[1]. 1) Loyola Univ Chicago-Stritch School of Medicine, Maywood, IL; 2) University of Minnesota Medical School, Duluth, MN.

The highly conserved COMPASS-like complexes recognize/bind specifically modified histone tails and carry out enzymatic histone modifications (methylation and demethylation of lysine residues). The complexes perform many essential functions in epigenetic gene control, including histone reader, writer and eraser functions, allowing for either gene activation or repression. The complexes are essential, with mutations in the fly and mammalian components directly linked to signaling pathway and stem cell defects, developmental and neurological disorders as well as a multitude of cancers. The COMPASS-like complexes are enriched on active, primed and poised enhancers and gene promoters to monomethylate lysine 4 of histone 3 (H3K4me1), considered the hallmark of transcriptional enhancers throughout the genome. We determined the COMPASS-like complex patterns of chromatin binding at various developmental stages in Drosophila using ChIP-seq analyses and identified potential transcription factors including nuclear receptors that collaborate with the complex in enhancer regulation. Genetic interaction studies incorporating null mutant alleles and RNAi combined with RNA-seq and gene specific ChIP analyses were used to examine effects of losing the complex on gene regulation and epigenetic modification. Our analyses revealed that the EcR hormone receptor is pre-engaged on the chromatin prior to ligand binding and that this engagement requires the stabilization by the COMPASS-like complex. Upon stimulation with hormone, the epigenetic modifications change in a precise manner representative of activation then enhancer 'closing' or poising for a subsequent round of activation, representing a form of enhancer pioneering.

D1501C The RNA paradox: can small RNA increase gene expression? *Nikita Deshpande*, Victoria Meller. Wayne State University, Detroit, MI.

Many species have dissimilar sex chromosomes. Dosage compensation is an essential process that modulatesexpression to equalize the ratio of X:A gene products in both sexes. Drosophila melanogaster achieves dosage compensation by transcribing male X-linked genes at twice the rate as females. This is accomplished by the Male Specific Lethal (MSL) complex, which binds the X, modifies chromatin and increases expression. The siRNA pathway contributes to X-localization of the MSL complex, but no RNAi components directly interact with the MSL complex. This suggests that the siRNA pathway acts through a novel and indirect mechanism. For example, an Ago2-containing complex could bind nascent RNAs from the X chromosome and recruit activities that alter epigenetic marks or chromatin architecture. This might facilitate MSL recruitment and spreading along the X-chromosome. To test this model, we used publically available databases to assemble a network of proteins that interact with Ago2. These formed the basis of a targeted screen that has identified several Ago2-interacting proteins that contribute to dosage compensation. Some of these are known to modify chromatin. I then used Chromatin ImmunoPrecipitation(ChIP) to demonstrate that members of the Ago2-interaction network maintain chromatin marks on the X chromosome. This analysis is now being extended to examine the interacting proteins themselves. This study addresses the molecular mechanism by which Ago2 contributes to X-recognition and will provide a useful model for how small RNA contributes to coordinated regulation of broad chromosomal domains in eukaryotes.

D1502A Exploring the chromatin regulation of an inner nuclear membrane Speg (CG9723). *C. Kuok*[1,2], H. Hou[1,3], R. Rosenfeld[1,2], Y. Tsatskis[2], A. Soltyk[4], T. Westwood[4], M. Wilson[1,3], H. McNeill[1,2]. 1) Department of Molecular Genetics, University of Toronto, Toronto, Canada; 2) The Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada; 3) Genetics and Genome Biology Program, SickKids Research Institute, Toronto, Canada; 4) Department of Biology, University of Toronto Mississauga, Mississauga, Canada.

Previously, we identified Speg (CG9723) as an inner nuclear membrane protein required for proper testis development. In *speg* mutants, flies have small testes that lack proper cyst structure and produce few late stage germ cells. To explore if *speg* functions in nuclear or chromatin organization, we examined *speg* null testes and enGal4-driven *speg* RNAi in wings with NLS-GFP. The nuclear envelope appears normal in *speg* mutants. Interestingly, heterochromatin protein 1 (HP1) is decreased in both *speg* mutant testes and wings. H3K4me3, H3K9me3, H3K27me3 and core histones are also decreased in *speg* mutant wings, suggesting a general requirement of *speg* for chromatin organization. We then performed DamID chromatin profiling in Kc cells. Bioinformatics analyses suggested that there are continuous Speg-associated domains spreading along *Drosophila* genome. The majority of Speg-associated domains are Black chromatin. Gene set enrichment analysis suggested that a subset of Speg-associated genes are significantly enriched in testis.

D1503B Regulation of metazoan DNA replication fork progression, stability and composition. *J. T. Nordman*[1], T. L. Orr-Weaver[2]. 1) Vanderbilt University, Nashville, TN; 2) Whitehead Institute; Massachusetts Institute of Technology, Cambridge, MA.

Genome duplication requires exquisite regulation to ensure accurate transmission of genetic information and to prevent chromosomal abnormalities associated with several diseases, including cancer. Further complicating the genome duplication process is the fact that replication forks, the molecular machines responsible for replication of the genome, must be able to stably navigate a dizzying array of replication impediments and chromatin subtypes that pose structural challenges to replication fork progression and stability. Very little is known, however, about how replication forks are able to facilitate progression through difficult-to-replicate regions of the genome present in all eukaryotic cells.

Purification of replication forks from distinct developmental contexts and identification of components provides a means to define plasticity at the fork, an approach for which Drosophila is ideal. In early Drosophila embryos, S phase is three to four minutes in length. Following the maternal-to-zygotic transition, S phase lengthens to approximately one hour. These rapid embryonic S phases are in contrast to the 10-12 hour S phase length in differentiated cell types. Importantly, rapid embryonic S phases of the Drosophila embryo are driven by increased numbers of replication forks per cell. We have developed an iPOND approach, coupled to quantitative mass spectrometry, to enrich and characterize

replication forks from Drosophila embryos. This methodology has identified factors that could be important for controlling replication fork progression and activity in development.

One example of how replication fork composition and activity can be modulated during development is the Suppressor of Underreplication protein, SUUR[1]. In Drosophila polyploid cells, DNA replication is repressed within specific regions of the genome, which results in chromosome fragility. SUUR mediates repression of DNA replication through direct inhibition of replication fork progression. SUUR is not always associated with replication forks, rather it is recruited to active replication forks in specific genomic regions to inhibit their progression. Given that SUUR associates with replication forks in specific developmental contexts, it provides an example of how replication fork composition and activity can be altered during development.

1. Nordman JT, et al. (2014) *Cell Rep.* **9**: 841–9.

D1504C Evaluation of genes required for telomere maintenance on *HipHop* dependent suppression of cell lethality after telomere loss. *Christopher Hendrix*, Miriam Snider, Ashley Hanson, Daniel Beck, Rebeccah Kurzhals. Southeast Missouri State University, Cape Girardeau, MO.

The telomere cap is a complex of proteins and nucleic acids found at chromosome ends which prevents the DNA terminus from being seen as a double strand break in need of repair. HP1, HOAP, HipHop, Ver, and Moi are components of the capping complex. In most cells the absence of a single telomere cap is sufficient to trigger apoptosis. Cells that do not die are likely to experience end-to-end fusions of uncapped ends, leading to gross chromosomal rearrangements and genomic instability. The apoptotic response to telomere loss or dysfunction is mediated by the DNA damage response pathway. However, even in a wildtype background, a small fraction of such cells manage to evade this apoptotic response.

We developed a technique that allows for controlled loss of a single telomere during development. We wish to understand how some cells survive telomere loss. Immunostaining for the telomere cap component HOAP revealed that in some somatic cells, non-telomeric ends can be healed by the addition of a new cap. To characterize this process, we misexpressed genes required for telomere maintenance, while simultaneously inducing telomere loss. We found that misexpression of *HipHop*, or its paralog *ms(3)K81*, resulted in increased survival of cells that lost a telomere. However, misexpression of *cav,* the gene encoding HOAP or *Su(var)205*, the gene encoding HP1, or *ver*, did not significantly increase cell survival. We suggest that HipHop has the ability to seed formation of new telomeres in somatic tissue.

In order to determine if proteins required for telomere maintenance are limiting for HipHop to suppress of cell death after telomere loss, we misexpressed *hiphop* while simultaneously inducing telomere loss in flies that are heterozygous for mutations in *tefu, mre11*, *nbs*, and *cav.* Initial results suggest that these genes do not significantly affect cell survival following telomere loss. We are currently testing other genes required for telomere maintenance. In the absence of misexpression of *hiphop*, cell survival is not significantly different in flies that are heterozygous for mutations in *tefu*, *mre11*, *nbs*, *cav*, *hiphop,* or *Su(var)205* following telomere loss. The telomere cap components tested to date do not appear to be limiting for HipHop's role in suppressing cell lethality after telomere loss.

D1505A Tip60/HDAC balance promotes neuroprotection of cognitive function in the neurodegenerative *Drosophila* brain. *Priyalakshmi Panikker*, Felice Elefant. Drexel University, Philadelphia, PA.

Appropriate histone acetylation homeostasis is critical for neural health and function and is maintained by the antagonistic activity of histone acetlytransferase (HAT) and histone deacetylases (HDAC). Disruption of this fine tuned epigenetic balance involving reduced levels of histone acetylation in the brain causes significant cognitive deficits that are a debilitating hallmark of most neurodegenerative disorders, including Alzheimer's disease (AD). Nevertheless, the HATS that generate these neuroepigenetic marks and their mechanisms of action remain largely unclear. We recently made the exciting discovery that Tip60 is critical for cognitive processes based on its role in neural epigenetic cognition gene control and remarkably, promotes neuroprotection for multiple cognitive neural circuits impaired in the brain during early AD associated neurodegenerative progression. Our findings support a model by which Tip60 promotes neuroprotection by epigenetically reprogramming gene sets that together protect and/or promote cognitive function. To test this model, we performed a gene expression analysis screen on 15 Tip60 cognition-linked target genes that have human homologs. Notably, we found that while expression for all 15 genes was repressed in the AD associated APP neurodegenerative fly brain, expression of 10 of these genes was restored by increasing Tip60 levels in the brain. We next tested possible epigenetic-based mechanisms for Tip60 transcriptional rescue under APP neurodegenerative conditions. Recent compelling studies show reduced histone acetylation levels and enhanced repressor HDAC2 activity in a variety of AD models and in the human AD brain. Thus, we selected 5 of the Tip60 rescued cognition genes and assessed levels of histone acetylation, Tip60 and HDAC2 enrichment using ChIP analysis with Abs to Tip60, Rpd3 (HDAC2), and acetylation marks at histone H4K5,12,and 16 in fly brains expressing APP versus APP and excess Tip60 levels. We found that all histone acetylation marks were reduced in the APP neurodegenerative fly brain and restored by increasing Tip60 levels. Conversely, repressor HDAC2 binding enrichment at these 5 cognition genes was enhanced in the APP fly brain and remarkably, significantly reduced in 3 out of the 5 genes tested upon increased Tip60 levels. Together, our results support a model by which increasing Tip60 in the neurodegenerative fly brain restores Tip60 binding and acetylation levels at cognition gene loci by displacing inappropriate HDAC2 binding, thus activating Tip60 cognitive gene expression to promote neuroprotection.

D1506B Regulation and scaling of developmental time during *Drosophila* embryogenesis. *C. Amourda*[1], J. Chong[1], T. E. Saunders[1,2,3]. 1) Mechanobiology Institute, Singapore; 2) Department of Biological Sciences, NUS, Singapore; 3) IMCB, A-star, Singapore.

Embryogenesis is a precisely regulated process that requires tight coordination of events in space but also in time. Despite being exposed to both endogenous and exogenous cues, embryos reliably develop to their final shape within a predictable timeframe. Perturbations have

subsequently little effect on embryonic development; i.e. the process of embryogenesis is able to account and to correct for environmental and intra-embryo noise. Despite the observation that development happens in a highly temporally coordinated manner there is little work focusing on how embryos are temporally robust.

Here, we develop procedures to analyze temporal robustness at the whole organism level. Using *Drosophila* embryos maintained at precise temperatures, we simultaneously image up to 60 embryos from early embryogenesis until larvae hatching. We time the stage of embryogenesis by identifying developmental landmarks. Due to the large embryo number and controlled environment, our setup enables us to study intra- and inter- embryo temporal variations. We analyzed temporal variation at temperatures from 16 to 30°C and found that there exists an optimal developmental temperature (21°C) at which the temporal variation between embryos is minimal. Furthermore, we show that temporal paths are highly correlated at high (>25ºC) and low (<19ºC) temperature (*i.e.* an embryo that develops quickly at early stages is also fast developing at later stages and *vice versa*).

In accordance with previous studies, embryos develop faster at higher temperatures, though with increasing temporal variability. Whilst this observation can be explained by the faster kinetics of various processes at increasing temperature, we are now investigating whether active temporal regulators are present and coordinate tissue growth with developmental time. Coupled with microarray analysis, our setup offers the unique opportunity to discover such unknown regulators. We performed miRNA arrays and we found that 22 miRNAs are differentially regulated at varying temperature during embryogenesis. These miRNAs have a broad spectrum of action. Despite being expressed during embryogenesis their absence does not prevent hatching. Hence, they may have a subtler role, possibly ensuring coordination between developmental processes. We are currently using miRNA knockout and miRNA overexpression to further understand their role in controlling developmental time.

Our work represents the first quantitative analysis of temporal variation in embryogenesis and the setup we have developed enables us to explore if – and if so, how – time is effectively regulated during embryogenesis.

D1507C RNA helicase Belle/DDX3 regulates transgene expression in Drosophila. *YC Huang*, PK Lo, WM Deng. Florida State Univ, Tallahassee, FL.

Belle (Bel), the Drosophila homolog of the yeast DEAD-box RNA helicase DED1 and human DDX3, has been shown to be required for oogenesis and female fertility. Here we report a novel role of Bel in regulating the expression of transgenes. Abrogation of Bel by mutations or RNAi induces silencing of a variety of P-element-derived transgenes. This silencing effect depends on downregulation of their RNA levels. Our genetic studies have revealed that the RNA helicase Spindle-E (Spn-E), a nuage RNA helicase that plays a crucial role in regulating RNA processing and PIWI-interacting RNA (piRNA) biogenesis in germline cells, is required for loss-of-bel-induced transgene silencing. Conversely, Bel abrogation alleviates the nuage-protein mislocalization phenotype in spn-E mutants, suggesting a competitive relationship between these two RNA helicases. Additionally, disruption of the chromatin remodeling factor Mod(mdg4) or the microRNA biogenesis enzyme Dicer-1 (Dcr-1) also alleviates the transgene-silencing phenotypes in bel mutants, suggesting the involvement of chromatin remodeling and microRNA biogenesis in loss-of-bel-induced transgene silencing. Finally we show that genetic inhibition of Bel function leads to de novo generation of piRNAs from the transgene region inserted in the genome, suggesting a potential piRNA-dependent mechanism that may mediate transgene silencing as Bel function is inhibited.

D1508A The activation of new *lawc* transcripts after the homologous long double-stranded RNA treatment in *Drosophila.* *O. Simonova*, R. Cherezov, J. Vorontsova, I. Mertsalov, D. Kulikova. Koltzov Institute of Developmental Biology, Moscow, RU.

The increasing of gene expression by small RNAs and long non-coding RNAs homologous to promoter or 3'-UTR gene region (RNA activation, RNAa) is opposite to phenomenon of RNA-interference (RNAi). Being opened in the human cell culture (Li et al., 2006) RNAa is considered to be an evolutionary conserved, but in ***Drosophila*** it has not been yet described although it is one of the most convenient model organism to study. Early we have discovered that long double stranded RNAs homologous to ***leg-arista-wing complex*** (***lawc***) gene Open Reading Frame caused activation of ***lawc*** gene expression in ***Drosophila melanogaster*** (Cherezov et al., 2013). Now we confirmed our finding in ***Drosophila*** Schneider 2 (S2) cell culture. We have cloned activated ***lawc*** RNAs and its subsequent analysis revealed that the activated RNAs were new long non-coding ***lawc*** transcripts. The mechanism of new ***lawc*** transcripts activation remained unclear but we proposed that in normal conditions its expression could be suppressed by a new set of microRNAs previously unstudied (Schertel et al., 2012).

D1509B Clueless is a ribonucleoprotein that binds the ribosome at the mitochondrial outer membrane. *R. T. Cox*, A. Sen. Uniformed Services University, Bethesda, MD.

Mitochondrial function is critical for cells. These organelles are central for many cellular functions, including synthesizing ATP, steroid biosynthesis, fatty acid beta-oxidation and apoptosis. Drosophila mitochondria contain their own DNA, mtDNA, that encodes the same suite of products as human mtDNA. However, the vast majority of proteins used in the many biochemical pathways carried out by mitochondria are encoded in the nucleus and must be imported. Since respiration is a source of reactive species that can cause mtDNA damage, it is important for the cell to be able to maintain mitochondrial quality control. Because loss of mitochondrial function gives rise to specific mitochondrial diseases as well as is common in neurodegenerative disease, we are identifying proteins responsible for supporting mitochondrial function.

Towards this end, we have characterized the protein Clueless (Clu). Clu is a large, multi-domain protein that is highly conserved. We showed loss of Clu causes a drop in ATP, increased oxidative stress, short life lifespans, mislocalized mitochondria and sterility. Clu peripherally associates with mitochondria because it can form a complex with the Translocase of the Outer Mitochondrial membrane (TOM) 20, as well as Porin. In addition, we showed that Clu genetically and physically interacts with the PINK1-Parkin quality control mitophagy complex. These

associations place Clu at the mitochondrial outer membrane and indicate that Clu's affect on mitochondria is direct. Recently, we have found that Drosophila Clu is a ribonucleoprotein that binds mRNA, and that the tetratricopeptide repeat domain is largely responsible for this. In addition, Clu is able to bind both large and small ribosomal proteins, and does so at the mitochondrial outer membrane. Mitochondrial protein import can occur either post- or co-translationally, however the mechanisms controlling co-translational import have not been well characterized. Our data support a model by which Clu binds mRNAs, the ribosome, and the outer membrane translocase to facilitate co-translational import, which we are now testing.

How would Clu's roles in protein import and mitochondrial quality control work together? We propose that Clu functions in protein import when mitochondria are healthy, but when the organelle is stressed and import no longer occurs, Clu contributes to PINK1-Park induced mitophagy in order to cull damaged mitochondria. By mediating both pathways, Clu may act as a sensor for mitochondrial quality.

D1510C Investigating a link between methyl-6 adenosine RNA and the dNab2 RNA binding protein in *Drosophila melanogaster*. *B. Jalloh*[1], R. Bienkoski[1], C. Rounds[1], C. Pak[2], S. Kelly[3], A. Corbett[1], K. Moberg[1]. 1) Emory University, Atlanta, GA; 2) Stanford University, CA, USA; 3) College of Wooster, OH, USA.

Intellectual disability is the most common developmental disorder, with an estimated worldwide prevalence of 1%. Although in many cases the causes of intellectual disability are complex, a number of types of intellectual disability are caused by mutations in a single gene. Loss of the *ZC3H14* gene, which encodes an evolutionarily conserved polyadenosine RNA binding protein, leads to a form of inherited autosomal recessive intellectual disability. Loss of the fly ZC3H14 ortholog, dNab2, within *Drosophila* neurons impairs behavior, short-term memory, and alters patterns of axon guidance in the brain. Intriguingly, these brains exhibit longer poly (A) tails and increased levels of m^6A (methylation of position-6 in the RNA base adenosine) expression as compared to control flies. The m^6A mark is an abundant and reversible RNA modification linked to post-transcriptional gene regulation of mRNA with poorly understood biological relevance. Biochemical data suggest that ZC3H14/dNab2 may interact physically with a metabolic enzyme known to deaminate adenosine monophosphate (AMP), leading to the hypothesis that this enzyme could act on m^6A in RNA as well. Our preliminary data reveal strong genetic interactions between dNab2 and the AMP deaminase in a retinal model (GMR-dNab2). These preliminary studies will be followed-up with biochemical and genetic experiments to test the hypothesis that dNab2 controls m^6A levels and expression of specific neuronal target RNAs by interacting with an AMP deaminase. These experiments could provide novel insight into how loss of ZC3H14 impairs brain function.

D1511A Investigating a link between methyl-6 adenosine RNA and the dNab2 RNA binding protein in *Drosophila melanogaster*. *B. Jalloh*[1], R. Bienkoski[1], C. Rounds[1], C. Pak[2], S. Kelly[3], A. Corbett[1], K. Moberg[1]. 1) Emory Universit, GA, USA; 2) Sanford University, CA, USA; 3) Wooster, OH, USA.

Intellectual disability is the most common developmental disorder, with an estimated worldwide prevalence of 1%. Although in many cases the causes of intellectual disability are complex, a number of types of intellectual disability are caused by mutations in a single gene. Loss of the *ZC3H14* gene, which encodes an evolutionarily conserved polyadenosine RNA binding protein, leads to a form of inherited autosomal recessive intellectual disability. Loss of the fly ZC3H14 ortholog, dNab2, within *Drosophila* neurons impairs behavior, short-term memory, and alters patterns of axon guidance in the brain. Intriguingly, these brains exhibit longer poly (A) tails and increased levels of m^6A (methylation of position-6 in the RNA base adenosine) expression as compared to control flies. The m^6A mark is an abundant and reversible RNA modification linked to post-transcriptional gene regulation of mRNA with poorly understood biological relevance. Biochemical data suggest that ZC3H14/dNab2 may interact physically with a metabolic enzyme known to deaminate adenosine monophosphate (AMP), leading to the hypothesis that this enzyme could act on m^6A in RNA as well. Our preliminary data reveal strong genetic interactions between dNab2 and the AMP deaminase in a retinal model (GMR-dNab2). These preliminary studies will be followed-up with biochemical and genetic experiments to test the hypothesis that dNab2 controls m^6A levels and expression of specific neuronal target RNAs by interacting with an AMP deaminase. These experiments could provide novel insight into how loss of ZC3H14 impairs brain function.

D1512B The level of nuclear 80S ribosomes increases during cell stress. *A. S. Abdullahi*[1,2], S. Brogna[2]. 1) Umaru Musa Yar'adua University Katsina, Katsina, NG; 2) University of Birmingham, Edgbaston, Uk.

A hallmark of translation initiation is joining of the small and large ribosomal subunits on the mRNA. Although the 40S and 60S subunits are synthesized and assembled in the nucleolus in eukaryotes, it is believed that there are mechanisms that kept them inactive, preventing 80S assembly and translation in the nucleus. The consensus is that translation occurs only in the cytoplasm. Contrary to this view, we have recently reported that translating 80S ribosomes are also present in the nucleolus and other nuclear sites in *Drosophila* (1). By employing our 80S reporter technique coupled to flow cytometer analysis of *Drosophila cells*, we have observed that nuclear 80S are most apparent during S phase. Furthermore, increased levels of nuclear 80S were observed upon serum starvation and other forms of cellular stress. Our observations suggest a role for nuclear translation during cellular stress.

1: Al-Jubran et al. Visualization of the joining of ribosomal subunits reveals the presence of 80S ribosomes in the nucleus. RNA. 2013.

D1513C Tet and epitranscriptomics in Drosophila. *F. Wang*[1], B. Delatte[2], S. Minakhina[1], F. Fuks[2], R. Steward[1]. 1) Waksman Institute, Piscataway, NJ; 2) Université Libre de Bruxelles, Brussels, Belgium.

The three vertebrate TET genes encode 5-methylcytosine (5mC) hydroxylases that catalyze the transition of 5mC to 5hmC, resulting in the elimination of the methyl mark on DNA. DNA methylation in Drosophila is controversial and recent genome-wide bisulfide sequencing did not uncover any methylated cytosine. However, Drosophila has one essential TET homolog that modifies RNA. We show that

hydroxymethylcytosine preferentially marks polyadenylated RNAs and is deposited by Tet in Drosophila. The transcriptome-wide hydroxymethylation landscape revealed hydroxymethylcytosine on transcripts of many genes expressed during neuron development. Tet and hydroxymethylated Cytosine mRNAs 5hmrC are most abundant in the larval brain, and Tet-deficient larvae have impaired locomotion behavior and brain development, accompanied by decreased RNA hydroxymethylation. This study highlights the distribution, localization, and function of cytosine hydroxymethylation and identifies central roles for this modification in Drosophila. Our findings open new research prospects in an emerging realm of biological regulation: epitranscriptomics.

D1514A The m^{6}A RNA modification controls neurogenesis and sex determination in *Drosophila* via its nuclear reader protein YT521-B. *T. Lence*[1], Junaid Akhtar[1], Marc Bayer[1], Katharina Schmid[2], Laura Spindler[3], Cheuk Hei Ho[4], Nastasja Kreim[1], Miguel A. Andrade-Navarro[1], Burkhard Poeck[3], Mark Helm[2], Jean-Yves Roignant[1]. 1) Institute of Molecular Biology, Mainz, DE; 2) Institute of Pharmacy and Biochemistry, Johannes Gutenberg University of Mainz, 55128 Mainz, Germany; 3) Institute of Zoology III (Neurobiology), Johannes Gutenberg University of Mainz, 55128 Mainz, Germany; 4) Kimmel Center for Biology and Medicine of the Skirball Institute, NYU School of Medicine, Department of Cell Biology, 540 First Avenue, New York, NY 10016.

N^6-methyladenosine RNA (m^{6}A) is the most abundant mRNA modification in vertebrates. While its functions in the regulation of posttranscriptional gene expression are beginning to be unveiled, precise roles of m^{6}A during development of complex organisms remain unclear. Here we carry out a comprehensive molecular and physiological characterization of the individual component of the m^{6}A methyltransferase complex as well as of the YTH reader proteins in *Drosophila melanogaster.* Components of the complex are ubiquitously expressed but show significant enrichment in the nervous system, which is consistent with the high level of m^{6}A in this tissue. Transcriptome wide m^{6}A profiling reveals that the modification is conserved, yet some unique features distinguish *Drosophila* from vertebrates. Surprisingly, mutant flies for the catalytic subunits are viable, but suffer from severe locomotion defects due to impaired neuronal functions, demonstrating the essential importance of m^{6}A in the *Drosophila* nervous system. Components of the m^{6}A methyltransferase complex also control the female-specific splicing of *Sex lethal* (*Sxl*) transcript and of its downstream targets, revealing a role for this modification in sex determination and dosage compensation. Remarkably, knock out (KO) of the nuclear m^{6}A reader YT521-B resembles the loss of the catalytic subunits, implicating this protein as a main effector of m^{6}A functions *in vivo*. Lastly, a screen to identify novel m^{6}A players revealed few conserved candidates that regulate m^{6}A levels and m^{6}A-dependent RNA processing, suggesting their role as *bona fide* components of the methyltransferase complex. Altogether, our study substantially extends our knowledge on m^{6}A biology, revealing the existence of novel components of the complex and demonstrating the crucial roles for this RNA modification in fundamental processes within the context of the whole animal.

D1515B Regulated epithelial microenvironment chip for whole organ studies in *Drosophila*. *C. Narciso*, N. Contento, T. Storey, D. Hoelzle, J. Zartman. University of Notre Dame, Notre Dame, IN.

The genetic mechanisms that lead to the precise size and pattering of tissues is an important question that has long fascinated researchers. Although the roles of many chemical signaling pathways have been elucidated, much work remains to understand the overall systems-level network that defines how basic cellular logic is determined as a result of both chemical and environmental factors in a tissue. More specifically, the impact of exogenous forces, such as mechanical compression, on the regulation of these genetic mechanisms to effect basic cellular processes such as proliferation and apoptosis remains unresolved. Although the mechanisms of regulation are unknown, mounting experimental evidence has increasingly implicated mechanical forces in the regulation of cell cycle and cell survival. However, probing these relationships experimentally remains problematic, owing to the unique challenges involved in mechanically manipulating tissues both *in* and *ex vivo*. Here we describe the fabrication and implementation of a scalable microfluidic culture chip (the **R**egulated **E**pithelial **M**icroenvironment **Chip**) for studying the impact of exogenous forces on development and gene expression in the *Drosophila* wing imaginal disc. The device consists of individually addressable culture chambers. Each chamber allows control over the chemical perfusion of culture media, in addition to the precise application of compressive forces exerted on the disc via a pneumatically operated membrane on the chamber's ceiling. Our results demonstrate that Ca^{2+} signaling is inhibited during mechanical compression, but once compression is removed a Ca^{2+} wave cascades throughout the disc. This response is dependent on the presence of specific serum conditions indicating synergy between chemical and mechanical factors. The observed wave is also qualitatively similar to observed waves of Ca^{2+} *in vivo* that are likely the result of larval motion. A quantitative understanding of how genetic and environmental factors interact is essential to decoding how basic cellular processes are dynamically regulated and can lead to cessation of growth and homeostasis.

D1516C Persistence of RNAi-mediated knockdown in *Drosophila* complicates mosaic analysis yet enables highly sensitive lineage tracing. *J. A. Bosch*, T. S. Sumabat, I. K. Hariharan. University of California - Berkeley.

RNA interference (RNAi) has emerged as a powerful way of reducing gene function in *Drosophila melanogaster* tissues. By expressing synthetic short hairpin RNAs (shRNAs) using the Gal4/UAS system, knockdown is efficiently achieved in specific tissues or in clones of marked cells. Here we show that knockdown by shRNAs is so potent and persistent that even transient exposure of cells to shRNAs can reduce gene function in their descendants. When using the FLP-out Gal4 method, in some instances we observed unmarked "shadow RNAi" clones adjacent to Gal4-expressing clones, which may have resulted from brief Gal4 expression following recombination but prior to cell division. Similarly, Gal4 driver lines with dynamic expression patterns can generate shadow RNAi cells after their activity has ceased in those cells. Importantly, these effects can lead to erroneous conclusions regarding the cell autonomy of knockdown phenotypes. We have investigated the basis of this phenomenon and suggested experimental designs for eliminating ambiguities in interpretation. We have also exploited the persistence of shRNA-mediated knockdown to design a sensitive lineage-tracing method, i-TRACE, which is capable of detecting even low levels of past

reporter expression. Using i-TRACE, we demonstrate transient infidelities in the expression of some cell-identity markers near compartment boundaries in the wing imaginal disc.

D1517A A *Drosophila* RNAi library modulates Hippo pathway-dependent tissue growth. *J. H. A. Vissers*[1,2], S. A. Manning[1,3], A. Kulkarni[1,2], K. F. Harvey[1,2,3]. 1) Peter MacCallum Cancer Centre, East Melbourne, Melbourne, Victoria, AU; 2) Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Melbourne, Victoria, AU; 3) Department of Pathology, University of Melbourne, Parkville, Melbourne, Victoria, Australia.

Libraries of transgenic *Drosophila melanogaster* carrying RNA interference (RNAi) constructs have been used extensively to perform large-scale functional genetic screens *in vivo*. For example, RNAi screens have facilitated the discovery of multiple components of the Hippo pathway, an evolutionarily conserved growth-regulatory network. Here we investigate an important technical limitation with the widely used VDRC KK RNAi collection. We find that approximately 25% of VDRC KK RNAi lines cause false-positive enhancement of the Hippo pathway, owing to ectopic expression of the Tiptop transcription factor. Of relevance to the broader *Drosophila* community, ectopic *tiptop (tio)* expression can also cause organ malformations and mask phenotypes such as organ overgrowth. To enhance the use of the VDRC KK RNAi library, we have generated a *D. melanogaster* strain that will allow researchers to test, in a single cross, whether their genetic screen of interest will be affected by ectopic *tio* expression.

Reference: Vissers *et al* (2016) Nat Commun. Jan 13;7:10368. doi: 10.1038/ncomms10368.

D1518B Hemophilia, direct diagnosis in Cuba suporting genetic counseling. *N. González*[1], M. Ramírez[2], Y. Cordero[2]. 1) Ramón González Coro hospital, Havana, Havana, CU; 2) National Center of Medical Genetics, Havana, Havana, CU.

Hemophilia A (HA) is an inherited bleeding disorder linked to the X chromosome caused by mutations in the gene for clotting factor VIII. Prenatal diagnosis in female carriers from families with hemophilia is vital to reduce the incidence of the disease. The main objective of our work is to describe the characteristics of a family with HA in which it was impossible to know the phase linkage and genetic counseling in the prenatal stage was conducted empirically. Subsequently, it could make a direct diagnosis confirmed the hypothesis for the advice and validated the decisions made by the couple together specialists.

D1519C Efficient targeted editing of genes with a modified Crispr/Cas9 strategy. *D. Li-Kroeger*[1,3], Y. He[3,4], H. Bellen[1,2,3,4]. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Neuroscience, Baylor College of Medicine, Houston, TX; 3) Neurological Research Institute, Houston, Texas; 4) HHMI, Baylor College of Medicine.

Here we present a Knock-in/Knock out approach to Crispr/Cas9 gene editing in *Drosophila* that allows simple dominant marker-based screening to delete DNA regions of interest. The insert cassette is engineered to allow precise and efficient replacement of the deleted segment with any DNA, allowing scarless swapping of engineered sequences for downstream applications. We created a modular toolkit for tagging any gene. We demonstrate the utility of these tools by creating dominantly marked loss-of-function alleles of several genes including the neuronal maintenance factor Nicotinamide Adenylyl Transferase (Nmnat). We then show using the Nmnat allele that we can efficiently swap the inserted cassette by creating Nmnat:GFP:Nmnat fusion proteins with either wild-type or mutated sequences. We find using multiple replicates of injection that greater than fifteen percent of vials from injected (G0) larvae contain Knock-in genes with seamless cassette swapping. In summary, we have created a toolkit and supporting reagents that allow genomic editing in *Drosophila* and permits scarless modification of regions of interest for downstream applications.

D1520A Curation of transcript models with all available public sequencing reads. *ZX Chen*, B. Busby, J. Fear, H. Yang, B. Oliver. National Institute of Health, Bethesda, MD.

A complete reference genome and accurate gene annotations are essential for all genetics and genomics research. The Berkeley *Drosophila*Genome Project (BDGP) release 6 of *D. melanogaster* genome has significantly improved completeness of the genome, especially for the Y chromosome and other heterochromatic regions. However, the current annotations were lifted over from release 5 and remain incomplete. To address this issue, we are taking a deeply data-driven approach to update the gene annotations. Here we use 13,020 runs of publically available RNA-seq data, with a total of 186 billion reads or 17 terabases from Sequence Read Archive (SRA) to re-construct gene models. Furthermore, we extracted associated metadata from SRA, Biosample, Gene Expression Omnibus (GEO), and publications to annotate each sample with tissue, sex, stage, genotype, cell-type and sample-type information. The samples come from a variety of tissues (whole organism [5,158], head [4,859], ovary [1,023], brain [121], testis [69]), stages (adult [8,896], embryo [2,422], larva [973], pupa [243]) and cell type (mix [10,889], S2 [1,122], Kc167 [150], OSC [118], neuroblast [57]). With a study of this scale we need to not only assess the quality of the individual samples, but also verify associated metadata. We subjected each of these datasets to a strict set of quality control metrics. First, all samples were mapped to the *D. melanogaster* BDGP release 6 genome with HISAT2. Alignments were used to measure strandedness, mappability, 5' or 3' bias, RNA integrity and gene expression abundance. For gene model annotation we selected only stranded libraries, for a total of 47 billion mapped reads. This dataset shows an expanded signal to noise ratio compared to single datasets such as modENCODE, that allows for better distinctions between intronic and exonic segments of gene models and provides clearer splicing evidence. Current FlyBase annotations are very good with only 13% of stranded SRA runs having more than 5% uniquely mapped reads in intergenic regions. However, there are still cases of unannotated genes, UTR extensions, novel splicing events, and antisense transcripts. For example, we found extended 5' UTR of the protein-coding gene *cut (ct)*, and an unannotated antisense transcript in 5' region of another protein-coding

gene *bazooka (baz)*. These results indicate that published sequencing data are rich resources for the curation of transcript models that will validate many of the outstanding current transcript models and add to the transcriptional complexity of the annotation.

D1521B Highly contiguous de novo genome assembly of a non-model metazoan using PacBio long reads. *Patrick F. Reilly*, Julie Z. Peng, Peter Andolfatto. Princeton University, PRINCETON, NJ.

Background: With the advent of population and comparative genomics research in non-model organisms, a highly contiguous and accurate reference genome has become paramount in assuring the validity of conclusions from such genome-wide analyses. In the last two years, the references assemblies of two model eukaryotes have been significantly revised using PacBio long read sequencing technology (*Saccharomyces cerevisiae* and *Drosophila melanogaster*). Here we extend these methods to a non-model Drosophilid, *D. yakuba*, in order to assess the practicality of PacBio long read assemblies for non-model metazoans, with the particular aims of rectifying previously characterized misassemblies, and producing a whole chromosome arm genome assembly for an insect genome within a reasonable budget.

Methods: We obtained ~100x of long reads from 20 SMRT cells, performed de novo assembly of the PacBio long reads using Celera Assembler and FALCON, meta-assembled these assemblies, and re-incorporated read information thrown out by the assemblers. Using an existing linkage map, we scaffolded the contigs into full chromosome arms.

Results: We generated meta-assembled contigs with an N50 of 12.2 Mb, three of which spanned at least 90% of a chromosome arm. Minimal scaffolding was necessary to generate chromosome arms (on the order of tens of contig joins across the genome). The contigs also corroborated evidence of and fixed two major (5 Mb and 3 Mb) misassemblies in the existing *D. yakuba* reference genome.

Significance: We have shown that PacBio-only assembly can generate a highly accurate whole chromosome arm genome sequence for non-model metazoans, a necessary prerequisite for reliable genomics analyses.

D1522C Library preparation effects on estimating satellite DNA abundance from short-read sequencing. *Sarah E. Sander*, Kevin H.-C. Wei, Andrew G. Clark, Daniel A. Barbash. Cornell University, Ithaca, NY.

Tandem repetitive DNA, also known as satellite DNA, is a major component of most eukaryotic genomes, including Drosophila and humans. Satellite DNAs can exist as selfish genomic parasites that propagate in the genome at the expense of the host, but can also form essential chromosome structures including centromeres, telomeres, and sub-telomeric regions. Despite these roles in chromosome structure, satellite DNA sequences differ widely in sequence, location, and abundance in the genome, even between closely related species. There is a basic molecular and theoretical understanding of how repetitive DNA can expand or contract within a genome. However, few studies test these models using genome-wide data, because the repetitive part of the genome is difficult to sequence accurately and to quantify with current technology.

Here we describe the effects of using newly developed PCR-free library preparation methods on the assessment of satellite DNA abundance from Illumina sequencing reads. We performed Illumina sequencing on replicate libraries constructed with PCR-free, 8 cycle PCR, and 12 cycle PCR methods from a single DNA extraction. We quantify satellite abundance from raw sequencing reads using our kmer-based algorithm, k-Seek. We then compare satellite abundances across library preparations using correlations, principal components analysis (PCA), and discriminant analysis of principal components (DAPC). We find that the different preparation methods produce libraries that are distinctly different from one another and that much of the differences between libraries are driven by satellite sequences that are underrepresented in conventional PCR-based library preps, such as the highly abundant AATAT satellite. These satellites are much better represented in sequencing reads derived from PCR-free libraries. Despite the PCR-induced bias, which skews absolute abundances, the bias appears to be quantitatively stable, so that contrasts across lines whose libraries are all constructed in the same way can still be reliable. Nevertheless, PCR-free methods are clearly preferred if there is interest in quantitative assessment of repeat composition.

D1523A Highly sensitive measurement of poly(A) tail by TAIL-seq2 reveals dynamic gene regulation via cytoplasmic polyadenylation during oogenesis. *Ahyeon Son*[1,2], Jaechul Lim[1,2], Mihye Lee[1,2], Hyeshik Chang[1,2], V. Narry Kim[1,2]. 1) Center for RNA Research, Institute for Basic Science, Seoul, KR; 2) School of Biological Sciences, Seoul National University, Seoul, KR.

The tail of eukaryotic mRNA is subject to intensive modifications and critically influences mRNA stability and translatability. To investigate RNA tails at the genomic scale, we previously developed a method called TAIL-seq, but its low sensitivity precluded its applications to minute amounts of biological materials. In this study, we present a new version of TAIL-seq (TAIL-seq2) by incorporating splint ligation, which strongly enhances the sequencing depth for mRNAs (~1000 fold compared to that of the previous version). The improved method enabled us to investigate the regulation of poly(A) tail in oocytes and embryos. We discover that the cytoplasmic polyadenylation takes place mainly during late oogenesis in Drosophila, prior to fertilization. Mutation of wispy, a noncanonical poly(A) polymerase, abolishes polyadenylation, indicating that Wispy is likely to be the major, if not the sole, poly(A) polymerase in oocytes. Only ~5% of maternal transcripts (~143 genes) escape from cytoplasmic polyadenylation. By comparing with ribosome profiling data, we further find that mRNAs with elongated poly(A) tail become translationally active upon egg activation. Thus, the dynamic control of poly(A) tail in maturing oocytes pre-shapes the translatomic landscape of initiating embryos.

D1524B Improving Genome Annotation across the Drosophila Clade. *T. D. Murphy*, the Eukaryotic Genome Annotation Team. NCBI/NLM/NIH, Bethesda, MD.

Comparative genomics research in the Drosophila clade has advanced considerably in the last decade with the availability of dozens of whole genome assemblies for various Drosophila species. However, many of these genomes have either old annotations that pre-date the use of RNA-seq evidence, or are from different pipelines that may complicate their use in cross-species analyses. The NCBI Eukaryotic Genome Annotation Pipeline (www.ncbi.nlm.nih.gov/genome/annotation_euk/) has been used to annotate over 300 organisms, ranging from Insects to Plants and Mammals. The pipeline generates alignment evidence including RNA-seq and cross-species protein alignments to predict gene models with Gnomon, an alignment- and HMM-based gene prediction program developed at NCBI. The pipeline also includes robust tracking logic to preserve gene, transcript, and protein identifiers with an annotation update, even with an update in the assembly.

We have used this pipeline to re-annotate many species in the Drosophila clade. In collaboration with FlyBase, these annotations have been used to update eight of the reference Drosophila annotations. This poster will discuss details of NCBI's pipeline, summarize details of the new annotation sets, and provide details on NCBI's future plans for providing annotations of other Drosophila assemblies currently available in GenBank. Some details of this presentation will also be covered in an NCBI workshop on the Saturday morning session.

D1525C Ilastik- and Matlab-based computational tools to analyze biological tubes in 3-D. *Ran Yang*, Eric Li, Madhav Mani, Greg J. Beitel. Northwestern University, Evanston, IL.

The architecture of biological tubes must be tightly regulated for an organism's survival. Understanding the mechanisms of tube size control requires the ability to quantify many aspects of tube structure such as tube length and diameter and cell shape. Our objective was to develop computational tools that could robustly measure differences in morphology of the Drosophila trachea, using open source and generally accessible software. Although programs to measure planar epithelia exist, few are able to process the more complex problem of a 3-D tube and measure cells on a highly curved surface. We acquired confocal images of the tracheal dorsal trunk and segmented the apical surface and overlying cells using Ilastik, an open source image analysis tool funded by the HHMI and Heidelberg Collaboratory for Image Processing. The segmentation data were imported to Matlab, where we used a marching cubes algorithm to create a tube skeleton, automatically detected branches, and mapped apical cell outlines on the tube surface. From this data, many features of interest can be calculated. Those that we focused on include tube length, luminal volume, cross-sectional regularity, and cell size and orientation relative to the local trunk axis. We are still optimizing and validating our code, but preliminary analysis of *src42A*$^{26\text{-}1}$ mutants, which have tube length defects and increased variation in cell orientation, produced results comparable to the previous studies done by Nelson et al. (2012) and Förster and Luschnig (2012). The major cell axes of *src42A*$^{26\text{-}1}$ mutants tend to be orthogonal to the tube axis, resulting in shorter dorsal trunks.

D1526A *REDfly*: The Regulatory Element Database for *Drosophila*. *M. S. Halfon*[1,2,3], M. Zia[1,2], S. M. Gallo[1,2]. 1) University at Buffalo, Buffalo, NY; 2) New York State Center of Excellence in Bioinformatics & Life Sciences, Buffalo, NY; 3) Roswell Park Cancer Institute, Buffalo, NY.

The REDfly database is a curated portal for *Drosophila cis*-regulatory data containing records for empirically validated *cis*-regulatory modules (CRMs, "enhancers") and transcription factor binding sites (TFBSs) reported in the published literature. REDfly's goal is to include all functionally tested sequences regardless of whether they have observable regulatory activity or have activity redundant with other, shorter regulatory sequences. Graphical views show the position of each CRM within its genomic locus, and the location of each CRM with respect to its associated gene is provided. Curation of TFBSs includes sites identified by electrophoretic mobility shift assay (EMSA, "gel shift"), DNAase I footprinting, and high-throughput yeast one-hybrid assays. REDfly currently covers more than 680 publications and contains more than 11,600 records of reporter constructs regulating over 550 genes, including over 5600 "minimal" CRMs, as well as over 2000 TFBSs. Extensive abilities exist for database searching and results filtering. In 2015 we released REDfly v4 including updates to release 6 genome coordinates, an option to download sequences in BED format, and numerous behind-the-scenes improvements. REDfly v5.0 was released in Spring 2016 with fully updated genome annotations and anatomical descriptions. Search has been updated to optionally retrieve all annotated CRMs within a user-defined distance of a locus, not just CRMs explicitly associated with a specific gene. Additional improvements scheduled for release in 2016 include further improved search and download capabilities, addition of ChIP-derived TFBSs, predicted CRMs, integration with Galaxy, and substantial new curation. REDfly provides a comprehensive source of *Drosophila cis*-regulatory data and is a powerful platform to facilitate high-throughput experimental and computational studies of gene regulation. REDfly is freely accessible at http://redfly.ccr.buffalo.edu and can be followed on Twitter at @REDfly_database.

D1527B DRSC Informatics Tools for Functional Genomics Studies, 2016 Update. *Claire Hu*, Arunachalam Vinayagam, Ankita Nand, Aram Comjean, Benjamin Housden, Ian Flockhart, Charles Roesel, Lizabeth Perkins, Norbert Perrimon, Stephanie Mohr. Harvard Medical School, Boston, MA.

A set of online informatics tools has been developed at *Drosophila* RNAi Screening Center (DRSC) to help scientists identify genes, select RNAi reagents, analyze high-throughput datasets and validate results. Here, we present recent updates to existing tools and new tools. DIOPT (flyrnai.org/diopt) was developed for query of predicted orthologs among common model systems by integrating 10 ortholog prediction approaches. We added Rat recently beside the 8 species we previously support. Our CRISPR sgRNA tools include a resource of pre-computed *Drosophila* sgRNA designs viewed in a genome browse context, as well as annotation of potential off-target locations and predicted efficiency. We recently added a Frameshift score, which predicts the likelihood of a frameshift mutation when repairing a CRISPR cut.

We launched three new resources. 1.) GLAD is a resource of high quality lists of functionally related *Drosophila* genes, e.g. based on protein domains (kinases, transcription factors, etc.) or cellular function (e.g. autophagy, signal transduction). 2.) DGET stores and facilitates search of

RNA-Seq based expression profiles available from the modEncode consortium and other public data sets for *Drosophila* genes. Using DGET, researchers are able to look up gene expression profiles, filter results based on threshold expression values, and compare expression data across different developmental stages, tissues and treatments. In addition, at DGET a researcher can analyze tissue or stage-specific enrichment for an inputted list of genes (e.g. 'hits' from a screen) and search for additional genes with similar expression patterns. 3.) MIST is a comprehensive resource of molecular interactions. MIST currently supports several species, including yeast, frog, worm, fly, fish, mouse and human. At MIST, users can mine known physical interactions and infer interactions using other supportive evidence as well as similar genes by correlation analysis. The web interface allows users to retrieve interacting or similar genes in table format as well as visualize these interactions as networks.

D1528C Model organism analysis using InterMine. *Rachel Lyne*[1,2], Karen Christie[5], Paul Davis[4], Jeff De Pons[7], Todd Harris[4], Kalpana Karra[3], Sheldon McKay[4], Howie Motenko[5], Paulo Nuin[4], Leyla Ruzicka[6], Julie Sullivan[1,2], Jennifer Smith[7], Sierra Taylor Moxon[6], Edith Wong[3], Mike Cherry[3], Joel Richardson[5], Mary Shimoyama[7], Lincoln Stein[4], Gos Micklem[1,2], Monte Westerfield[6,8]. 1) Cambridge Systems Biology Centre, University of Cambridge, Cambridge, UK; 2) Department of Genetics, University of Cambridge, Cambridge, UK; 3) Department of Genetics, Stanford University, Stanford, CA; 4) Ontario Institute for Cancer Research, Toronto, ON, Canada; 5) The Jackson Laboratory, Bar Harbor, ME; 6) ZFIN, University of Oregon, Eugene, OR; 7) Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI; 8) Institute of Neuroscience, University of Oregon, Eugene, OR.

Ultimately the aim of studies in model organisms is to further understanding of human biology and disease and eventually facilitate the translation of research into clinical practice. The InterMOD consortium provides a framework for accessing model organism data through the InterMine data integration system (http://intermine.org). InterMine-based databases have been developed for budding yeast (http://yeastmine.yeastgenome.org), rat (http://www.ratmine.org), zebrafish (http://zebrafishmine.org), nematode (http://im-dev.wormbase.org/tools/wormmine), mouse (http://mousemine.org) and fruitfly (http://flymine.org) (known as the MOD-InterMine databases) together with a complementary InterMine database containing human data (http://humanmine.org). The result is a unified interface for data access, search and exploration, through which data from different model organism can be related to each other and to human data through orthology, and likewise model organism data can become more accessible to medical researchers.

The web interface includes a useful identifier resolution system, sophisticated query options and interactive results tables that enable powerful exploration of data, including summaries, filtering, browsing and support for lists. Graphical analysis tools provide a rich environment for data investigation including statistical enrichment of sets of genes or other biological entities.

The powerful, scriptable web service API includes client libraries in multiple widely used languages, including Python, Perl, Ruby, Java and JavaScript, allowing programmatic access to data and facilitating creation of bioinformatic workflows.

Building on this framework, and as part of the NIH BD2K program, we are creating tools that will interrelate human genes and their MOD orthologs, allowing users to examine similarities and differences across the range of model organisms, and therefore increasing the ease with which biomedical researchers can exploit MOD data. A pilot Gene Ontology Analysis tool will be presented. Please come by for a demonstration to the poster/exhibition hall, to booth 403..

D1529A Enhanced orthology data in FlyBase. *Steven Marygold*[1], David Emmert[2], Victor Strelets[3], Josh Goodman[3], Jim Thurmond[3], Claire Hu[4], Thom Kaufman[3], the FlyBase Consortium. 1) University of Cambridge, Cambridge, UK; 2) Harvard University, Cambridge, MA; 3) Indiana University, Bloomington, IN; 4) Harvard Medical School, Boston, MA.

FlyBase has recently incorporated orthology data from the DRSC Integrative Ortholog Prediction Tool (DIOPT). This dataset integrates ortholog predictions between 8 species (*D. melanogaster*, *H. sapiens* and 6 other model organisms) from 10 individual tools (Compara, Homologene, Inparanoid, Isobase, OMA, OrthoDB, orthoMCL, Phylome, RoundUp and TreeFam). The DIOPT approach provides a comparison of orthology predictions originating from different algorithms based on sequence homology, phylogenetic trees or functional similarity. DIOPT data are now searchable directly in FlyBase through the new 'Orthologs' tab of our QuickSearch tool, and are shown explicitly within the 'Orthologs' section of *D. melanogaster* Gene Reports.

The new Orthologs QuickSearch tool is quick and intuitive to use: simply select an input species, enter one or more gene symbols/IDs, then select one or more output species. When the input species is *D. melanogaster*, there is a choice between searching the new DIOPT data or our existing OrthoDB-derived orthology calls. The OrthoDB-specific dataset comprises ~40 species, biased towards those that are closely related to *D. melanogaster*, and will be particularly relevant to researchers interested in orthology relationships between *Drosophila* species or related Diptera, for example.

The results page of a QuickSearch Orthologs search shows the list of ortholog predictions arranged by species, and includes links to report pages at the relevant species databases, NCBI, Ensembl and/or OMIM. For DIOPT-based searches, the number and list of individual ortholog prediction tools that support a given orthologous gene-pair relationship is given; links are also provided to an alignment between orthologous gene-pairs on the DIOPT site, and to FlyBase Gene Reports where a non-*Drosophila* gene has been expressed transgenically in flies. A similar presentation is used for DIOPT-based data within the 'Orthologs' section of *D. melanogaster* Gene Reports.

In addition to serving the core *Drosophila* community, our new orthology search tool will provide a convenient portal to FlyBase data for non-*Drosophila* researchers - they can search with a human/mouse/worm (etc) gene of interest and quickly access the available knowledge and reagents for the fly ortholog(s). The provision of extensive links within the results page to orthologous gene reports at external websites will further aid inter-species/database navigation.

D1530B *flyDIVaS*: A database for genus- and genome- wide divergence and selection in Drosophila. *C. E. Stanley*, R. J. Kulathinal. Temple University, Philadelphia, PA.

Almost a decade ago, the 12 Drosophila Genomes project and the associated development of community-based resources including the AAA site (Assembly/Alignment/Annotation), transformed D. melanogaster into a premiere comparative model (Clark et al. 2007). However, the analysis sets made available for evolutionary inference and biological discovery have become outdated. Here, we provide an updated and upgradable comparative genomics resource of Drosophila divergence and selection, flyDIVaS, based on the latest genomic assemblies, curated FlyBase annotations, and recent OrthoDB orthology calls. flyDIVaS is an online database containing D. melanogaster-centric orthologous gene sets, sequence alignments, divergence estimates (% gaps, dN, dS, dN/dS), and codon-based tests of positive selection. Out of 14K protein-coding D. melanogaster genes, ~80% have one aligned ortholog in the closely related species, D. simulans, and ~50% have 1-1 alignments in the original 12 sequenced species. Genes and their orthologs can be chosen from four taxonomic datasets differing in phylogenetic depth and coverage density, and visualized via interactive alignments and phylogenetic trees. Computationally-savvy users can also batch download entire comparative datasets. A preliminary functional survey finds conserved mitotic, cell cycle, and neural genes, highly diverged immune and reproduction-related genes, strong signals of divergence across tissue-specific genes, and an enrichment of positive selection among highly diverged genes. We encourage researchers to regularly use this resource as a tool for biological inference and discovery, as well as in their classrooms to help train the next generation of biologists to creatively use such genomic big data resources in an integrative manner. flyDIVaS will be annually updated and is freely available at www.flydivas.info.

D1531C A Comprehensive and Precise Genome Duplication Kit in *Drosophila melanogaster*. *G. Mardon*, M. Fa, Y. Tan, R. Chen. GenetiVision Corporation, Houston, TX.

We have generated a new set of transgenic fly stocks that carry >80 kb BAC genomic clones tiling the majority of the autosomal portion of the *Drosophila melanogaster* genome. As a new fly stock collection carrying molecularly-defined genomic duplications, it is a very useful tool for rescue and complementation testing, genetic fine mapping, and can form the basis for further genomic engineering. Specifically, a set of about 1,200 overlapping BACs that tile the entirety of chromosomes 2 and 3 was selected and inserted using site-specific integration. This collection covers >99% of the genes in the autosomal genome. Together with the existing genomic duplication collections for the X and 4th chromosomes, the vast majority of the *Drosophila* genome is now covered. Due to the precise nature of this new collection where the end points of each BAC and the genomic insertion site are precisely known, additional applications are enabled, such as clonal analysis. Furthermore, this collection can serve as the starting point for other manipulations such as gene tagging, generating point mutations, and structure-function analyses. Transgenic stocks are now available through a web-based searchable database (http://genetivision.com/duplication.html).

D1532A Do not let your money fly away: Cost-effective strategies for new investigators operating a fly lab. *A. Auge*, W. J. Kim. University of Ottawa, Ottawa, ON, CA.

According to the NIH director, early-career researchers in the biomedical sciences currently face the worst financial environment of the past half century. Thus, the cost-effective use of start-up packages is extremely important. Here, we present our experience from the past year in setting up a new low-cost fly lab in Canada.

General strategies: Be creative; traditional purchasing techniques are not the most cost-effective.
1) Ask the experts in your department for used items: incubators, microscopes and Petri dishes. Collaborate with facility managers who know about abandoned equipment. Having certain items manufactured at a local machine shop saves money. Our custom fly pads—which are bigger than commercial fly pads—were made in this fashion, at a similar cost.
2) Take advantage of sales that vendors offer to new investigators.
3) Buy scientific supplies at non-scientific stores. Amazon.com, eBay, and dollar stores offer similar supplies for a lower price
4) Sign up for a lab account with vendors and don't forget to negotiate before you regularly order in bulk. Vendors usually offer a lower price than what they advertise. Below are strategies we followed to establish four fly stations and one fly cooking room and a description of how we saved money maintaining twelve trays of stock fly lines with weekly experiments at a low cost.

Equipment:
1) Use websites such as LABx.com for trading used items. There, cheap equipment can be purchased on sale.
2) Browse Amazon.com for cooking room supplies (oven plate, pots, spoons, scoops and strainers), fly station materials (tubing, connectors, paintbrushes and spatulas), general lab equipment (carts and bag holders), and immunostaining items (glass well dishes, Pyrex dishes, micro scissors, and tweezers).
3) Organizers, containers, and storage boxes can be purchased at dollar stores and department stores. For example, purchasing gooseneck lamps from IKEA instead of a vendor's microscope lighting will save 200 CAD per unit.

Consumables:
1) Most fly-food ingredients can be found at a local grocery or bulk food store.
2) Vials, bottles and plugs should be negotiated as regular bulk orders for a discount with sales representatives. For small-scale and short-term use, for example, cotton balls from Amazon.com fulfill the same purpose as conventional plugs.

In **summary**, by setting up a fly lab using these cost-effective methods, new researchers can obtain supplies of equal or higher quality. We saved 2,500 CAD per fly station and 1,000 CAD on the cooking room compared to purchasing from conventional vendors. A further 20,000 CAD per year is saved on consumables (vials, food, bottles, plugs) for maintaining and experimenting with flies. Don't let your money fly away.

D1533B A New Protocol to Visualize Dopamine in Whole Mount *Drosophila* Brains. *Y. Liu*[1], K. Cichewicz[1], E. Garren[1], J. Hirsh[1], A. Tito[2], S. Zhang[2]. 1) University of Virginia, Charlottesville, VA; 2) University of Texas Health Science Center at Houston, Houston, TX.

Dopamine is a neurotransmitter critical in mediating reward pathways, locomotion, and learning and memory. Dopamine visualization is crucial for studying dopamine localization, release, and subcellular quantitation in the brain. We have developed a new immunostaining protocol to directly detect dopamine (DA) in brains of *Drosophila melanogaster* with high signal to noise ratio. The DA antibody stains dopamingergic neurons and terminal projections in wild-type flies while DA deficient brains lack staining, showing specificity of the antibody. The common method to infer DA in the brain utilizes immunostaining of tyrosine hydrolyase (TH), the rate limiting enzyme for DA synthesis. Here, we show the flaw in using this marker, since detectable levels of TH produced by poorly translated TH protein can produce normal amounts of dopamine. Reduced DA staining in the terminal region of a vesicular transporter mutant tell us that stained dopamine is contained in vesicles. This new immunodetection protocol will allow accurate and direct visualization of changes in subcellular localization of DA as a function of other genetic and behavioral state alterations.

D1534C What's new at the Bloomington Drosophila Stock Center. *A. L. Parks*, K. Matthews, K. R. Cook. Bloomington Drosophila Stock Center, Indiana University, Bloomington, IN.

The Bloomington Stock Center added over 5200 stocks in 2015, bringing our total number of stocks to almost 59,000. In 2015 we added >1600 TRiP RNAi stocks, >1000 Mi{MIC} stocks and we began acquisition of the InSITE collection. InSITE insertions, like Mi{MIC}s, carry attP sites that allow you to swap in cassettes carrying your DNA of choice. We also brought in a variety of other exciting new stocks, including UAS-mir and UAS-mir-sponge stocks, Rab knockout lines, transcriptional calcium sensors, Mi{MIC}-based Recombinase-Mediated Cassette Exchange lines expressing tagged fusion proteins under their own power and more. We will provide an overview of these and other new acquisitions and highlight some important sets of pre-existing useful stocks. As always, we hope we will give you ideas for experiments or new ways to explore biological processes. We welcome all suggestions and questions so come by and see us!

Mouse Genetics 2016 Conference

Poster Session Abstracts

M5001A Oncogenic Role of BRE (BRCC45) by USP7-mediated CDC25A Deubiquitylation. *K. Biswas*[1], S. Philip[1], S. Chang[1], B. Martin[1], S. Burkett[1], S. North[1], S. Sharan[1]. 1) National Cancer Institute, Frederick, MD; 2) National Cancer Institute, Frederick, MD; 3) National Cancer Institute, Frederick, MD; 4) National Cancer Institute, Frederick, MD; 5) National Cancer Institute, Frederick, MD; 6) National Cancer Institute, Frederick, MD; 7) National Cancer Institute, Frederick, MD.

BRCA2 has an essential role in DNA repair, and therefore primary cells exhibit severe proliferation defect or fail to survive in its absence. Yet, *BRCA2*-deficiency results in tumorigenesis. It is believed that mutations in genes like *TP53* contribute to the viability of *BRCA2*-deficient cells that subsequently undergo neoplastic transformation due to their unstable genome. To identify other genes that contribute to cell survival in the absence of *BRCA2*, we have undertaken an insertional mutagenesis screen using the Murine Stem Cell Virus (MSCV). Here we describe one of the genes identified in the screen, brain and reproductive organ expressed (TNFRSF1A modulator) (*BRE*), also known as BRCC45. BRE is a component of the BRCA1-complex that specifically recognizes the Lys-63-linked ubiquitinated histones H2A and H2AX at the site of DNA damage. We found that overexpression of BRE can rescue the lethality of *Brca2* null mES cells. Furthermore, our findings revealed that its overexpression results in perturbation of DNA damage induced degradation of CDC25A phosphatase, a key cell cycle regulator and an oncogene, by facilitating deubiquitylation through ubiquitin specific peptidase 7 (USP7). The oncogenic role of BRE is demonstrated by its effect on the growth of BRCA2-deficient tumor cells in mice and the positive correlation between BRE overexpression and CDC25A levels is confirmed in human breast tumors. Altogether, our findings demonstrate a novel oncogenic role for BRE that is independent of its known role in BRCA1-mediated tumor suppression.

M5002B Genes, Orthologs, and Human Diseases: How Model Organism Databases and the Gene Ontology Empower Knowledge Discovery. *J. A. Blake*. The Jackson Laboratory, Bar Harbor, ME.

The Model Organism Databases (MODs) and the Gene Ontology (GO) systems are core data resources for the study of human biology and disease. The MODs having been continuously funded by since the initiation of the Human Genome Project in recognition of the power inherent in the use of these key model organisms for knowledge discovery. They provide gold standard integration of multiple genetics and genomics data, and have been instrumental in the rapid advances in our understanding of the how biological systems work and how genetic variation impacts human biology and contributes to human diseases. The 2016 TAGC meeting brings together communities from Ciliates, Yeast, Mouse, Zebrafish, Drosophila and *C. elegans* to share genetics discovery stories and advance our common objectives.

I will discuss how the MODs and GO communities work together in their common objectives to capture and integrate genetics and genomics data at surprising depth and complexity. I will demonstrate how these communities provide these data to a variety of research communities. I will use the Mouse Genome Database and the Gene Ontology Consortium as the primary examples of how the shared and complementary efforts of experimental scientists, bioinformatics analysts, and bioinformatics specialists together advance science in our current research environment that features very large heterogeneous data sets and rapidly advancing technologies that build on existing understanding of biology and disease.

This work is funded by NIH grants HG 000330 to the Mouse Genome Database (PIs, JA Blake, CJ Bult and JT Eppig) and HG 002273 to the Gene Ontology Consortium (PIs JA Blake, JM Cherry, SE Lewis, PW Sternberg and PD Thomas).

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M5003C Neonatal Exposure to UV-Radiation and NER Pathway Deficiencies Enhance Melanomagenesis In A Novel Transgenic K5-*Edn3* Mouse Model. *D. Cardero*[1*], A. P. Benaduce[1], D. Batista[1], G. Grilo[1], K. Jorge[1], C. Milikowski[2]. 1) Florida International University , Miami, FL; 2) University of Miami, Miami, FL.

Melanoma is the most aggressive form of skin cancer and results from transformed melanocytes. Melanomagenesis is influenced by both genetic and environmental factors. Exposure to Ultra Violet Radiation (UVR) is accepted as one of the most common environmental factors leading to melanomagenesis. The Nucleotide Excision Repair (NER) pathway is very significant during UVR exposure because it is responsible for repairing UVR-induced DNA damage. Xeroderma Pigmentosum (XP) is an autosomal recessive disorder resulting from deficiencies in any of the eight XP complementation group genes, which code for many proteins indispensable to the NER pathway. XP patients exhibit extreme sensitivity to UVR and a high predisposition to developing skin cancers, including melanoma. Upon being exposed to UVR, *Xpa*^{tm1Tnka}-deficient mice, which share many characteristics of XP patients, develop skin cancers, but not melanoma. The endothelin 3 (Edn3) pathway is critical for the development, proliferation, survival and migration of melanocyte precursor cells. The aim of this study was to develop a novel transgenic UVR-induced melanoma mouse model deficient in XPA in conjunction with overexpression of the EDN3 pathway. Three groups of transgenic mice that overexpress the EDN3 pathway under control of the keratin 5 promoter Tg(KRT5-rtTA)1Glk Tg(tetO-Edn3,-lacZ)Kosl (aka K5-Edn3) and have *Xpa*^{tm1Tnka} heterozygous or homozygous mutations (*Xpa*-/- K5-Edn3, *Xpa*+/- K5-Edn3 and *Xpa*+/+ K5-Edn3) were either exposed to a single suberythemal neonatal dose of UVR at 3.5 days, two doses of UVR (at 3.5 days and 6 weeks), or a single dose of UVR at 6 weeks during adulthood. Only transgenic K5-Edn3 mice developed melanomas, and animals exposed to one dose of UVR at 6 weeks of age did not develop any melanomas. *Xpa*-/- K5-Edn3 mice showed increased penetrance (60%, n=10) and earlier disease appearance, when compared to *Xpa*+/- K5-Edn3 (46%, n=13) and *Xpa*+/+ K5-Edn3 (*19%, n=16*). These results suggest that neonatal UVR exposure along with the over-activation of the EDN3 pathway is sufficient for melanomagenesis in mice, and is enhanced by deficiencies in the NER pathway.

M5004A *In Vivo* Modeling of Heritable Dopamine Transporter Dysfunction Associated with Neuropsychiatric Disorders. *G. L. Davis*[1,2], R. D. Blakely[2]. 1) Graduate Neuroscience Program, Vanderbilt University, Nashville, TN; 2) Department of Biomedical Sciences, Florida Atlantic University, Jupiter, FL.

Attention-Deficit Hyperactivity Disorder (ADHD) is the most common childhood psychiatric disorder, and one that, left untreated, is associated with low socio-economic status, addiction and/or incarceration. Our lab has created a construct valid mouse model based on the

Slc6a3^{tm1Rbl} (DAT Val559) coding variant to elucidate neurobiological mechanisms supporting ADHD and other DA-linked comorbid disorders. With these 129;B6-*Slc6a3*^{tm1Rbl} animals and their wildtype adolescent littermates, we sought evidence for cognitive, attention and impulsivity alterations using the 5 choice serial reaction time task (5-CSRTT) and used progressive ratio and sucrose preference tasks to examine changes in motivation and hedonic valuation. In the 5-CSRTT, mice were trained to a baseline performance with a 5 second delay followed by a 2 second stimulus presentation. Premature, incorrect, and correct responses were recorded, as well as, omissions. Once animals met predetermined criteria for task completion, the paradigm was manipulated to probe for different aspects of attention and impulsivity. This effort consisted of increasing the delay between the start of the trial to the presentation of the stimulus from 5 seconds to 15 seconds. Mice were also tested under a variable delay condition where four different delay times (2, 5, 10, and 15 seconds) were used throughout the session in a randomized order. DAT Val559 mice showed several key differences in the task, including faster acquisition of the task, increased impulsivity under the long delay condition, yet improved performance with a variable delay. We hypothesize that with the proper motivation, DAT Val559 may hyperfocus on the parameters driving task acquisition and differentially utilize the temporal information presented during training sessions, being less reliant on absolute timing expectations. An enhancement in motivation for reward in the DAT Val559 mice was suggested by an increase in the progressive ratio break point. Interestingly this response became dysregulated under a devalued condition (i.e. DAT Val559 mice will continue to nose-poke for reward despite home cage satiation). This alteration is likely not due to a difference in hedonic valuation as we found no genotype difference in sucrose preference. Ongoing studies seek to determine the cellular and circuit level plasticities that derive from lifelong expression of the DAT Val559 variant and that lead to the observed changes in impulsivity and motivation. Supported by NIH Awards MH107132-02 (GLD) MH105094 (RDB).

M5005B KDM1A inhibition may contribute to MAPT (tau)-mediated neurodegeneration in Alzheimer's disease. *A. K. Engstrom*, R. A. Moudgal, M. A. Christopher, D. A. Myrick, B. G. Barwick, A. I. Levey, D. J. Katz. Emory University, Atlanta, GA.

Alzheimer's disease (AD) is an irreversible, progressive brain disorder caused by massive neuronal cell death in the frontal and temporal cortices and the hippocampus. AD is associated with the accumulation of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated MAPT (tau) (NFTs). However, how NFTs contribute to neuronal cell death remains unclear. Recent data from our lab has demonstrated that the histone demethylase KDM1A(LSD1) is mislocalized with NFTs in AD cases. In addition, loss of LSD1 systemically in adult mice is sufficient to recapitulate many aspects of AD. These data raise the possibility that neurofibrillary tangles contribute to neuronal cell death, in AD, by interfering with the continuous requirement for LSD1 to repress inappropriate transcription. Here we further test this model by removing one copy of *Kdm1a* from Tg(Prnp-MAPT*P301S)PS19Vle (P301S Tau) mice, which contain a human transgene overexpressing an aggregation-prone mutant of tau. Our preliminary results show these mice exhibit a much faster and more severe neurodegeneration phenotype. This suggests that aggregated tau functions genetically through the loss of LSD1. As a result, it may be possible to target this step therapeutically to block the progression of AD.

M5006C Degenerative transformations in the Liver and Gonads of male Wister albino rats by *Irvingia gabonensis* (Aubery-Lecomte ex O'Rouke) Seed extract. *H.Edim Etta*[1*], E. Victor Ikpeme[2], C. Chinomsos Olisaeke[1], E. Eneobong Eneobong[2]. 1) Cross River University of Technology, Calabar, Cross River, Nigeria; 2) University of Calabar, Calabar, Cross River State, Nigeria.

The effect of seed extract of *Irvingia gabonensis* -African Bush Mango (ABM)- seeds, on the liver and gonads of male Wister albino rats (*Rattus norvergicus*), was investigated. This investigation was prompted by the increasing international acceptance of the seeds of the ABM (test substance) as being an effective weight reducing herbal medication. Male Wister albino rats, were treated with 50mg/kg bw, 100mg/kg bw and 150mg/kg bw of ABM seed extract. The extract was administered by oral intubation for fourteen (14) days, after which the organs, to be investigated were collected for histological processing. Photomicrographs of organs showed mild to severe degenerative effects on the histology of the organs investigated. The seed extract of *Irvingia gabonensis* had a dose-dependent effect on the organs. Some of the histopathological effects observed in the liver were, sinusoidal dilations (SD), hemorrhaging of the central vein (HCV), inflammation (I) and abnormal distribution of hepatocyte (ADH). Others were, macrosteatosis(M) and macrovesicular fatty infilterations (MFI). In the testes, arrested spermatogenesis (AS), degenerated germinal epithelium (DGE), absent interstitial cells (AIC), hardened basal cells (HBC) and empty seminiferous tubules(EST) were some of the effects of the seed extract observed. This indicates that seed extracts of *Irvingia gabonensis* could cause degenerative transfomations of both liver and testes in the male mammal at accumulated high concentrations.

M5007A Embryo and neonate phenotyping identifies new genes essential for mammalian development. *A. Flenniken*[1], J. Ellegood[2], L. Nutter[1], L. Bower[3], D. Rowland[3], L. Lanoue[3], D. Clary[3], F. Benso[4], S. Newbigging[1], M. Henkelman[2], C. McKerlie[1], K. Lloyd[3], The DTCC-KOMP2 Consortium. 1) The Centre for Phenogenomics, Toronto, Canada; 2) Mouse Imaging Centre, Toronto, Canada; 3) University of California, Davis, USA; 4) Charles River Laboratories, Wilmington, USA.

Identifying the structural basis of developmental anomalies using imaging, gross morphology, and histology has led to new insights into gene function. In the last 5 years, the DTCC Consortium, one of three members of the NIH KOMP2 project and a part of the IMPC, has generated knockout mouse lines for 850 unique genes selected for having little to no pre-existing functional annotation. Mutant lines that survive post-weaning are tested in a multi-system phenotyping pipeline from 9 to 16 weeks of age, while lines that fail to give birth to homozygous offspring or survive post-weaning at 3 weeks of age undergo an alternative pipeline. To identify developmental abnormalities leading to death before (embryo lethal) and after (subviable) birth, we screened mutants for viability at different developmental stages to determine the window of lethality and imaged the animals using sensitive methods for each stage. Our screen uses optical projection tomography (OPT) for embryos identified as homozygous lethal at embryonic day (E)12.5 but viable at E9.5, micro-computed tomography (micro-CT) for lines viable at E15.5 but lethal before birth, and high resolution magnetic resonance imaging (MRI) of whole brains of postnatal day (P)7 mice for lines that are

viable at birth but lethal before weaning. DTCC uses an automated image analysis system that is applied to 8 biological replicates comprised of either sex per mutant line. A mutant population average scan is overlaid for comparison to an age-matched wild type population average 3D atlas to identify and quantitate differences with <5% false discovery rate. Automation eliminates bias, increases throughput, and enhances sensitivity to detect differences. Our analysis also includes evaluation and annotation of gross morphology in freshly dissected embryos using a structured set of e-Mouse Atlas Project (EMAP) and Mammalian Phenotype (MP) ontology terms. Staining for lacZ expression analysis is completed at E12.5 for all embryo lethal and neonate subviable lines that have been analyzed at E15.5. Histological assessment of paraffin-embedded sections is done on embryos and placentas for lines analyzed at E9.5 or E15.5 and on brains and whole bodies of neonates analyzed at P7. Examples of pleiotropic, quantified, and characterized gross and subtle phenotypes from each of the imaging modalities and histology will be presented. To date, our pre-weaning viability screen of 688 mutant lines has found 197 lines (29%) either lethal (114 lines; 17%) or subviable (83 lines; 12%). These morphological findings from image analysis with histology of mutant embryos and neonatal mice have provided new and informative insights into gene function essential for mammalian development. Supported by NIH grants U42OD011175 and U54HG006364.

M5008B Gene expression and regulation in food restricted mice. *D. J. Guarnieri*, T. Nguyen, V. Pat, R. Ruh, S. Terhaar, P. Sohal. Saint Bonaventure Univ., Saint Bonaventure, NY.

While it has been widely recognized that food restriction enhances learning and motivation, the neural mechanisms underlying this adaptation are not well defined. It is likely that changes in gene expression underlie the behavioral response to food restriction. Previously, microarray analysis was conducted on animals that had been food restricted (75% of normal caloric intake) over the course of five days (FR-5). Changes in gene expression were confirmed by quantitative PCR (qPCR) within the hypothalamus, as well as three brain regions within the meso-cortico-limbic circuitry including the medial prefrontal cortex (mPFC). The purpose of our experiments is to assess the role of the glucocorticoid receptor (GR) in mediating observed changes in gene expression, with a focus on *Cdkn1a, Arrdc2,* and *Mertk*. We hypothesized that similar molecular adaptations to FR-5 may also occur in peripheral tissue, and show that all three genes are up-regulated in the mouse kidney. We also hypothesized that some adaptations may occur in an acute restraint stress model and tested this by assessing gene expression in mice that were immobilized for 30 minutes. We found that *Cdkn1a* is up-regulated in the mPFC while *Arrdc2* and *Mertk* are unchanged 2.5 hours after the stressor. Additionally, we show that *Mertk* is up-regulated in the male but not the female kidney, while *Arrdc2* shows adaptation in both sexes (FR-5). In order to address whether these genes represent direct targets of the GR, we use bioinformatic approaches to identify relevant GBRs (glucocorticoid binding regions) and plan to use reporter assays in mammalian cells to assess whether they are functional GREs (glucocorticoid responsive elements). These ongoing studies will better characterize the transcriptional response to mild food restriction and will determine the generality of the response across stress models, tissue types and in males versus females. Ultimately, we anticipate that these studies will allow us to address the role of these molecular responses in mediating long-term behavioral changes after mild and chronic stress.

M5009C The role of *Arid1a* as a suppressor of spontaneous mammary tumors in mice. *N. Kartha*, L. Shen, C. Maskin, J. Schimenti. Cornell University, Ithaca, NY.

Human cancer genome studies have identified the SWI/SNF chromatin remodeling complex member *ARID1A* as one of the most frequently altered genes in several tumor types. Its role as an ovarian tumor suppressor has been functionally demonstrated in compound knockout mice. In my primary thesis work, I have found genetic and functional evidence that *Arid1a* is a bona fide breast cancer tumor suppressor, using the *Mcm4*^{chaos3} mouse model of sporadic mammary carcinogenesis. Nearly all mammary tumors that form in these mice contain a deletion removing one *Arid1a* allele, while the remaining intact allele is silenced or downregulated in many cases. The epigenetically silenced allele could be reactivated by treatment with the cytosine methyltransferase inhibitor 5-azacytidine. Restoration of *Arid1a* expression in a *Chaos3* mammary tumor line greatly impaired its ability to form tumors following injection into cleared mammary glands, indicating that ARID1A insufficiency is crucial for maintenance of these tumors. Transcriptome analysis of tumor cells before and after re-introduction of *Arid1a* expression revealed alterations in growth signaling and cell-cycle checkpoint pathways, suggestively in a TRP53-dependent manner. These preliminary results provide *in vivo* evidence for a tumor suppressive and/or maintenance role in breast cancer. My current thesis work involves the generation of a mammary-specific conditional knock-out mouse model of *Arid1a*, which will help determine whether loss of ARID1A is an initiating driver of mammary tumors. I am also attempting to induce expression of the remaining *Arid1a* allele present in multiple *Chaos3* mammary tumor cell lines in a locus-specific manner, using CRISPR-activation (CRISPRa) technology. If successful, this would indicate a potential opportunity for therapeutic intervention in ARID1A-deficient human breast cancer subtypes.

M5010A Mitochondrial Fetal Drive in Response to Nutritional Stress During Gestation. *Robert Kesterson*[1], Tonia Schwartz[2], Laura Lambert[1], Larry Johnson[1], Daniel Kennedy[1], David Allison[1], Scott Ballinger[1]. 1) University of Alabama at Birmingham, Birmingham, AL; 2) Auburn University, Auburn, AL.

Mitochondrial and nuclear genome interactions play a role in many diseases, and are of increasing concern with the advent of mitochondrial transfer techniques to create “three-parent babies”. During gestation in mammals, the placenta regulates growing energetic demands of developing offspring which may have long-term consequences on fertility, fecundity, and fetal programming. In this respect, we were curious about the impact of maternal-offspring mtDNA mismatch upon fetal development and response to nutritional stress. Mitochondrial-Nuclear eXhange (MNX) mouse strains (containing the nucleus from C57BL/6J and the mitochondrial genome from C3H/HeN mice) were used to assess sources of genetic variation that regulate placental (dys)function, fetal programming, and fetal development in response to nutritional stress. Ten two-cell embryos of either mismatched (MNX) or matching (C3H) mitochondrial and nuclear backgrounds were transferred to the ostia of

oviducts in C3H pseudopregnant mice and developed to term. Females from each combination were randomly assigned to either ad lib (AL) diet or 30% caloric restriction (CR) during gestation [n=6 surrogate-embryo genotype combination (AL MNX, CR MNX, AL C3H, CR C3H)]. At day 18 of gestation (near full-term) fetuses and placentas were removed by cesarean section and total number of pups, pup body weight and placental weight recorded.

Results: While neither placental weight (0.1007±0.0059g AL MNX, 0.0931g CR MNX, 0.1034±0.005g AL C3H, 0.09722±0.0171g CR C3H), nor average weight (1.049±0.1410g AL MNX, 0.8739g CR MNX, 1.047±0.1342g AL C3H, 0.7020±0.2664g CR C3H) of pups developed to full term were significantly different per group, only one full term pup was recovered from the CR MNX cohort of 60 embryos transferred (1.67%) compared to 9 in the AL MNX (15%) group. The ad libitum groups produced similar numbers (31.67% MNX and 33.3% C3H) of pups that developed normally to embryonic day 18.

Conclusions: In comparing dietary restriction relative to ad lib, fetuses with mismatched mtDNA relative to the nuclear genome were at higher risk of miscarriage. Further studies are ongoing to determine the effects mtDNA mismatch on maternal physiology; however, these data indicate differences in survivability in response to nutritional stress between embryos of matching or mismatched nuclear and mitochondrial genomes, demonstrating that variation in the fetal mitochondrial genome alters placental and fetal development in response to gestational dietary restriction.

M5011B A spontaneous mutation of neurexin III in the 129S1/SvImJ strain of mice enhances empathic fear behavior. *S. Keum*, A. Kim, H.-S. Shin. Institute for Basic Science (IBS), Daejeon, KR.

Empathy is an important emotional process that involves the ability to recognize and share emotions with others. We previously developed an observational fear learning (OFL) behavioral assay to measure empathic fear in mice. In the OFL task, a mouse is conditioned for fear to the context where it observes a conspecific demonstrator receiving aversive stimuli. We have recently reported that empathic fear response is highly variable among 11 inbred mouse strains, and innate differences in conditioned fear, anxiety, locomotor activity, sociability and preference for social novelty are not significantly correlated with OFL among those strains. However, the genetic factors underlying variability in empathic fear remain to be determined. Intriguingly, we have found that mice of the 129S1/SvImJ (129S1) strain, exhibit a marked increase in OFL, as compared with another 129S substrain, 129S4/SvJaeJ (129S4). Through genetic and molecular analyses, a nonsynonymous mutation of arginine to tryptophan (R498W) in neurexin III (*Nrxn3*) was identified as the causative variant. This mutation occurs at a residue that is well conserved among mammalian species and is predicted to be deleterious to the protein by *in silico* databases. We have further determined that a deletion of *Nrxn3* in the anterior cingulate cortex leads to a decrease in OFL. Knock-in mice with the R498W mutation by the CRISPR/Cas9 system are currently being tested. Taken together, we propose that *Nrxn3* is an important regulator in neural circuits of OFL. These works also demonstrate the validity of the approach to utilize substrains to identify genes and alleles regulating social behaviors.

M5012C Animal Models in Diabetes Research. *K. G. Kumar*, Ana Perez. Taconic Biosciences Inc, Hudson, NY.

Diabetes is a disease characterized by hyperglycemia due to lack of insulin. Animal models are useful to study the pathophysiology and complications of diabetes. The studies shown below provide some characterization parameters on monogenic, multigenic and diabesity models that are critical for choosing the best models.

Goto-Kakizaki rat is an inbred model bred selectively for insulin resistance. As this model is not bred in a classical way of inbreeding, some genetic heterogeneity is expected. We observed variability in hyperglycemia and tested if this is due to genetic heterogeneity by selective improvement of phenotype. We found a significant improvement in the hyperglycemia within a generation (pre-selection vs post-selection glucose levels - 160.0+1.1 vs 174.8+1.6 mg/dl; $p<0.001$; n=3990). The data suggests that the genetic heterogeneity for hyperglycemia is prevalent in this model and could be exploited for identification of candidate genes for hyperglycemia.

NOD model is also an inbred model to study type I diabetes, it develops diabetes due to insulitis starting at about 8 weeks of age. There are many gene-environment interactions influencing the penetrance of diabetic phenotype in this model. We assessed whether environmental variation due to change in health status can alter the penetrance of Diabetes in NOD/MrkTac model. Our data shows that the health status variation may not be an influencing factor for the disease as the colonies show no variability in diabetic prevalence in 2 different health statuses.

Diet induced obesity (DIO) in C57BL/6NTac strain is being used for testing many drugs against diabesity and associated complications. This model have functional nicotinamide nucleotide transhydrogenase (*Nnt*) gene that is absent in the "J" strain. NNT is a major source of mitochondrial NADPH and its functional loss results in mitochondrial redox abnormalities. Our phenotypic study shows that DIO Tac model is heavier, have higher cholesterol and are more insulin resistant than age matched DIO Jax model.

Leptin receptor deficiency in C57BLKS genetic background is an inbred monogenic model, which shows sustained rise in blood sugar as the animal ages (200 mg/dl at 4 weeks to 500 mg/ dl at 9 weeks of age) due to depletion of beta cells.

We will present data that will compare and contrast the use of these models and their parameters that need to be considered when breeding these for studies. Ideally, diversity seen in human patients can be studied using more than one model.

M5013A Integrated analysis of the Jackson Laboratory Knockout Mouse Project 2 (KOMP2) data. *Vivek Kumar*, Donghyung Lee, Vivek Phillip, James Clark, Karen Svenson, Robert E. Braun, Stacey Rizzo, Elissa J. Chesler, Vivek Kumar. The Jackson Laboratory, Bar Harbor, ME.

The Knockout Mouse Project at The Jackson Laboratory has characterized over 300 mouse knockout lines in 28 assays including 10 behavioral and 18 metabolic/physiology assays. This public dataset represents one of the largest using a classical rapid test battery approach. Here we present a comprehensive analysis of this data. We have statistically modeled the environmental and genetic contributions to phenotype variation using multiple statistical approaches. By modeling environmental variables captured in individual test metadata fields, we are able to

increase the sensitivity and precision with which mutants can be detected. By modeling across behavioral, metabolic and physiological phenotypes we provide rich phenotypic resource for the mouse genetics community.

M5014B Identifying Causal Variants for an Allergen-Induced Inflammation QTL. *L. Laudermilk*, S. Kelada. UNC Chapel Hill, Chapel Hill, NC.

Introduction: In asthma, neutrophil levels in sputum are correlated with disease severity and lung function, hence a better understanding of the genes and pathways that affect neutrophilic inflammation in these patients may offer new insights into disease pathogenesis. To identify genes that control neutrophilic inflammation, our lab applied a house dust mite model of asthma to incipient lines of the Collaborative Cross (CC). We identified a quantitative trait locus (QTL) for neutrophils and an associated chemokine, CXCL1, on Chr 7 and subsequently identified *Zfp30* as a candidate gene. In aggregate, our results indicated that a *Zfp30* cis-eQTL underlies variation in CXCL1 and neutrophil recruitment. However, the specific causal variant(s) remain to be identified. We sought to identify the causal variants that regulate *Zfp30* expression level as a way to reveal important regulatory mechanisms of inflammation and disease-related phenotypes. **Methods:** Gateway cloning, luciferase assays, qPCR, and site-directed mutagenesis were used to clone candidate regulatory regions of *Zfp30* from representative mouse strains and test the ability of these regions to recapitulate *in vivo* expression patterns. **Results:** We found that *Zfp30* is differentially expressed in both CC lines and founder strains of the CC. *Zfp30* expression levels in mice from the preCC correlate with the haplotype of the 5' region of the gene, resulting in three expression groups (high, medium, and low). Using a combination of publically available SNP genotype data and DNAseI footprinting data, we identified a ~500 bp candidate regulatory region of the *Zfp30* promoter which contains a single putative causal variant (rs51434084). Using reporter assays with haplotypes from representative CC strains, we show that this region of the *Zfp30* promoter is sufficient to recapitulate *in vivo* patterns of expression at both the mRNA and protein level. Furthermore, mutagenesis of rs51434084 conferred a significant change in expression levels, successfully capturing the expression difference between high and medium *Zfp30* expression groups. **Conclusion:** These results indicate that rs51434084 plays a significant role in the *Zfp30* cis-eQTL and therefore likely affects CXCL1 levels and neutrophil chemotaxis. Future experiments will determine the mechanism of the variant's impact on gene expression and characterize the mechanism by which ZFP30 regulates CXCL1 and other targets.

M5015C Identification of Genetic Modifier Loci that Affect Early Sudden Death in a Mouse Model of Accelerated Heart Aging. *S. Lewis*, H. Higuchi, T. Takimoto, W.-H. Lee, S. Ikeda, A. Ikeda. University of Wisconsin-Madison, Madison, WI.

Purpose: The purpose of this research is to identify genetic modifiers that interact with the transmembrane protein 135 (*Tmem135*) gene to cause early sudden death in a transgenic (TG) mouse model overexpressing *Tmem135* that exhibits heart abnormalities similar to those in the aged heart. Finding genes that interact with *Tmem135* will help elucidate the role of TMEM135 in the early onset and accelerated progression of aging in the heart and death by heart failure.

Introduction: *Tmem135* encodes a transmembrane protein that regulates mitochondrial dynamics. Overexpression of *Tmem135* causes fragmentation of the mitochondria both *in vitro* and *in vivo* in the myocardium. *In vivo*, *Tmem135* overexpression leads to phenotypes in the heart including hypertrophy, collagen accumulation, heart dysfunction, and early adult death, which are similar to abnormalities observed in aged hearts. Furthermore, the gene expression profile of TG hearts is similar to that of the aged mouse hearts. While backcrossing TG mice generated in the FVB background onto the C57BL/6 background, we found that a number of mice die suddenly around postnatal day 21. Increasing the C57BL/6 background increases death in TG mice, suggesting that either the C57BL/6 alleles of the modifiers interact with *Tmem135* to cause early sudden death or FVB alleles are protective against sudden death by heart failure.

Methods: To find genetic modifiers contributing to early sudden death, we conducted quantitative trait locus (QTL) analysis in a semi-quantitative fashion using mice generated by the FVB -C57BL/6 intercross and backcross to C57BL/6. A total of 35 mice, 16 of which suffered sudden early death, were genotyped across the genome using 779 markers to differentiate C57BL/6 and FVB alleles. Mice were categorized as either 0, for survival, or 1, for sudden death. Using R/QTL, we conducted a whole-genome scan with 1000 permutations to determine thresholds for selective candidate QTLs.

Results/Conclusion : QTL analysis identified 2 loci on Chromosomes 2 and 17 that affect early sudden death (p =0.014 and p=0.02, respectively). On Chromosome 2, the peak lod score was 4.6 at 51.215 cM. This QTL has a 95% confidence interval between rs4223268 (68.2 cM) and rs1347690 (80.332 cM). On Chromosome 17, the peak lod score was 4.25 at 28.138 cM. This QTL has a 95% confidence interval between rs3696834 (14.38 cM) and rs3657117 (35.6 cM). Based on our results, genes within our identified loci likely interact with *Tmem135* to affect sudden death by heart failure in young mice.

M5016A Resistance mitigating effect of *Artemisia annua* on *Plasmodium berghei* ANKA and *Plasmodium yoelii*. *Kangethe Lucy*[1,2,3], Sabah Omar[2], Nganga Joseph[3], Kinyua Johnson[3], Hassanali Ahmed[4]. 1) Technical University of Kenya, Nairobi, Kenya; 2) KEMRI, Nairobi ,Kenya; 3) JKUAT, Nairobi, Kenya; 4) Kenyatta University, Nairobi, Kenya.

Resistance of *Plasmodium falciparum* to antimalarial drugs is a major problem in malaria eradication. Artemisinin derivatives particularly in combination with other drugs are thus increasingly used to treat malaria.The study was designed to demonstrate resistance mitigating effect of *Artemisia annua* phytochemicals on *Plasmodium berghei* and *P. yoelii*. Swiss albino mice were inoculated with the murine plasmodia and thereafter treated for 4 days with the *A. annua* and with the pure artemisinin. After 4 days parasitemia was determined and effective doses 50 and 90 were calculated. The doses were utilised to determine concentrations to be used in resistance development.The ED_{50} AND ED_{90} with *P. berghei* were 1.43 and 7.18 mg/kg.day respectively. After 10 exposures and 20 exposures the ED_{50} were determined again and relative sensitivity index (RSI) was calculated as new ED_{50} /ED_{50} initial.

The RSI increased in artemisinin exposed parasites but the same was not true with *Artemisia annua* exposed parasites. Moreover on removal of drug pressure the acquired resistance was not stable and the parasites were more sensitive once more.

M5017B Long-term exercise positively benefits body composition and metabolism during aging in a sex-dependent manner. *R. C. McMullan*[1], S. A. Kelly[2], K. Hua[1], J. Faber[1], F. Pardo-Manuel de Villena[1], D. Pomp[1]. 1) University of North Carolina-Chapel Hill, Chapel Hill, NC; 2) Ohio Wesleyan University, Delaware, OH.

The aging process is associated with declining exercise abilities and unhealthy changes in the ratio of lean body mass to fat. Exercise, even at reduced levels, can ameliorate the physiological changes associated with aging. Exercise is well-known to protect against many chronic diseases and to extend life expectancy. Despite these benefits, willingness to engage in exercise – and physiological responses to exercise – vary widely in human populations. Unfortunately, long-term exercise is difficult and costly to measure in human cohorts. Furthermore, the physiological effects of aging in humans are confounded with important changes in lifestyle and environment that are often difficult to quantify. We used the common inbred mouse strain C57BL/6J to examine long-term patterns of exercise during aging, as well as the resulting physiological consequences of long-term exercise, in a well-controlled environment. Male (n=30) and female (n=30) mice that were already 1 year old (reflecting approximately 40 year old humans) were randomized to treatments of exercise (access to voluntary running wheels) or no exercise until they were 2 years old (reflecting approximately 70 year old humans). Body composition and metabolic traits were measured prior to, throughout, and at the end of the treatment period. Wheel running levels (distance, speed, duration) were greater in females compared to males and declined with age. Sex-dependent differences in body-composition trajectories were observed during the aging process. Long-term exercise significantly reduced body mass and percent body fat, while maintaining percent lean mass; however, the magnitude of these effects was also sex-dependent. This study provides robust evidence that long-term exercise can be used as a preventative measure against age-related weight gain and changes in body composition that have negative implications on health. Furthermore, we demonstrate that inbred mouse strains can be robustly used to characterize the effects of long-term exercise and factors (e.g. sex, age) modulating these effects. This will facilitate more detailed studies on the relationships between exercise and health in aging populations, including the potential impact of genetic predisposition.

M5018C Mutations in PI(3,5)P_2 biosynthesis and neurological disease in human and mouse. *Miriam Meisler*, Guy Lenk. Univ Michigan, Ann Arbor, MI.

Positional cloning of a spontaneous insertional mutation of the gene *Fig4* in 2007 revealed the mammalian role of this endolysosomal gene first studied in yeast. The FIG4 protein functions in a protein complex with VAC14 and PIKFYVE to generate the signaling lipid PI(3,5)P_2. Mammalian neurons are particularly susceptible to reduced levels of PI(3,5)P_2, which result in cell vacuolization and neurodegeneration. Knockout of FIG4 specifically in neurons results in neurodegeneration, and expression specifically in neurons rescues the null mouse. Through a combination of screening and exome sequencing, several human neurological disorders with mutations in this pathway have been identified, including the peripheral neuropathy Charcot-Marie-Tooth Disease and a form of polymicrogyria with seizures. Studies of mice carrying a common human mutation revealed a defect in myelination that is being studied as a model of neuron/glial interaction. The cellular vacuolization phenotype is being used in genetic and chemical screens for modifiers of severity. We have also explored the effects of strain background on *Fig4* phenotypes. The *ingls* mutation of mouse *Vac14* resembles the *Fig4* mutant mouse, and analysis human variants of *VAC14* is in progress. The interplay between human and mouse genetics has been essential to our growing understanding of the function of PI(3,5)P_2 signaling in mammalian cells.

M5019A Systematization of the regulation of mammalian chromosome biology with evolutionary genetics & OMICs: A synopsis - 2016. *F. S. Nallaseth*[1,2,3,4,5,6,7,8,9], R. Felder - Gibbions[10], Z. S. Guo[5,11], J. Ceci[4,12], I. S. Han[13], R. DeLisio[6], M. J. Dewey[4], D. Woodbury[10], L. A. Schein[10], M. L. Tracey[8], J. B. Whitney III[3]. 1) LISNJ, Belle Mead, NJ 08502; 2) CBSA, Boston, MA; 3) MCG, Augusta, GA 30912; 4) USC, Columbia, SC 29208; 5) Huffington CA, MCB, Baylor CM, Houston, TX 77030; 6) RIMB, Nutley, NJ 07110; 7) FHCRC, Seattle, WA 98109; 8) FIU, Miami, FL 33199; 9) FAU, Boca Raton, FL 33431; 10) RWJUH, Piscataway, NJ 08854; 11) U Pittsburgh Cancer Inst, Pittsburgh, PA 15213; 12) HJKRI, U Buffalo, NY 11427; 13) U Ulsan, Seoul, Korea.

Application of principles regulating evolutionary genetics, testis determination (TD) & chromosome biology confirm induction of Y rearrangements & epigenetic modifications in interspecific (IS) combinations of mouse genomes & Y chromosomes. Chimeric adult males with >90% XX somatic & testicular cells confirm *Sry* dispensability. This & 3 methods confirmed genetically modulated rearrangements in the direct & inverted repeat (DR/IR) dense ~200 kb TD *Sxr*-region spanning the 34 kb IR *Sry* locus/gene of fertile males, at high frequency in all tissues, germlines & species. This is consistent with the spectrum of high frequency serendipitous subversions of chromosome biology (SHFSSCB) reported in IS mice from natural hybrid zones (NHZ) & lab matings. Induction by unbiased, evolutionary divergence, rather than by drug or heteroduplex selection, retains the pathological relevance of errors in endogenous sequences, native loci & developmental contexts - with systematization & control of inducing networks by mouse genetics. Also isolated is a putative retrotransposon encoded fusion ORF: SRY-HMG (splice site?)-LINE-1 transposase. *Sry* & yeast (*S.c.)* IR are similar as known sites of double strand breaks (DSB) processed into double Holliday Junctions (dHJ) & their rearrangement products. In *S.c.* (& mouse?) the choice between dissolution (& repair) or resolution (& deletions) of dHJ is a consequence of the interactions of the Sgs1 helicase/Top3 topoisomerase/Rmi1 heteromer with Rad51. EMSA & 'Pull Down' assays confirm that Rmi1 & Top3/Rmi1 preferentially (>10x) bind HJ over 10 synthetic recombinational or replicational structures. The dimer but neither monomer, stimulates Sgs-1N/HJ complex formation. Complexes with HJ have ~6x> affinity than with pseudoreplication forks (pRF) & so this choice between the 2 activities of Sgs1 at stalled forks may be imposed by DNA structure & the dimer. Structural similarity with unstable replication slow zones (RSZ)/fragile sites (FS) & *cis* suppressors of viral *Oris* may also predict *Sxr* instability. These results are a basis for an unbiased & integrative approach to systematizing, surveilling & controlling mammalian genome biology, preempting (disease) mutations & shifting therapeutic paradigms!

M5020B A Novel Mouse Model of Leptomeningeal Melanocytic Disease Based on the Overexpression of GRM1 (mGluR1). *Joseph W. Palmer*, Juliano Freitas, Lida Kos. Florida International University, Miami, FL.

Leptomeningeal disease (LMD) is a rare form of cancer that forms in the two inner most layers that surround the central nervous system (CNS) called the leptomeninges. The leptomeninges is made up of the dura mater and the arachnoid layer, and is responsible for the circulation of cerebral spinal fluid (CSF) throughout the CNS. The occurrence of primary LMD is extremely rare, and there are limited case studies involving the pigment producing cells called melanocytes that naturally reside in the leptomeninges. When these melanocytes become oncogenic they can produce pigmented lesions in the CNS called melanocytomas. Recent evidence indicates that leptomeningeal melanocytic tumors in adult patient's harbor mutations in GNAQ and GNA11, two G proteins that are activated downstream of certain G protein coupled receptors(GCPRs) such as metabotropic glutamate receptor 1, GRM1 (mGluR1). Transgenic mice that overexpress *Grm1* exclusively in melanocytes under the regulation of the dopachrome tautomerase promoter, B6-Tg(Dct-Grm1)ESzc, spontaneously form cutaneous melanocytic neoplasia in the ears and tails. The goal of this study was to investigate if the overexpression of *Grm1* in leptomeningeal melanocytes also lead to the formation of LMD. Tg(Dct-Grm1)ESzc mice showed a significant increase in the accumulation of ectopic pigment in the posterior region of the brain specifically along the transverse sinus between the cerebellum and cortex. The transgenic mice also presented large melanocytic lesions on the cranial floor. 73.9% of mice examined (n=23) exhibited bilateral or unilateral ectopic pigment distribution in the posterior region of the brain. While in control mice no pigment was found (n=5). These results show that the upregulation of *Grm1* increases the distribution of pigment found associated within the CNS of mice. With limited treatment available to patients, the significance of this study lies in the development and characterization of a reliable mouse model for this rare tumor type so as to provide insight into the mechanism at work and possible treatment approaches.

M5021C One RING to Rule Them All: RNF212 Regulates The Size of The Ovarian Follicle Pool. *H. Qiao*, H. Neil. UC Davis, Davis, CA.

A key determinant of female reproductive lifespan is the size of the resting oocyte pool following extensive oocyte elimination during fetal and perinatal periods. Excessive oocyte culling occurs in response to excessive transposon activity, defects in meiotic prophase, and various forms of DNA damage. For example, in mice lacking SPO11 or MSH4, two key recombination factors, normal numbers of oocytes are present at birth, but most oocytes die within a few days. *Rnf212* encodes a RING-family E3 ligase that promotes protein modification by the ubiquitin-like molecule, SUMO. We previously showed that RNF212 acts at recombination sites to promote chromosomal crossing over. Unexpectedly, we now show that *Rnf212* mutation restores the resting oocyte pools of both *Spo11* and *Msh4* mutants. These data implicate RNF212-mediated SUMOylation as a novel aspect of the meiotic checkpoint-signaling pathway that leads to oocyte apoptosis.

M5022A Effect of ketogenic diet on endurance running performance in males and females of two genetically distinct mouse strains. *Andreea Radulescu*[1], William Barrington[2], David Threadgill[2]. 1) University of Surrey, Guildford, Guildford, GB; 2) Texas A&M University, College Station, Texas, USA.

Proper nutrition is a key component of athletic performance. Traditionally, endurance athletes have used high-carbohydrate diets to maximize glycogen stores and fuel their performance. Low-carbohydrate, high-fat ketogenic diets, however, have recently gained popularity among endurance athletes to boost performance and fat loss. Athletes assert that adaptation to a low-carbohydrate diet can improve the body's ability to utilize fat stores by increasing its ketone utilization. The few human studies that analyzed the impact of low-carbohydrate diets on endurance performance show mixed results. However, these studies have very low sample sizes and only examined males. We found that a ketogenic diet can greatly increase endurance running performance in a genetic background- and sex-dependent manner. The study compared forced running performance of five C57BL/6J and FVB/NJ mice of each sex fed ad libitum Western or ketogenic diets for three months. Ketogenic diet-fed C57BL/6J female mice increased their running distance by 89% versus Western diet-fed mice. Male C57BL6/J mice, however, did not have a significant diet effect. FVB/NJ mice of both sexes improved running distance. Ketogenic diet-fed female FVB/NJ mice increased running distance by 70% and males by 49%. The results were independent of fat mass of the mice. Current studies are investigating whether sex-specific hormones are involved in the disparate effects of ketogenic diet on running performance in male and female C57BL/6J mice by ovariectomizing and castrating mice and comparing their forced running phenotype to sham surgery controls. Assessing the role of sex hormones in adaptation to a low-carbohydrate, high-fat diet will help identify the cause of the sex differences on endurance running performance leading to a better understanding of the role of ketogenic diets on exercise performance and the improving ability to identify individuals who might benefit from a ketogenic diet.

M5023B IMPC metabolic phenotyping: Systemic search for new gene functions associated with disturbances in energy balance regulation and glucose homeostasis. *Jan Rozman*[1,10], Robert Brommage[1], Birgit Rathkolb[1,2,10], Manuela Oestereicher[1], Stefanie Leuchtenberger[1], Martin Kistler[1], Valerie Gailus-Durner[1], Helmut Fuchs[1], Eckhard Wolf[2,10], Martin Klingenspor[3,4], Monica Campillos[5,10], Aakash Chavan Ravindranath[5,10], Thomas Werner[6,7], Christine Schuett[1], The IMPC Consortium[9], Martin Hrabe de Angelis[1,8,10]. 1) German Mouse Clinic, Inst. of Experimental Genetics, Helmholtz Zentrum Muenchen, Germany; 2) Inst. of Molecular Animal Breeding and Biotechnology, Gene Center, Ludwig-Maximilians-Universitaet Muenchen, Germany; 3) Technical University Munich, Else-Kroener-Fresenius Center for Nutritional Medicine, TUM School of Life Sciences Weihenstephan, Freising, Germany; 4) Technical University Munich, ZIEL - Institute for Food and Health, Freising, Germany; 5) Systems Biology of Small Molecules, Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum Muenchen, 85764 Neuherberg, Germany; 6) Departments of Internal Medicine and Nephrology, University of Michigan, Ann Arbor, Michigan, USA; 7) Genomatix GmbH Muenchen, Germany; 8) Chair of Experimental Genetics, Center for Nutrition and Food Sciences Weihenstephan, Technische Universitaet Muenchen, Germany; 9) The International Mouse Phenotyping Consortium, MRC Harwell, Oxfordshire, UK; 10) German Center for Diabetes Research, Helmholtz Zentrum Muenchen, 85764 Neuherberg, Germany.

The International Mouse Phenotyping Consortium (IMPC) aims to generate, phenotype, and offer a knock-out mouse model for every protein-coding gene. Disturbances in energy balance regulation or glucose homeostasis result in diseases such as obesity and type 2 diabetes mellitus. We are especially interested in genes linked to these metabolic disorders. Therefore, IMPC phenotyping data were used to identify novel genotype-phenotype associations. From the IMPC adult phenotyping pipeline we selected 7 clinically relevant parameters: (1) blood glucose levels after overnight fasting, (2) area under the curve during the glucose tolerance test, (3) non-fasting triglycerides, (4) body mass, (5) metabolic rate and (6) oxygen consumption both normalized to body mass, and (7) respiratory exchange ratio. Depending on the completeness of uploaded data, phenotyping data were obtained from 329 mutant lines (for metabolic rate) to 1649 mutant lines (for body mass). Phenodeviants were selected based on the ratio of absolute mean mutant divided by absolute mean wildtype (wt) parameter values, resulting in a new index value that ranged between 0.2 (i.e. 20% of wt) and 2.8 (i.e. 280% of wt) depending on the parameter. In brief, we could both confirm published knowledge regarding functional associations to metabolism (*Mrap2* and *Cpe*) as well as identify novel genes that could be linked to human disorders. The combination of more than one parameter ratio (e.g. strong deviations in glucose clearance, fasted blood glucose, and triglyceride levels in 1.4 % of the genes) also helped to detect new disease models. The dataset may be useful for analysis of metabolic pathways and identification of novel regulatory networks.

M5024C Extracellular matrix perturbations in the urinary tract of mouse model of vesicoureteral reflux. *F. Tokhmafshan*[1], I. Gupta[2]. 1) McGill Univ., Montreal, Canada; 2) McGill Univ., Montreal, Canada.

Background: Anomalous development of the ureterovesical junction (UVJ), which joins the ureter to the bladder, is associated with vesicoureteral reflux (VUR): the abnormal retrograde flow of urine from bladder towards the kidneys. VUR is prevented by the muscular layer of the ureters and the bladder that function like a valve to occlude the UVJ. Human studies have demonstrated that refluxing UVJs have elevated levels of fibrillary collagens and degeneration in the smooth muscle layer. This suggests that the integrity of the ECM is crucial for preventing VUR.

Hypothesis: Perturbations in the expression and assembly of fibrillary collagens and elastic fibres, as well as the smooth muscle composition of the developing urinary tract result in a UVJ that refluxes.

Results: The inbred C3H/HeJ (C3H) mouse line has been previously shown by our laboratory to be a fully penetrant model of recessively inherited VUR with a UVJ defect. We have mapped VUR in the C3H mouse to the *Vurm1* locus (LOD=7.4) spanning 22Mb on the proximal end of Chromosome 12. Within this locus we have identified putative disease-causing variants in a number of ECM-related genes such as *Itgb8, Itgbp1, Lmb1, Fbln5*. The C3H as well as the non-VUR C57Bl/6J (B6) mouse line have been used to study the above mentioned ECM components throughout urinary tract development (embryonic day (E)15, newborn, adult) using special stains: Sirius Red (fibrillary collagens), Masson's trichrome (collagen), Verhoeff's Van Gieson (elastic fibres), as well as immunohistochemistry and western blot analysis. Type I and type III collagens are detected in the musculature layer of the ureter and the bladder at E15, and they are also observed in the lamina propria starting at the newborn stage. Collagen fibres grow in length and thickness with age with a preponderance of type I collagen noted in adult mice. Elastic fibres are sparse and interwoven between collagen fibres in the lamina propria and the musculature of the ureter and the bladder in newborn and adult mice. Starting at the newborn stage, the relative amount of fibrillary collagens in the ureter and the bladder of C3H mice is significantly higher than in B6 mice. The musculature of the bladder in C3H mice exhibits degenerative changes at the newborn stage that continue to adulthood. While there are no qualitative differences in elastic fibres in the ureter and bladder of C3H vs. B6 mice, quantitative analysis is ongoing. The increase in the fibrillary collagen content of the bladder and ureter in C3H mice could result in a stiffer bladder and UVJ that prevents the occlusion of the UVJ and results in VUR.

Conclusion: Our results suggest there is a link between ECM perturbations and VUR.

M5025A Translating between human and mouse genetics and phenotypes using the Human-Mouse: Disease Connection. *M. Tomczuk*, S. M. Bello, C. L. Smith, J. A. Kadin, J. E. Richardson, J. T. Eppig, the MGI Staff. The Jackson Laboratory, Bar Harbor, ME.

The mouse is an excellent translational model for human disease research, providing rich phenotype-to-genotype data for studying disease mechanism, as well as evoking candidate genes for human diseases and suggesting new mutant model development in mice. Mouse Genome Informatics (MGI, www.informatics.jax.org), has developed the Human-Mouse:Disease Connection (HMDC, www.diseasemodel.org) portal for improved access to and visualization of these data. Search results in the HMDC are displayed in interactive grids and tables that are dynamically linked to MGI data such as detailed gene and phenotype information, pertinent disease model references, and available mouse model resources through repositories via the International Mouse Strain Resource (IMSR). Here we use human Myelodysplastic Syndrome (MDS) as an example of how MGI search tools aid in identifying available mouse models of human disease, quickly provide access to information on genes known to cause the disease, and identify human candidate disease genes and possible treatment targets.

MDS is a group of hematopoietic disorders in which the bone marrow does not produce sufficient blood cells. To identify experimental mouse models of MDS, investigators can search for disease terms in the HMDC. The results of a search on 'Myelodysplastic Syndrome' show that mutations in the *ASXL1* human gene are implicated in MDS and that mutations in the *Asxl1* mouse homolog show analogous symptoms. This model may be explored for possible therapy targets. The table indicates seven other mouse genes (*Atg7*, *Bap1*, *Crebbp*, *Mybl2*, *Polq*, *Samd9l, Srsf2*) with mutations that are reported as MDS models that are not yet associated with the human ortholog as causative genes of the disease in human and may be new potential candidate disease genes. Three human genes (*GATA2*, *SF3B1*, *TET2*) are implicated in this condition in which orthologous mouse mutants have not yet been reported to model this syndrome. Mutations targeting these mouse genes may be interesting to evaluate as potential murine disease models. In addition, the Phenotypes and Alleles Query form can be used to search by phenotypic terms, such as 'abnormal myelopoiesis' and 'anemia' to obtain a comprehensive list of mutant mouse alleles presenting phenotypes similar to human disease symptoms.

Coming in August 2016, the HMDC will see some exciting changes, including a simplified query form and the incorporation of the Human Phenotype Ontology so that mouse phenotypes can be more directly compared with human disease phenotypes, allowing for easier translation between mouse and human data. Supported by NHGRI grant HG000330.

M5026B Identifying enhancers that regulate genes critical in mouse spermatogenesis. *T. Tran*, John Schimenti. Cornell University, Ithaca, NY.

Enhancers are non-coding DNA fragments that increase the concentration of active transcriptional machinery at promoter sites, therefore to positively drive gene expression. Furthermore, enhancers are known to reside in regions of open chromatin, range from 50 bases to 20 kilobases long, and contain sequences necessary for transcription factor and/or coactivator binding (Li et al., 2016). Beyond these characteristics and a handful of previously identified enhancers, there is much more to learn. A good way to start is by identifying enhancers sequences at a genome-wide level.

We aim to identify enhancers that regulate meiotic genes, specifically genes involved in spermatogenesis. Spermatogenesis is the male version of meiosis, where primordial germ cells undergo two rounds of cellular division to produce four haploid spermatozoa. It is critical that all genes necessary for spermatogenesis are transcribed at the correct time and place, otherwise viable sperm cannot be produced and the organism is infertile. To identify enhancer sequences involved in spermatogenesis, we will use two genome-wide sequencing techniques, ATAC-seq and PRO-seq, on mouse spermatocyte DNA. Both techniques allow us to map the chromatin state, nucleosome patterning, and RNA polymerase positioning across the entire spermatocyte genome. Along with the fact that enhancers are situated within 50KB of a gene's transcription start site (TSS), we can compare our list of spermatogenesis genes and their known TSS loci to the genomic profile and predict enhancer sequences.

Loss-of-function mutants are usually associated with having point mutations or indels in the gene's coding sequence. Therefore, translation goes awry and growing peptide chains have incorrectly incorporated amino acids or truncation due to early stop codons. However, an alternative hypothesis is that disrupted gene expression is due to mutations in the gene's non-coding sequence, like an enhancer. A future aim is to incorporate previously discovered non-synonymous, single nucleotide polymorphisms (SNPs) into identified enhancer sequences and look at the effects on gene transcription.

M5027C Investigating the phenotype of *Lyplal1* knockout mice. *R. A. Watson*, A. S. Gates, F. E. Calvert, W. E. Bottomley, Sanger Mouse Pipelines, C. J. Lelliott, I. Barroso. Wellcome Trust Sanger Institute, Cambridge, GB.

Several GWAS studies have found an association between variants close to the lysophospholipase-like 1 (*Lyplal1*) locus and metabolic traits, including central obesity, fatty liver and waist-hip ratio (where the effect is more significant in females than males). LYPLAL1 is also upregulated in the adipose tissue of obese patients, and a mutation has been identified in a lipodystrophic patient with no other known mutation. However, the physiological role of LYPLAL1 is not fully understood. We investigated the role of LYPLAL1 using a knockout mouse model.

Lyplal1 knockout mice (C57BL/6N-*Liplal1*$^{tm1b(KOMP)Wtsi}$, from the KOMP repository) were initially characterised on a normal chow diet by the Sanger Mouse Pipeline, with no phenotype observed. The mice were then challenged by being placed on a high fat diet (HFD) at 6 weeks of age. The phenotype of these mice was investigated up until 28 weeks of age using various tests, including DEXA body composition analysis, glucose and insulin tolerance tests and indirect calorimetry. *Lyplal1*$^{-/-}$ mice were indistinguishable from wild type mice in all *in vivo* tests performed. Several tissues were collected for further *ex vivo* analysis. *Lyplal1* knockout was confirmed by Western blot. However, no effect of *Lyplal1* knockout was observed on organ weight or on the expression of a range of metabolic genes. Further investigations including tissue morphology analysis and RNAseq are ongoing, to gain a better understanding of the biological role of LYPLAL1.

M5028A Analysis of odor identification in B6;129-*Psen1*^{tm1Mpm} Tg(APPSwe, tauP301L)1Lfa/Mmjax mice. *Lisa S. Webb*, Stephen Fink, Quinton Pace, Rachel Schendzielos, Nazifa Khan, Benjamin Genovese, Katie Whitcomb, Emma Croushore, Harold J. Grau, Darlene A. Mitrano. Christopher Newport University, Newport News, VA.

Alzheimer's Disease (AD) is a progressive neurodegenerative disease affecting a significant and growing proportion of the elderly population. There are several mouse models exhibiting a variety of AD phenotypes on a multiple genetic backgrounds available to study AD. The model we use is the B6;129-*Psen1*^{tm1Mpm} Tg(APPSwe,tauP301L)1Lfa/Mmjax (3xTg-AD) mouse, a triple transgenic model that develops both the amyloid-b plaques and neurofibrillary (tau) tangles. Our study aims to provide more information regarding the various cognitive and biochemical characteristics of the 3xTg-AD model compared to the background and parental strains. Here we report our preliminary data buried food tests examining olfactory effects, specifically the ability to *identify* (rather than discriminate between) odors.

M5029B SIK1 is a key regulator of adipose mass, glucose and lipid metabolism in mice. *D. West*[1], S. Roy[1], AJ Nava[1], M. Adkisson[1], K. Lloyd[2]. 1) Childrens Hospital of Oakland Research Institute, Oakland, CA; 2) University of California, Davis, CA.

SIK1 is a serine/threonine kinase belonging to a three-member AMP-activated protein kinase family. Both SIK2 & SIK3 reportedly have roles in lipid and glucose metabolism in adipose tissue and liver respectively, while a functional role for SIK1 in adipose tissue or liver function has not been described. In a preliminary study, we found decreased adipose depot mass in both female and male *Sik1*$^{tm1(KOMP)Vlcg}$*-/-* mice at ~50-days-of-age mutants with normal body weight. In a follow-up study conducted as part of the KOMP2 adult phenotyping pipeline (http://www.mousephenotype.org/impress), we found significant differences in body composition, glucose & lipid metabolism (all $p < 0.0001$; Mixed Model; n=8 F *Sik1-/-* , n=246 F *Sik1+/+*, n=8 M *Sik1-/-*, n=239 M *Sik1+/+*). Compared to control mice, *Sik1*$^{tm1.1(KOMP)Vlcg}$*-/-* mice exhibited dramatically reduced body fat and body fat/body mass ratios (fat mass/total mass: F-/- = 0.13 ± 0.01; F+/+ = 0.186 ± 0.002; M-/- = 0.114 ± 0.01; F+/+ = 0.220 ± 0.003; Mean ± SEM), and a significantly increased lean mass/body mass ratio. In addition, there were significant decreases in

circulating total cholesterol, HDL-cholesterol, and insulin relative to controls, and a markedly improved area under the glucose tolerance test compared to controls. At 50 days of age, *Sik-/-* male mice had significant alterations of gene expression in liver, muscle, and adipose tissue consistent with a derangement of lipid and glucose metabolism. These data support a role for SIK1 in the regulation of adipose mass, glucose and lipid metabolism. Supported by NIH grants U42OD011175, U54HG006364, DK085124.

M5030C High-throughput multi-system phenotyping identifies pleiotropy and novel gene function. *D. West*[1], I. Morse[2], C. McKerlie[3], K. Lloyd[4], The DTCC-KOMP2 Consortium[1,2,3,4]. 1) Children's Hospital of Oakland Research Institute, Oakland, CA; 2) Charles River Laboratories, Wilmington, MA; 3) The Center for Phenogenomics, Toronto, Canada; 4) University of California, Davis CA.

Our ongoing project's goal is to discover and relate poorly-annotated genes to informative functional information and identify novel phenotypes associated with human disease as part of a high-throughput mouse production and phenotyping pipeline. Adult phenotyping data are now available for ~680 uniquely targeted mutant mouse strains produced and phenotyped by the DTCC Consortium, one of three members of the NIH KOMP2 project and a part of the International Mouse Phenotyping Consortium (IMPC). Mutants were produced from genes prioritized on the basis of interest expressed by the scientific community and selected for having little to no pre-existing functional annotation. Mice for this pipeline were generated by microinjection of targeted C57BL/6N stem cells, chimeric founders were backcrossed to C57BL/6NCrl mice, and target-confirmed heterozygous mutant mice were intercrossed to produce homozygotes for phenotyping. A total of 197 lines were either embryo lethal or partially subviable prior to weaning as homozygotes, and for a subset of these lines adult phenotyping was completed in heterozygous mice. The phenotyping pipeline uses 16 mice (8 females; F, 8 males; M) per strain and co-raised wild type controls. Mice enter the standardized IMPC comprehensive adult phenotyping pipeline (http://www.mousephenotype.org/impress) at ~9 weeks of age, with weekly tests ending in necropsy and blood collection at week 16. We found one or more phenotypic difference compared with controls in ~60% of the mutant lines ($p < 0.0001$; Mixed Model Analysis). Individual phenotypes (not including viability) were assigned to 16 broad categories. The most frequent phenodeviants were found in tests assessing metabolism (clinical chemistry, glucose tolerance test, and indirect calorimetery), behavior, the nervous system (auditory brain stem response and prepulse inhibition), and the musculoskeleton system (bone mineral content and bone morphology). Examples of pleiotropic, statistically validated, and characterized phenotypes from each of these categories will be presented. These findings provide significant novel functional annotation for many genes of interest to the scientific community. Data are readily available at: http://www.mousephenotype.org/; and mutants can be recovered from cryopreserved germplasm at https://www.komp.org/. Supported by NIH grants U42OD011175, U54HG006364. We thank the outstanding efforts of the DTCC Production and Phenotyping Teams.

M5031A Newly formed heterotopic bone in Fibrodysplasia Ossificans Progressive still requires Activin A for maintenance and expansion. *LiQin Xie*, Lily Huang, Nanditha Das, Xialing Wen, Lili Wang, Genevieve Makhoul, Andrew Murphy, Vincent Idone, Aris Economides, Sarah Hatsell. Regeneron Pharmaceuticals, Tarrytown, NY.

Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disorder characterized by episodic but cumulative heterotopic ossification (HO). The HO in FOP develops by an endochondral process that involves a cartilage intermediate and which overall appears to mirror normal endochondral bone formation. As this heterotopic bone develops, it connects to the normal skeleton (by a process that is currently not understood), and these connections eventually start to bridge joints, resulting in progressive immobility, and eventually death. FOP is caused by mutations in the type I BMP (bone morphogenetic protein) receptor ACVR1, the most mutation altering arginine 206 to histidine ($ACVR1^{R206H}$). We have generated a genetically and physiologically accurate mouse model of FOP – 129;B6N-$Acvr1^{tm2Vlcg}$ ($Acvr1^{[R206H]FlEx}$)/+ Gt(ROSA26)$Sor^{tm3.1(cre/ERT2)Vlcg}$/+. In this model, the HO process is initiated by treating the mice with tamoxifen, that effectively converts the conditional allele – $Acvr1^{[R206H]FlEx}$ – to its mutant counterpart – $Acvr1^{R206H}$ – thereby generating mouse cohorts with the same genotype as FOP patients. Using this model, we demonstrated that there is an absolute requirement for activin A to kickstart the process of HO. This requirement for activin A was demonstrated *in vivo* using neutralizing antibodies to activin A. However, in these experiments the anti-activin A antibody was administered at the same time as initiation of HO, and hence this setting only addressed the requirement of activin A at the initial stages of HO, prior to the formation of cartilage. We explored whether activin A continues to play a role after the endochondral process has started and even when partly mineralized heterotopic lesions could be observed. Our results indicate that inhibition of activin A with a fully human monoclonal antibody completely blocks formation of HO in this model of FOP when the antibody is administered 9 days after initiation of the model. To further test delayed treatment's effect on already formed – yet still nascent – HO, anti-activin A Ab was injected at 3 weeks post initiation of the model. With 3-weeks treatment, existing HO volume (lesion size) decreased by 37±41 mm^3 in activin A Ab treatment group ($p<0.05$), while it increased by 19±45 mm^3 in isotope control group. In summary, these results demonstrate a continued role for activin A even after mineralized heterotopic bone lesions have formed and indicates that anti-activin A may be able to reverse the progression of nascent (and not fully mineralized) heterotopic bone.

M5032B A trypsin-like protease from *Alternaria alternata* allergens promotes airway inflammation through activation of protease-activated receptor-2/β-arrestin signaling. *M. C. Yee*[1], H. L. Nichols[1], K. Pal[1], D. Polley[2], K. J. Lee[1], M. Ming[1], M. D. Seigler[1], M. D. Hollenberg[2], M. O. Daines[3], S. Boitano[3], K. A. DeFea[1]. 1) University of California, Riverside, CA; 2) University of Calgary, Calgary, AB, Canada; 3) University of Arizona, Tucson, AZ.

Fungal *Alternaria alternata* exposure is correlated with increased morbidity and a higher risk of fatal asthma attacks in asthmatic patients. *Alternaria* allergens contain serine proteases capable of activating coagulation factor II (thrombin) receptor-like 1, F2RL1, [proteinase-activated receptor-2 (PAR_2)] and promote airway inflammation that is dependent upon protease activity. We have previously shown that PAR_2-dependent airway inflammation is mediated through the β-arrestin 2 activated pathway. Thus, we hypothesized that proteases from *Alternaria*

filtrates will promote activation of the β-arrestin 2 (ARRB2) dependent cellular signaling pathways that mediate airway inflammation in murine models of *Alternaria*-induced asthma. We identify a single alkaline serine protease, with trypsin-like characteristics, AASP (Alternaria Alkaline Serine Protease) that can activate PAR_2, as demonstrated by recruitment of β-arrestin 2 to PAR_2 and mobilization of intracellular Ca^{2+} in cultured cells. Using histological and flow cytometric analyses, we demonstrate that *Alternaria*-induced airway inflammation requires both PAR_2 and ARRB2, as demonstrated by the reduced recruitment of leukocytes (particularly eosinophils and CD4+ T-cells) into the lung, goblet cell hyperplasia, and thickening of the lung epithelium in B6-*F2rl1*^{tm1Jkaw}/*F2rl1*^{tm1Jkaw} or B6-*Arrb2*^{tm1Rjl}/*Arrb2*^{tm1Rjl}, compared to wild-type mice. These inflammation parameters are also reduced by treatment of mice with Soybean Trypsin Inhibitor (SBTI) during the administration of *Alternaria* allergens, indicating that AASP is likely necessary for the inflammation induced by *Alternaria*. These are the first experiments demonstrating that the PAR_2-β-arrestin 2-dependent signaling axis is important for asthma induced by household allergens. Our studies also suggest that targeting this pathway via biased antagonism with a PAR_2-targeted ligand could be a new avenue of therapeutic intervention for asthma.

M5033C An OVA-sensitized and MCh-challenged mouse phenotyping screen for new genes involved in lung function and respiratory disease. *Y. Zhu*[1], Mohammad Eskandarian[1], Ann Flenniken[1], Amy Woroch[1], Lily Morikawa[1], KC Kent Lloyd[2], Frank Benso[3], Colin McKerlie[1], The DTCC-KOMP2-Phase1 Consortium. 1) The Centre for Phenogenomics, Toronto, ON, CA; 2) Mouse Biology Program, University of California, Davis, USA; 3) Charles River Laboratories, Wilmington, USA.

This project's goal was to discover and relate poorly-annotated genes to respiratory function and inflammatory lung disease as part of a high-throughput mouse production and phenotyping pipeline. Sixty strains of knockout mice were produced, expanded, and screened using 16 mice (8 females; F, 8 males; M) per strain. Twelve mice (6F, 6M) were homozygous knockouts and 4 mice were co-raised wild type controls (2F, 2M). All mice were sensitized with an inert allergen (ovalbumin; OVA) over 21 days. On day 22 mice were challenged with a non-allergen airway agonist (Methacholine; MCh) followed by measurement of enhanced pause (Penh), respiratory rate, tidal volume, peak expiratory flow (PEF), and expiratory time (Te) using unrestrained whole-body plethysmography (WBP) to assess respiratory function, lung development, and airway hyperreactivity. Measurements were taken at baseline and at concentrations of 0, 12.5, 25, and 50 mg/ml MCh. In addition serum immunoglobulin (OVA-specific IgG_1 and IgE) was measured by ELISA and standardized semi-quantitative histopathology scoring was used to assess allergic airway disease and acquired immune response/TI hypersensitivity. Scoring evaluates airway wall thickening, inflammatory cell infiltration, fibrosis, and Clara cell hyperplasia characterized by hyper secretion, mucus metaplasia, and airway mucus plugging.

The 60 strains of knockout mice had cumulative histopathology scores between 1.25 and 2.94 compared to the wild-type average of 1.93 (n=231). The 5 genes which caused the highest scores (2.31 – 2.94) were: *Svs2*, *Slc47a2*, *Adipoq*, *Fbxl22*, and *Arap1*. These 5 strains also had either significantly increased Penh ($p \leq 0.05$), or decreased PEF ($p \leq 0.05$), or increased Te ($p \leq 0.05$), or a combination of increased Penh and decreased PEF compared to wild type mice. The 5 knockout strains with the lowest histopathology scores had no abnormal WBP measurements.

We report a standardized and validated method to screen presumptive null mutant mouse strains with poorly annotated genes for abnormal phenotypes associated with respiratory function and inflammatory lung disease. Strains with high histopathology scores correlated with abnormal lung function results. One strain with decreased tidal volume and Penh (airway hyperreactivity) after sensitization may be particularly interesting as a potential drug target. All of the mouse strains used in this screen are freely available to the research community and may provide new models for hypothesis-driven mechanistic studies or purpose-driven drug target research.

M5034A Investigating Operative DNA Damage Response Pathways in Mouse Primordial Germ Cells. *J. C. Bloom*, J. C. Schimenti. Cornell University, Ithaca, NY.

The ability of organisms to pass their genetic information onwards to subsequent generations is crucial for survival and propagation of a species. In mice, primordial germ cells (PGCs) are set aside very early in development to become the germline lineage. PGCs are first distinguishable as a group of 45 cells in the epiblast of 6-6.5 day old embryos (E6-6.5). These cells then simultaneously migrate and proliferate to the genital ridges (the location of the future gonads) at E10.5 and by E13.5 the population of PGCs reaches its ultimate peak of approximately 25,000 cells.

Because DNA replication associated with rapid PGC proliferation is subject to spontaneous errors, and because PGCs carry the genetic information that will be passed down to the next generation, mechanisms exist to avoid the propagation of these mutations. In accordance with the desire to maintain genomic integrity in the germline, studies have revealed that PGCs are, to a greater extent than somatic cells, highly sensitive to genetic defects and environmental perturbations affecting DNA. While studies of cultured somatic cells and single-celled eukaryotes such as yeast have elucidated DNA damage response pathways, cell cycle checkpoints, and DNA repair mechanisms, our understanding of these processes in the mammalian germline is much less clear. To investigate how genomic integrity is maintained in the mammalian germline, we are characterizing DNA damage response mechanisms in primordial germ cells. We found that mutations affecting two different DNA damage response pathways: Fanconi Anemia that respond to errors in DNA replication and MCM9 being involved in double strand break repair, trigger cell cycle slowdown rather than apoptosis. Double mutant analyses suggested that canonical checkpoint pathways are not involved in responding to these defects in PGCs. To further explore these findings, we are conducting studies to examine mutational burden in mice defective for certain checkpoint pathways, as well as using CRISPR/Cas9 genome editing to develop *in vivo* DNA damage pathway sensors.

M5035B *Top3b*-null Mice Show Defective Neurogenesis, Synaptic Plasticity and Increased Anxiety. *Y. Joo*, W. Peng, Y. Wang, S. Ghosh, K. Fisbein, R. Spencer, H. van Praag, M. Mattson, W. Wang. NIH, Baltimore, MD.

Topoisomerase 3b (TOP3B), the first RNA topoisomerase, interacts with FMR1 (FMRP), the disease gene product in fragile X mental retardation syndrome. Increasing evidence suggests that TOP3B regulates RNA metabolism and promotes synapse formation. A recent study also shows that individuals carrying deletion of *TOP3B* gene are at increased risk of developing schizophrenia and intellectual disability. However, the functional role and pathologic mechanism of TOP3B in mental disorders are unclear. Here we show that *Top3b*-deficient mice have increased anxiety and intensified fear conditioned memory compared with wild type mice in several behavioral tests. In addition,*Top3b*-deficient mice display enlarged ventricles, a phenotype commonly observed in schizophrenia patients. These enlarged ventricles could be due to reduced proliferation and differentiation of adult neural stem cells in subventricular zone (SVZ) and hippocampus of *Top3b*-deficient mice. Furthermore, we show that two forms of protein synthesis-dependent synaptic plasticity, long-term depression (LTD) and long-term potentiation (LTP) that involve activation of metabotropic glutamate receptors (mGluRs), are impaired in the hippocampus of *Top3b*-deficient mice. Mechanistically, TOP3B binds to a group of mRNAs, which are crucial for adult neurogenesis and newly developed tissue structure; and may regulate an emotional condition including anxiety level and fear memory. Our data demonstrate that TOP3B is required for adult neural genesis and synaptic plasticity, and provides a mechanism for how its mutation can lead to neurodevelopmental disorders.

M5036C Investigating how cytoskeletal protein mutations cause Amyotrophic Lateral Sclerosis disease using neuronal cells differentiated from mouse embryonic stem cells. *Kim Nguyen*, Lisa Schneper, Kathryn Sheldon, Zhonghua Gao, James Broach. Penn State Hershey Coll of Medicine, Hershey, PA.

Amyotrophic Lateral Sclerosis (ALS) affects 15,000 to 21,000 Americans at any one time (ALS association). Unfortunately, this destructive motor-neurodegenerative disease still remains untreatable largely due to a lack of knowledge in the disease mechanisms and a lack of model organism for drug screen. Currently, known causative genetic mutations of ALS only account for ~6% of ALS cases despite the total ALS heritability being 50%. Not only is there a tremendous knowledge gap in the genetic basis of ALS, but also the associated clinical phenotypes are complicated and disconnected to the known genetics. Therefore, the goal of our study is to *identify and characterize the genetic factors underlying the disease and specifically correlating these to various groups of ALS clinical features*. To understand genetic mechanism underlying ALS disease, we have recruited and exome-sequenced more than one hundred ALS patients. In addition to rare damaging variants in ALS causative genes such as *SOD1* and *TARDBP (TDP43)* we have found *de novo* compound heterozygous mutations in cytoskeletal-related proteins such as DNAH2, DYNC2LI1, STARD9 and NRP2. The *ex vivo* functional characterization of mutations were established in mouse embryonic stem cells (mESC) by CRISPR knockout technology with the presumption that compound heterozygous mutations abolish protein function. Two CRISPR targets per gene were generated and transfected into neuronal-fluorescent mESC. Single positive knockout cell were FACS-sorted and propagated for validations. Validated knockout clones then are used for differentiation into motor neurons, astrocytes, and oligodendrocytes. Phenotypes of neuronal cell viability and neurite outgrowth length and numbers are then accessed by live-cell staining, fixed-cell staining and qRT-PCR. The results of the *ex vivo* characterization will establish the roles of cytoskeletal-related proteins in ALS and will also allow us to generate mouse models that could be great contributions to the ALS field.

M5037A Androgen receptor plays distinct roles in prostate basal and luminal cells and is required for rare stem cell activities in both compartments. *Z. Wang*[1], Q. Xie[1], T. Cai[2], H. Corrigan[1], S. Joshua[1]. 1) University of California Santa Cruz, Santa Cruz, CA; 2) National Institute of Biological Sciences, Beijing, China.

Androgen is an important steroid hormone regulating prostate physiology, and its signal through the androgen receptor (AR) plays critical roles in prostate development and cancer progression. It has been shown that prostate stromal AR and epithelial AR have different functions in organogenesis. Yet the role of epithelial AR in prostate homeostasis and cancer initiation remains controversial. Recent studies have provided a clearer picture of the cell lineage relationships in the prostate epithelium, but how AR regulates them is unclear.

Here, using genetic lineage tracing, we carefully investigated the role of AR in different epithelial cell types of the adult prostate. We show that AR has a heterogeneous expression pattern in adult basal cells, and is dispensable for basal cell maintenance. However, AR is cell-autonomously required for the luminal differentiation capability of rare basal stem cells *in vivo*. In contrast, AR loss in luminal cells induces a brief period of cell proliferation and disrupts their normal columnar cell morphology and polarity, but it does not affect luminal cell survival or androgen-mediated regression-regeneration. Interestingly, we find that during regeneration AR is selectively required for the multipotency of castration-resistant *Nkx3-1*-expressing luminal stem cells (CARNs), as it prevents their differentiated daughter cells from apoptosis. Finally, we show that PTEN loss can override AR-loss defects in both basal and luminal compartments to initiate prostate tumors. Our data reveal the multifaceted roles of AR in different prostate epithelial cell types for orchestrating tissue homeostasis *in vivo*, and highlight the distinct mechanisms utilized by AR in rare basal and luminal stem cells. Our results also argue for a less prominent role of epithelial AR in early prostate cancer initiation.

M5038B Mammalian Retinal Regeneration in Response to an α7 nAChR Agonist. *M. K. Webster*, H. B. Bach, C. L. Linn. Western Michigan University, Kalamazoo, MI.

Irreversible vision loss due to disease, age or damage reduces quality of life worldwide and is a substantial burden on national healthcare. Previous studies in this lab have shown that α7 nicotinic acetylcholine receptor agonists are capable of providing neuroprotection in a glaucoma model. We seek to understand if and how the selective α7 nicotinic acetylcholine receptor agonist, PNU-282987, leads to proliferation of adult mammalian retinal neurons in Long Evans rats. To determine if PNU-282987 triggers proliferation of retinal neurons, animals were treated with 1 mM PNU-282987 and 1 mg/mL BrdU twice a day for varying amounts of time. Antiboides against BrdU revealed

that new retinal neurons were generated in a dose and time dependent manner and double labeling using antibodies against specific retinal neurons demonstrated evidence of new photoreceptors, retinal ganglion cells as well as BrdU positive cells in the inner nuclear layer.

A well-established source of new retinal neurons in other vertebrate model organisms is the Müller glia. To determine if Müller glia are the source of new BrdU positive cells in PNU-282987 treated rat eyes, Müller glia and progenitor cells were examined for BrdU colabeling. This demonstrated that Müller glia are in fact BrdU positive and express the proliferation marker PCNA. Further, nestin, a neural progenitor cell marker, and PCNA positive cells were observed in the inner nuclear layer. This data supports the hypothesis that Müller glia give rise to new retinal progenitor cells and that these are the cells that then differentiate and gave rise to different types of mature neurons between 7 and 28 days after treatment with PNU-282987.

To begin to understand the gene pathways responsible for proliferation mediated by the selective α7 nicotinic acetylcholine receptor agonist, mRNA-seq was performed on animals treated with PNU-282987. Our pathway analysis demonstrated that after only 3 days of treatment there was significant changes in a number of key signaling pathways known to be involved in development and retinal regeneration in other vertebrate systems. As adult mammalian neurons do not typically regenerate or proliferate, we conclude that PNU-282987 is somehow reversing this process. Our results will have important implications for uncovering mechanisms to induce dedifferentiation and subsequent regeneration of the mammalian retina.

M5039C A Transgenic Mouse Model for Understanding *cis* and *trans* Mechanisms of lncRNA *Jpx in vivo*. *S. Carmona*, B. Lin, T. Chou, S. Sun. University of California, Irvine, Irvine, CA.

Mammals experience a gene dosage imbalance between males and females due to their differing number of X chromosomes. The contrasting levels of X-linked gene products are resolved by X-Chromosome Inactivation (XCI), in which the long-noncoding RNA (lncRNA) *Xist* is activated in females and coordinates long-term gene silencing of one X Chromosome. *Jpx*, another lncRNA expressed from a locus just proximal to *Xist*, is able to activate *Xist* expression by binding to and removing CTCF protein from the *Xist* promoter. While a *Jpx* deletion is lethal in differentiated female mouse embryonic stem (mES) cells, *Jpx* overexpression causes ectopic *Xist* expression in both male and female mES cells. This project aims to understand how well our knowledge of *in vitro* lncRNA mechanisms remain true *in vivo* (in a live animal), specifically for *cis* and *trans* gene regulatory mechanisms. Thus, we used a BAC transgene to produce mouse lines overexpressing *Jpx* [Tg(Jpx)#Shsn], or *Jpx* and *Xist* together [Tg(Jpx, Xist)#Shsn]. We found that transgenic animals are viable, visibly normal, and fertile. Both male and female parents are able to transmit the transgene to their offspring; however, in certain lines we observed a significant lack of transgenic mice derived from the male parent. We also found that *Jpx* and *Xist* expression tend to increase together when located proximally to each other (in Tg (Jpx, Xist) mice), indicating that *Jpx* has a *cis* preference *in vivo*. Our data suggest that *Jpx* holds a consistent mechanism *in vitro* and *in vivo*, and that*Jpx* may have an unstudied role in spermatogenesis and imprinting.

M5040A The cytosine methylase DIM-2 and the H3K9 methylase DIM-5 mediate clustered mutation of repetitive DNA sequences in *Neurospora crassa*. *E. Gladyshev*, N. Kleckner. Harvard University, Cambridge, MA.

In somatic cells of mammals, plants and filamentous fungi, repetitive DNA is normally silenced in the form of heterochromatin with the contaminant occurrence of methylated cytosines (5mC) and methylated histone H3 lysine 9 residues (H3K9me2/3). The basis for the selective silencing of repetitive sequences has been proposed to involve aberrant products of DNA replication and repair, sequence-specific binding proteins, and RNA-based mechanisms. The pathway of heterochromatin assembly has been elucidated in particular detail in the filamentous fungus *Neurospora crassa*. In this organism, DNA methylation is established by DIM-2, a classical cytosine methylase, which is recruited to chromatin by the physical association with HP1 that recognizes H3K9me3 created by DIM-5, a Su(var)3-9 lysine methylase. Here we show that DIM-2 can promote clustered cytosine-to-thymine mutations in the germline (premeiotic) cells of Neurospora. This process is specifically dependent on the presence of repetitive DNA and requires DIM-5. Our previous studies have shown that repetitive sequences are designated for mutation in the absence of Rad51, and that this process involves the pattern of homology recognition expected for direct interactions between intact, co-aligned DNA duplexes. Taken together, our findings suggest that direct interactions between intact DNA duplexes may serve as the fundamental trigger of heterochromatin assembly on repetitive sequences. A mechanism for occurrence of clustered mutations is also of interest for the etiology of cancer, where extensive chromosomal duplications and clustered mutations are prominent features.

M5041B Histone H3R17me2a Mark Recruits TET3 to Initiate Active DNA Demethylation in mouse Zygotes. *Y. Hatanaka*[1*], N. Shimizu[2], K. Morita[2], M. Satoh[2], A. Honda[1,3], M. Hirose[1], S. Kamimura[1,4], N. Ogonuki[1], T. Nakamura[5], K. Inoue[1,4], Y. Hosoi[2], T. Nakano[6], K. Matsumoto[2], A. Ogura[1,4,7]. 1) RIKEN BioResource Center, Ibaraki, JP; 2) Kinki Univ., Wakayama, JP; 3) Miyazaki Univ., Miyazaki, JP; 4) Tsukuba Univ., Ibaraki, JP; 5) Nagahama Institute, Shiga, JP; 6) Osaka Univ., Osaka, JP; 7) Univ.of Tokyo, Tokyo, JP.

[**Introduction**]The parental genomes in zygotes undergo 5-methylcytosine (5mC) oxidation by a dioxygenase TET3, which converts 5mC to 5-hydroxycytosine (5hmC). However, its interacting proteins have not been identified yet. We have reported that maternal gonad specific expression gene (*1700011E24RIK* or GSE) is essential for 5mC oxidation in mouse zygotes. To understand how GSE protein is involved in 5mC oxidation, we sought to identify the maternal factors interacting with GSE in mouse zygotes. [**Methods**]To investigate the interaction of GSE-TET3 and identify its interacting protein, we performed yeast two-hybrid system using cDNA library of MII oocytes and ovaries and co-immunoprecipitation using PN3 zygotes. We performed immunostaining of 5mC and 5hmC and methylated DNA immunoprecipitation (MeDIP) and hydroxymethylated DNA immunoprecipitation (hMeDIP) analysis in *Line1* retrotransposon sequence using knockdown (KD) by injecting the siRNA and knockout (KO) zygotes at PN3 and 5 stages. We treated TBBD, a specific inhibitor of methylation at H3R17, in growing oocytes and collected zygotes by performing *in vitro* maturation and intra cytoplasmic sperm injection (ICSI).[**Results and Discusion**]We showed that GSE interacted with TET3 and maternal methyltransferase like 23 (METTL23), a novel arginine methyltransferase that catalyzed asymmetric

dimethylated histone H3 arginine 17 (H3R17me2a). Knockout of GSE or KD of METTL23 resulted in significant reduction of 5mC oxidation, as demonstrated by an increased 5mC level and a decreased 5hmC level in the male pronucleus. This was most likely due to deficient pronuclear localization of TET3. The impaired 5mC oxidation was confirmed by MeDIP and hMeDIP in *Line1* in zygotes derived from maternal GSE- or METTL23-KD. Treatment with TBBD reduced the H3R17me2a level in the both pronuclei, and caused higher 5mC and lower 5hmC levels in the male pronucleus. Thus, TET3-dependent 5mC oxidation in zygotes is regulated by specific histone modification H3R17me2a.

M5042C Downregulation of MBD2, a Mi-2/NuRD Chromatin Remodeling Complex Component, Potentiates Erythroid Terminal Differentiation and Hemoglobin Synthesis by Allowing the DNA Binding of TFCP2 (CP2c) TF Complexes. *Chul Geun Kim*[1], Min Young Kim[1], Ji Sook Kim[1], Hea Young Eum[1], Dae Hyun Ha[1], Seung Han Son[1], Mi-Ae Park[1], Yea Woon Kim[2], Eun Jung Baek[3], AeRi Kim[2], Ji Hyung Chae[1]. 1) Hanyang University, Seoul, KR; 2) Pusan National University, Pusan, KR; 3) Hanyang University, Guri-si, KR.

Regulation of erythropoiesis and globin gene expression had been intensively investigated, as an effort to improve the management of common but devastating genetic hemoglobinopathies, leading to breakthroughs for the understanding of regulation of eukaryotic gene expression. A heterohexameric ternary transcription factor (TF) complex of TFCP2 (CP2c), CYP27B1 (CP2b), and PIAS1 (CBP) binds to the α-globin promoter to induce transcriptional activation of α-globin in erythroid cells (Kang et al., Nucleic Acids res, 2010), while CP2c homotetramer is known to charge in most cases. We found that GATAD2A (p66α), a component of the Mi-2/NuRD chromatin remodeling complex (CRC), interacts with CP2c/CP2b per se and represses CBP-mediated α-globin expression by modulating both their DNA binding and cellular protein levels. Interestingly, the p66α protein level was not changed during erythroid differentiation *in vitro* and *in vivo* and, instead, MBD2/MBD3, other components of the Mi-2/NuRD CRC and known to directly interact with p66α, was dramatically reduced in their expression: the transcriptional activity of CBP is inversely correlated with expression levels of MBD2 and MBD3. We have studied how the interplay between CP2c TF complexes and Mi-2/NuRD members potentiates terminal erythropoiesis and globin gene expression using multi-disciplinary tools of methods, and found that MBD2/3 directly regulates p66α function to CP2c TF complexes and thus downregulation of MBD2 potentiates erythroid terminal differentiation and hemoglobin synthesis by allowing the DNA binding of CP2c TF complexes. Our findings open up a new mode of CRC action on transcriptional regulation in general and a molecular execution mechanism of erythropoiesis at the terminal stage by interplay between CRC and TFs, leading to transcriptional induction of globin gene expression.

M5043A A Targeting Small Molecule Inhibitor of MBD2-GATAD2A Interaction Induces Myeloid Leukemia Cell-specific Cell Death. *Chul Geun Kim*[1], Min Young Kim[1], Insung Na[2], Young Su Lim[1], Ji Sook Kim[1], Yu Chen[2], Arjan van der Vaart[2], Eun Jung Baek[4], Young Yeol Lee[3], Buom-Yong Ryu[5], Vladimir N. Uversky[2]. 1) Hanyang University, Seoul, KR; 2) University of South Florida. Tampa, USA; 3) Hanyang University College of Medicine, Seoul, KR; 4) Hanyang University College of Medicine, Guri-si, KR; 5) Chung-Ang University, Ansung, KR.

Mi-2/NuRD (Nucleosome Remodeling and Deacetylase) chromatin remodeling complex (CRC), an assemblage of proteins that combine key epigenetic regulators necessary for histone deacetylation and demethylation, is known to be an epigenetic reader of DNA methylation that regulates genes involved in normal development and neoplastic diseases. To our surprise, we have found that MBD2 and MBD3 are downregulated, with no disruption of the integrity of the Mi-2/NuRD complex, during terminal differentiation of MEL cells *in vitro* as well as normal erythropoiesis in the bone marrow. In further, we confirmed that MBD2 downregulation potentiates terminal erythroid differentiation and transcriptional activation of both α- and β-globin genes via recruitment of TFCP2 (CP2c) TF complexes to their promoters. In accordance with the previous report that MBD2 recruits the CHD4 nucleosome remodeling protein to the complex via a coiled-coil interaction with GATAD2A (p66α), spontaneous differentiation of MEL cells occurred by arbitral disruption of MBD2-p66α interaction or MBD2 knock down. Along with previous report that MBD2 knock out mice are viable and fertile, our data indicate that MBD2-p66α interaction is involves in gene silencing in general, but is dispensable in normal differentiation of some types of cells, i.e., erythroid lineage cells and also strongly suggest that disruption of MBD2-p66α interaction induces normal differentiation or cell death of some types of leukemia, like MEL cells which are arrested at the proerythroid stage. Based on this, we identified small molecules disrupting MBD2-p66α interaction using a computer aided molecular docking and tested their usefulness as an anticancer drug in various available cancer cell lines. We found that a drug 086567 shows a specific anticancer effect to myeloid leukemia cells with IC50 = ~20 μM, and also confirmed its anticancer effect in the mouse xenograft model of MEL cells. Mechanistically, this drug causes cell cycle arrest at various stages, leading to chromosomal aneuploidy and apoptotic gene expression.

M5044B Genome wide analysis of transcriptional profiles of *Usp22* mutant placentas reveal impaired cancer signaling cascades. *E. Koutelou*[1], A. Schibler[1], L. Wang[1], H.-P. Chao[1], X. Kuang[1], A. Salinger[1], Y. Lu[1], Y.-C. Chen[3], M. Wilson[2], B. Atanassov[1], D. Tang[1], S. Dent[1]. 1) UT-MD Anderson Cancer Center, Science Park, Smithville, TX; 2) UT-MD Anderson Cancer Center, Houston, TX; 3) Baylor College of Medicine, Houston, TX.

USP22, a component of the SAGA complex, is overexpressed in highly aggressive cancers, but the normal functions of this deubiquitinase are not well defined. We determined that loss of USP22 function in mice results in embryonic lethality between E12.5 and E14.0 due to defects in extraembryonic placental tissues that result in a failure to establish proper vascular interactions with the maternal circulatory system. These defects correlate with abnormal gene expression patterns that reflect defective receptor kinase signaling, including TGF-beta and RTK pathways. As these same pathways are often hyperactivated in cancer, our data provide the first insights to UsP22 functions that may be tied to oncogenesis.

M5045C Imprinted DNA methylation status can be reconstituted by combining activity of distinct *H19* ICR elements in mice. *H. Matsuzaki*, K. Tanimoto. University of Tsukuba, Tsukuba, Ibaraki, Japan.

Allele-specific DNA methylation at differentially methylated regions (DMRs) is critical for controlling imprinted genes expression and early embryogenesis in mammals. We previously demonstrated that the *H19* imprinting control region (ICR), a DMR in the *Igf2/H19* locus, was capable of acquiring paternal-allele-specific DNA methylation (imprinted methylation) when ectopically introduced into mouse genome (transgenic mouse; TgM). By using this genetic approach, we have dissected the *H19* ICR activity and identified a couple of *cis* elements essential for protecting the fragment from undesired methylation on the maternal allele. To test if these elements, when combined and introduced into heterologous CG-rich sequences, are sufficient to generate imprinted methylation status, we conducted reconstitution experiments. We employed a bacteriophage lambda DNA fragment as a scratch sequence, generated its TgM, and found that the fragment acquired DNA methylation regardless of its parental origin. Then, we introduced CTCF binding sites and Sox-Oct motifs of the mouse *H19* ICR into the lambda DNA (termed "LCb") and found that transgenic LCb fragment in mouse exhibited unmethylated state irrespective of their parental origin.

In this study, to search for *cis* elements essential for depositing paternal-allele specific methylation, we generated multiple TgM lines carrying a series of 5'-truncated *H19* ICR fragments and identified the 118-bp region as a candidate element carrying such an activity. When these sequences were internally deleted from the intact *H19* ICR transgene (2.9-kb) in mouse, the mutant fragment failed to acquire DNA methylation after paternal transmission. Finally, we appended the 118-bp sequences to the LCb fragment (termed "LCb118") and tested its ability to instruct imprinted methylation in TgM. The fragment became capable of acquiring DNA methylation only when paternally inherited. These results demonstrated that the 118-bp region was necessary and sufficient for acquisition of paternal allele-specific DNA methylation in TgM. Furthermore, combination of specific DNA sequences, rather than general property of the local DNA sequence, such as GC-content, determines identity of a DMR.

M5046A On the role of the epigenetic factor PRDM9 in meiosis of the wild mouse. *Z. Trachtulec*[1], P. Flachs[1], F. Pratto[2], C. L. Baker[3], R. Sedlacek[1], K. Paigen[3], R. D. Camerini-Otero[2], P. M. Petkov[3], O. Mihola[1]. 1) Division BIOCEV, Institute of Molecular Genetics ASCR, Prague, Czech Republic; 2) National Institute of Diabetes, Digestive, and Kidney Diseases, NIH, Bethesda, MD, USA; 3) Center for Genome Dynamics, The Jackson Laboratory, Bar Harbor, ME, USA.

The histone-3-lysine-4-trimethyl(H3K4me3)transferase PRDM9 (PR domain containing 9) determines the sites of the double-stranded breaks (DSBs) initiating meiotic recombination in the mouse, human, and cattle by binding at specific DNA sequences known as hotspots. The rapid evolution of the DNA-binding domain of the PRDM9-encoding gene is a response to the consequences of the meiotic paradox (the most often used hotspots disappear most quickly from the population) that leads to new hotspots. Deletion of the *Prdm9* gene from the laboratory mouse induces a shift of the DSBs to other H3K4me3 sites including promoters and a complete arrest of meiotic prophase I. Some organisms, such as dogs, birds, and baker´s yeast that lack PRDM9, have recombination hotspots that are conserved in closely related species and often located near promoters. We therefore investigated if PRDM9 is essential for meiosis in the wild mouse, using two types of *Prdm9* mutations and two wild-derived mouse strains. We phenotyped these mice by indirect immunofluorescent labeling of spread nuclei to assess synapsis and DSB repair, finding that mutant phenotypes depend on genetic background. To reveal hotspot positions, the products of anti-H3K4m3 and anti-DMC1 (a DSB-repair protein) testicular chromatin immunoprecipitations were sequenced and analyzed. We will present the phenotyping results of these *Prdm9*-deficient mice including the epigenomic analyses.

This work was supported by the Czech Science Foundation (14-20728S), the Academy of Sciences of the Czech Republic (RVO 68378050), the project BIOCEV (CZ.1.05/1.1.00/02.0109), and the Czech MEYS (LM2011032, LQ1604). CLB, KP, and PMP were supported by NIH P01 grant GM099640 to KP and R01 grant GM078452 to PMP.

M5047B Full length transcript sequencing of wild derived mouse strains identifies strain specific novel gene structures. *Monica I. Abrudan*[1], Anne Czechanski[2], Laura Reinholdt[2], Marcela Sjoberg[1], Thomas M. Keane[1]. 1) Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, GB; 2) The Jackson Laboratory, Bar Harbor, ME.

The mouse gene annotation is currently based on the C57BL/6J strain, whereas there is much less evidence for gene structures in genetically distant strains such as CAST/EiJ, PWK/PhJ, and SPRET/EiJ. These distant strains are important as they include founders for recombinant panels such as the Collaborative Cross (CC) and the Diversity Outbred Cross (DO). Short read RNA-Seq has previously been generated and used to explore the splicing landscape of these strains. Full length transcript reconstruction from short reads is difficult and there has not yet been a systematic effort to generate full length cDNA sequencing for the wild-derived strains. In this experiment, we generated matched long read Pacbio cDNA and Illumina RNA-Seq for two tissues (liver and spleen) and four strains (C57BL/6J, PWK/PhJ, CAST/EiJ, and SPRET/EiJ). The vast majority of the Pacbio reads contain full length transcripts. We find that spleen has a significantly richer transcriptional landscape. The PacBio cDNA reads capture on average 23% more unique protein coding genes in spleen than in liver, across all strains, despite the fact that there exist similar total numbers of reads in the two tissues. Using a conservative approach, in liver Pacbio cDNA we found 2760, 2812, 3352 and 3460 novel splice junctions in C57BL/6J, CAST/EiJ, PWK/PhJ, and SPRET/EiJ respectively. Intersecting the splice junctions extracted from the PacBio cDNA reads, we find 91 splice junctions specific to wild derived strains only, absent from GENCODE and from the C57BL/6J cDNA reads. From the analysis of the Illumina RNA-Seq reads, we find on average 1189 new splice junctions in each sample, across all strains, in liver and 1328 in spleen. Using the PacBio cDNA reads, we identify a potential new gene belonging to the PHD Finger family, specific to SPRET/EiJ, located on Chromosome 14. This novel transcript was also confirmed by the AUGUSTUS gene prediction pipeline. In a similar fashion, we identified a potential new isoform of a protein phosphatase, *Ppp2r3d*, also specific to SPRET/EiJ, that has also been predicted by AUGUSTUS.

M5048C Integration of heterogeneous cross-species functional genomics data in GeneWeaver.org *J. A. Bubier*[1], G. Sutphin[1], M. A. Langston[2], E. J. Baker[3], E. J. Chesler[1]. 1) The Jackson Laboratory, Bar Harbor, ME; 2) University of Tennessee, Knoxville, TN; 3) Baylor University, Waco, TX.

The use of model organisms to understand mechanisms of phenotypic diversity, development, aging, health and disease has been highly productive but faces challenges due to the unique nature of each model organism and its characteristics. The widespread application of whole genome functional studies and the diversity of sequenced genomes has created the critical mass of data necessary for efficient large-scale and cross-species data integration. The conservation of underlying pathways across species enables us to identify generalized mechanisms of disease. GeneWeaver.org is a database and suite of tools that allows users to integrate, query and analyze heterogeneous data from 10 supported species, with a variety of research applications. User submitted gene sets from individual or bulk uploads are seamlessly integrated and analyzed in light of a database containing sets of genes corresponding to Gene Ontology, Mammalian Phenotype Ontology, Comparative Toxicogenomics Database Chemicals, OMIM, MeSH annotations as well as pathway based gene sets from resources such as Pathway Commons, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Molecular Signatures Database (MSigDB). The available tools use statistical methods and graph-based algorithms to perform set-set matching operations on gene sets and network edges. The results are presented in a dynamic graphical output. As an example, we applied GeneWeaver to a set of 73 gene sets derived from diverse experimental types related to aging across six species (yeast, worm, fly, rat, mouse and human). The most highly connected gene among the cross species gene sets was identified as the tetraspanin transmembrane protein family member *tsp-7* (*Cd63* in mice). Experimental validation of *tsp-7* using bacterial-fed RNAi in *C. elegans* demonstrated a 10.2% extension of mean lifespan compared to empty vector (p=0.009, n=627). This example illustrates how aggregating experimental evidence of a variety of data types (differential RNA expression, QTL, proteomic, etc.) enables the discovery of novel genes common to conserved processes.

M5049A High throughput screening of International Knock-out Mouse Consortium leads to novel gene-phenotype annotations. *J. Clark*, C. Smith, K. Svenson, B. Braun, J. Eppig, E. Chesler. The Jackson Laboratory, Bar Harbor, ME.

The Jackson Laboratory

The Knock-Out Mouse Project and The International Mouse Phenotyping Consortium was formed to perform characterization of a comprehensive collection of mammalian genes through the systematic perturbation and phenotypic analysis of single gene disruptions performed by the International Knock-out Mouse Consortium. To date, The Jackson Laboratory has 444 fully characterized mutant strains examined with a wide range of phenotypic assays. Genes were prioritized by the research community, often based on information of phenotypic importance in one or more domain areas. However, the breadth of the phenotyping platform provides many additional new annotations even for these known genes. Each new phenotypic annotation generated by IMPC analysis is reported to Mouse Genome Informatics, and these annotations can be compared to existing information on known phenotypic alleles. A preliminary analysis of these annotations reveals that mutant strains generated and phenotyped by The Jackson Laboratory, 1478 phenotype annotations (or approximately 55% of the 2,674 total JAX strain phenotype annotations) for 243 unique genes were novel. Of the remaining 199 genes, 4,804 annotations were derived from MGI literature and submitted data curation, and 1,196 mostly novel phenotype annotations resulted from The Jackson Laboratory phenotyping pipeline. Additional analysis including targeted curation of genes and phenotypes characterized by the IMPC, and evaluation of the structural relations among annotations across phenotypic alleles will enable more precise quantitation of the knowledge gained by this consortium.

Support: NIH Grant# U54 HG006332.

M5050B New exome sequencing of wild derived inbred strains of mice significantly improves power to link phenotype and genotype. *Matt Dean*[1], Peter Chang[1], Emily Kopania[1], Matt Salomon[1], Lorraine Provencio[1], Rachel Mangels[1], Sara Keeble[1], Brent Young[2], Annie Orth[2], Francois Bonhomme[3], Jeffrey Good[1]. 1) University of Southern California, Los Angeles, CA; 2) Université Montpellier; 3) University of Montana.

The mouse has proven to be a powerful system to dissect the genetic and environmental influences on phenotypic variation, even informing the study of diseases in humans. In spite of a wealth of discoveries, most modern mouse strains are related to each other in complex ways, and have captured only a small fraction of genetic variation known to segregate in their wild progenitors. Wild-derived mice have the potential to increase power to connect phenotype to genotype by introducing novel single nucleotide polymorphisms (SNPs) to science. Here, we enrich exomes from 26 wild derived strains (the so-called Montpellier strains) and perform high throughput sequencing to an average coverage of 20 X. SNP's were called using an extremely conservative customized pipeline. We identified 1.14 million SNPs in our dataset, approximately 20% of which are not currently known in existing databases of mouse genetic variation. Simulations show that these new genetic variants increase mapping resolution. In addition, there are over 300 genes with an early stop codon segregating in the first 50% of the protein, providing potential alternatives to existing knockout resources. The novel genetic variation discovered here promises to increase the power of mouse genetics.

M5051C Deep genome sequencing and variation analysis of 13 inbred mouse strains defines candidate phenotypic alleles, private variation, and homozygous truncating mutations. *A. G. Doran*[1], K. Wong[1], J. Flint[2], D. J. Adams[1], K. W. Hunter[3], T. M. Keane[1]. 1) Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, GB; 2) The Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford OX3 7BN, UK; 3) Laboratory of Cancer Biology and Genetics, NCI, NIH.

Background

The Mouse Genomes Project is an ongoing collaborative effort to sequence the genomes of the common laboratory mouse strains. In 2011, the initial analysis of sequence variation across 17 strains found 56.7M unique SNPs and 8.8M indels. We carried out deep sequencing of 13 additional inbred strains (BUB/BnJ, C57BL/10J, C57BR/cdJ, C58/J, DBA/1J, I/LnJ, KK/HiJ, MOLF/EiJ, NZB/B1NJ, NZW/LacJ, RF/J, SEA/GnJ and ST/bJ) cataloging molecular variation within and across the strains. These strains include important models for immune response, leukemia, age-related hearing loss and rheumatoid arthritis. We now have several examples of fully sequenced closely related strains that are divergent for several disease phenotypes.

Results

Approximately, 27.4M unique SNPs and 5M indels were identified across these strains compared to the C57BL/6J reference genome (GRCm38). The amount of variation found in the inbred laboratory mouse genome has increased to 71M SNPs and 12M indels. We investigate the genetic basis of highly penetrant cancer susceptibility in RF/J finding private novel missense mutations in DNA damage repair and highly cancer associated genes. We use two highly related strains (DBA/1J and DBA/2J) to investigate the genetic basis of collagen induced arthritis susceptibility and identify several candidate genes and biological pathways with potential roles in collagen induced arthritis pathogenesis.

Conclusion

This study significantly expands the catalog of fully sequenced laboratory mouse strains and now contains several examples of highly genetically similar strains with divergent phenotypes. We show how studying private missense mutations can lead to insights into the genetic mechanism for a highly penetrant phenotype.

M5052A Functional annotation of proteoforms in the Mouse Genome Database using the Protein Ontology. *H. J. Drabkin*[1], K. R. Christie[1], C. N. Arighi[2], C. H. Wu[2], J. A. Blake[1]. 1) The Jackson Laboratory, Bar Harbor, ME; 2) University of Delaware, Newark, DE.

The concept of one gene/one polypeptide suggested in the 40's and 50's was dispelled in the late 70's with the discovery of splicing. A single eukaryotic gene can encode multiple protein isoforms due to the usage of alternate promoters or polyadenylation sites, alternative splicing of the primary transcript to generate different mRNAs, and/or selection of alternative start sites during translation of an mRNA. A protein can be further subjected to a single or multiple of post-translational processing including proteolytic cleavage as well as protein amino acid modifications. The functioning or cellular location of these different protein entities (proteoforms) can often be quite different.

The Protein Ontology (PRO, http://proconsortium.org) is a resource that supplies unique identifiers to specific proteoforms resulting from expression of a gene. These forms are organized in an ontological framework that explicitly describes how these entities relate. The ontology currently has over 68,000 isoforms and 2,200 modified proteoforms, which are either imported from high-quality sources or added via literature-based annotation by PRO curators.

The Mouse Genome Informatics (MGI, http://www.informatics.jax.org) is the international database resource for the laboratory mouse. It provides integrated genetic, genomic, and biological data to facilitate the study of human health and disease. MGI uses the Gene Ontology (GO, http://www.geneontology.org) for functional annotation of mouse genes. The GO defines concepts used to describe gene product functioning, location, and participation in biological processes, as well as relationships between these concepts. At MGI, when GO literature-based manual annotation applies to a specific proteoform this is indicated using PRO. These annotations are grouped according to the encoding gene, and can be displayed at MGI, as well as at the Amigo database of the Gene Ontology Consortium (http://amigo.geneontology.org/amigo). The annotations are also provided to the PRO website, where they can be viewed in the context of other proteoforms.

Supported by NIH Grants HG000330, HG002273, and GM080646.

M5054C Rapid evolution of co-amplified X and Y chromosome genes and genomic structures in mice. *A. Kruger*, J. Mueller. University of Michigan, Ann Arbor, MI.

The mammalian X and Y chromosome have diverged considerably since their evolutionary origins as autosomes. The mouse X and Y Chromosome, however, have undergone convergent evolution by independently acquiring and co-amplifying three gene families not present on the human sex chromosomes. Here we examine the evolutionary dynamics of these three X-Y co-amplified gene families and their genomic structures in rodents. We find that they were acquired via transposition and retrotransposition events followed by subsequent amplification. Our between species comparison of the three X-Y co-amplified gene families reveals they are among the most rapidly evolving genes on the rodent sex chromosomes. Moreover, comparison of their gene family members sequence divergence within mice highlights distinct protein regions under strong positive selection. Together with the rapid evolution of their protein-coding sequences, the genomic structures within which these gene families are harbored vary significantly in copy number. By tracking the evolutionary trajectory of the three X-Y co-amplified gene families we are gaining insights into the coevolution of their sequence diversification and massive amplification.

M5055A The future of reference assembly updates. *V. A. Schneider*, on behalf of the Genome Reference Consortium and NCBI Annotation Team. NIH/NCBI, Bethesda, MD.

An organism's reference genome assembly provides a standard coordinate system for reporting, a substrate for annotation and serves as the basis for analyses ranging from the study of individual genes to population genomics. As a result, the quality and content of a reference assembly is critical to research success. The Genome Reference Consortium (GRC) is responsible for updates to the mouse and zebrafish reference genome assemblies, including closing gaps, correcting path and sequencing errors and adding sequence to represent diversity. The chromosomes of the clone-based reference genome assemblies for these species each represent a single strain, C57BL/6J and Tü, respectively. However, due to inter-strain variation, some genomic regions are insufficiently represented by a single strain. Historically, the GRC has provided sequence representations for additional mouse strains at divergent regions with alternate loci scaffolds that are also comprised of finished, clone-based sequence. However, costs associated with mapping and sequencing genomic clones have limited this effort. As sequencing costs continue to fall and read lengths grow longer, though, ongoing and planned efforts by various investigators to sequence and assemble high quality genomes from different mouse and zebrafish strains provide the GRC with new opportunities to represent diversity and correct errors. We will present examples of recent curations to the mouse and zebrafish reference assemblies, as well as plans for curation in the context of multiple high quality assemblies. NCBI provides bioinformatics and database support for the GRC and annotates these reference assemblies, including the alternate loci, as part of its genome annotation pipeline. Annotation features include genes, RefSeq transcripts, genomic clone placements, repeats and genomic sequences not included in the assembly. Additionally, the GRC provides annotations that provide information about assembly quality and curation efforts. These annotations can be viewed in the NCBI Map Viewer, which permits the simultaneous display of genomic maps with different coordinate systems, as well as the Genome Data Viewer, a browser that supports the upload of user data, enabling it to be viewed alongside NCBI annotations. We will demonstrate how to use these browser resources to evaluate GRC-curated reference assemblies. The GRC welcomes public feedback on the mouse and zebrafish assemblies and displays information about regions under review on its website (http://genomereference.org).

M5056B Identifying genetic factors associated with extinction of strains in the Collaborative Cross. *J. R. Shorter*, F. Pardo-Manuel de Villena. University of North Carolina, Chapel Hill, NC.

The Collaborative Cross (CC) is a research population of inbred mouse strains derived from an eight way cross using five classical inbred strains and three wild-derived strains representing three subspecies. The CC is genetically diverse because it combines together the genomes of the eight strains; A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, NZO/HlLtJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ. This genetic diversity is essential for trait mapping studies, as classical inbred mouse strains can only capture a fraction of genetic variation found in the CC. However, this high level of genetic diversity may also act as a double-edged sword. The Dobzhansky-Muller model of hybrid incompatibility predicts that crosses between closely related species may produce inviable or infertile offspring. This is the result of allelic incompatibles across different loci that have evolved during the process of speciation. We observe an unusually high level of extinction in the CC strains, over 90%, which is much higher than comparable inbred reference panels. To investigate this high extinction rate, we are taking several different approaches. First, we will use living and extinct CC strains to find significant genotype ratio distortion sites that indicate allelic incompatibilities. We will also investigate genotype ratio distortion in the Diversity Outbred (DO) population that was created from 160 CC strains, but does not have the same infertility and inviability issues. We also will collect approximately 1000 pairs of productive and non productive mice that represent the last two generation of each extinct CC strain. This data set will be used in a mapping study to identify genetic factors that led to strain infertility. Finally, we will validate candidate allelic incompatibilities by using selective crosses of living DO and CC strains.

M5057C The genomes of *Mus caroli* and *Mus pahari* uncover the evolutionary dynamics of the mouse lineage. *D. Thybert*[1,2], The Caroli - Pahari genome consortium. 1) The Genome Analysis Center, Norwich, GB; 2) European Bioinformatic Institut, Hinxon-Camrbidge, GB.

The lack of genomes from intermediate species between the *Mus musculus* and the rat has hindered the study of genomic mechanisms involved in the divergence of the mouse from the other mammals. Here, we present the complete genome sequences of *Mus caroli* and *Mus pahari* which diverged 3 MYA and 6 MYA from *Mus musculus*. The genomes of these two species enable an analysis of evolutionary dynamics within the Muridae lineage that uncovers the mechanisms which fashioned the specificity of the mouse genome. For instance, our analysis shows both that a wave of inter-chromosomal rearrangement occurring between 3 and 6 MYA shaped the karyotype of the laboratory mouse ancestor and that genome integration of retrogenes has been increased in the *Mus* lineage. By taking advantage of the comparable divergence time of *Mus caroli* and *Mus pahari* from the laboratory mouse with chimp and gorilla from human, we can unravel the evolutionary mechanisms specific to *Muridae* from evolutionary mechanisms common to both mammalian clades to better understand the evolutionary mechanisms that shaped the mouse genome.

M5058A Genetic pest management technologies to control invasive rodents. *D. M. Kanavy*, D. W. Threadgill. Texas A&M University, College Station, TX.

Many strategies exist to manage invasive pests, ranging from poison to trapping, with varying degrees of success. Genetic technologies are increasingly being applied to insect pests, but not vertebrates. We propose to implement a genetic strategy to eradicate invasive mouse populations as another tool for pest control.

Mus musculus, the common house mouse, is one of the most widespread invasive species. Mice threaten human health, agriculture, and biodiversity on many islands, most specifically seabirds. The Farallon Islands are one example of a threat against biodiversity, with the ashy storm petrel being endangered by the predation of burrowing owls, which are attracted to the island due to the large population of invasive mice. Rodenticides are the most common method of eradicating mice, but their use leads to poisoning of non-target species and has limited

efficacy against mice. An approach that could eliminate non-target species disruption would be to combine genetically engineered mice, which for example produce a daughterless phenotype, with a gene drive system that could make the genetic technique self-sustaining. There are naturally occurring and artificial gene drive systems. For this project, we have investigated exploiting a naturally occurring gene drive, the t-complex. Using the t^{w2} haplotype of the t-complex, which is a full t-haplotype lacking the embryonic recessive lethality found in many other t-complex alleles, we observed the t^{w2} haplotype being transmitted to offspring with a transmission distortion ratio of 92.4%. We are currently exploring combining this natural gene drive system with genetic alterations that would produce daughterless offspring as an approach to effectively crash an invasive population without adversely affecting other species.

M5059B Unexpected translation reinitiation by on-target CRISPR-Cas9 genome editing. *S. Makino*, R. Fukumura, Y. Gondo. RIKEN BioResource Center, Tsukuba, Ibaraki, JP.

Translation initiation is emerging as one of the key mechanisms in regulation of gene expression. Translation is usually initiated from the first 5' ATG codon with the certain flanking sequence, e.g. Kozak sequence. In the presence/occurrence of nonsense mutation near 5' region in ORF, translation is sometimes reinitiated from a downstream inframe ATG codon, producing mutant protein lacking an N-terminal region. Such N-truncated protein has been reported as a responsible genetic factor for several human diseases. Some shortened proteins act as a hypomorphic protein and others act as a dominant negative protein. However, it has been known at all neither what extent translation reinitiation universally occurs nor how we may generally assess even if it may exist.

Here we show the concrete case of translation initiation *in vivo* and how to validate the event *in vitro* by using a dual-tagged expression vector. In the process of CRISPR-Cas9 genome editing, we have succeeded in introducing several out-of-frame indel mutations biallelically to knockout the endogenous *Gli3* gene. Unexpectedly, almost full length of the GLI3 protein was expressed in the homozygous mutants *in vivo*. The dual-tagged vector revealed that the expressed mutant GLI3 proteins lacked N-terminal residues but had intact C-terminal.

Thus, translation reinitiation indeed occurred *in vivo*. The *in vivo* assessment system with the dual-tagged vector is practically applicable for any genes, e.g., for the diagnosis of human diseases as well as for designing genome-editing vectors.

Moreover, translation reinitiation has been also found without mutations; e.g., upstream open reading frames (uORFs) regulate gene expression in response to environmental conditions. Thus, the time has come to investigate if in general translation reinitiation has a critical regulatory role of gene expression as a new paradigm in the central dogma.

M5060C Optimisation and high-throughput production of CRISPR/Cas9-mediated knockout mouse strains. *E. J. Ryder*, B. Doe, B. Rosen, J. Bottomley, G. Duddy, K. Boroviak, M. Thomas, E. Brown, D. Gleeson, D. von Schiller, D. Sethi, M. Woods, R. Ramirez-Solis, Sanger Mouse Genetics Project. Wellcome Trust Sanger Institute, Cambridge, GB.

The creation of targeted mutations by the use of CRISPR/Cas9 gene editing has revolutionised the process and possibilities of altering the function of genes in a wide variety of animals and plants. We describe the challenges of transitioning this technology into a high-throughput mouse production environment with the generation and characterisation of over one hundred new mutant mouse strains.

The majority of our CRISPR-mediated knockout strains are the result of whole exon deletions in which two guide RNAs are designed per flanking region to maximise the chance of a successful double-strand break. The gRNAs and Cas9 mRNA are introduced into 1-cell mouse zygotes by cytoplasmic injection and the G0 mice screened by a rapid PCR/qPCR method to determine the level of mosaicism and the percentage of putative deletion events. The mutation is then sequence-verified in the next generation to ensure that complete deletion of the exon has occurred.

We have found that the use of the CRISPR/Cas9 system has significant advantages in both the speed and level of generation of new strains, and has allowed us to significantly reduce the numbers of animals needed to achieve germline transmission.

M5061A Elevated canonical Wnt signalling disrupts development of the embryonic midline and may underlie cases of *ZIC3*-associated Heterotaxy. *R. Arkell*, A. Alzahrani, K. Diamand, J. Ahmed, K. Barratt. Australian National University, Canberra, ACT, AU.

Heterotaxy is a congenital abnormality where the internal thoraco-abdominal organs demonstrate abnormal arrangement across the left-right (L-R) axis of the body. It can affect the development of the heart, liver, lungs, intestines, and spleen. The L-R embryonic axis is established early in embryogenesis when unidirectional signals emanate from a specialised structure at the embryonic midline, called the node, to initiate distinct molecular pathways on the left and right sides of the developing embryo. The gene most commonly mutated in human cases of Heterotaxy is the X-linked *ZIC3*, but the mechanism by which the ZIC3 transcription factor prevents Heterotaxy remains unknown. A genetic screen for mutations that affect murine embryogenesis identified a novel null allele of *Zic3*, called katun (*Ka*). The mutant embryos exhibit Heterotaxy and also incompletely penetrant, partial (posterior) axis duplications and anterior truncation. These latter two phenotypes are redolent of elevated canonical Wnt signalling and analysis of *Ka* embryos reveals ectopic expression of direct targets of Wnt/β-catenin mediated transcription in mutant embryos. ZIC3 is a member of the Zic family of transcriptional regulators and previous work has shown that ZIC proteins can inhibit Wnt/β-catenin mediated transcription when overexpressed in cell lines. This raises the possibility that dysregulated Wnt signalling may contribute to Heterotaxy. We have investigated this notion by analysis of the murine batface (*Bfc*) gain-of-function allele of β-catenin that results in elevated Wnt/β-catenin signalling. We find this strain exhibits incompletely penetrant defects of L-R axis formation and synergises with the *Zic3 Ka* allele to produce an increased incidence of L-R axis defects. In both the *Ka* and *Bfc* strains, the node of homozygous embryos is misshapen and contains patches of non-ciliated cells that express endoderm genes. Moreover we find that human *ZIC3*-Heterotaxy associated mutations encode proteins that are defective in their ability to inhibit Wnt/β-catenin mediated transcription. Overall this provides strong evidence that Wnt dysregulation may underlie cases of ZIC3-associated Heterotaxy.

M5062B HOX proteins are essential for motor neuron subtype differentiation and connectivity by regulating the expression of *Ret/Gfra* genes. *C. Catela*[1], M. M. Shin[1], D. H. Lee[1], J. P. Liu[2], J. S. Dasen[1]. 1) NYU School of Medicine, New York, NY; 2) University of Virginia School of Medicine, Charlottesville, VA.

Precise regulation of gene expression is fundamental for the generation of neuronal diversity and circuit assembly during development. However, the transcriptional mechanisms that establish patterns and levels of gene expression in a given neuronal population remain largely elusive. Here we show that HOX/HOX cofactor interactions in motor neuron (MN) subtypes are essential for their specification and connectivity through regulation of the expression of *Ret/Gfra* surface receptors, essential for MN organization, axon branching and guidance. HOX and MEIS transcription factors control the levels of *Ret* and define the expression pattern of *Gfra1* and *Gfra3* in MN subtypes. Deletion of *Ret* and *Gfra3* in mice results in MN organization and innervation defects similar to those observed in HOX mutants. Finally, ectopic expression of *Ret* and *Gfra1* is sufficient to cause MNs to respond to limb derived signals, circumventing the necessity for *Hox* genes. These results show that one of the strategies of *Hox* genes is to regulate the levels and expression pattern of cell surface receptors, gating the ability of MNs to respond to limb signals. Future experiments will aim to uncover the full spectrum of effector genes downstream of HOX networks, providing novel insights into the transcriptional strategies employed by Hox proteins during MN subtype development.

M5063C Genetic studies of large mammalian sex chromosome palindromes harboring testicular germline genes. *Q. Ellison*, J. Mueller. University of Michigan, Ann Arbor, MI.

In placental mammals, the sex chromosomes are enriched for large (>10kb), nearly identical (>99% nucleotide identity), palindromes, which harbor genes expressed during testicular germ cell development. Despite their abundance, the molecular function of these palindromes has not been explored, in part due to their repetitive nature. Y chromosome palindromes are thought to have evolved to facilitate gene conversion between palindrome arms, allowing for the removal of deleterious mutations on the otherwise non-recombining Y chromosome. On the freely recombining X chromosome, an additional function for palindromes is suggested. I propose that the structure of large palindromes may facilitate transcriptional activation of their associated genes during testicular germ cell development. This proposition is based on the finding that genes associated with sex chromosome palindromes are expressed predominantly or exclusively in post-meiotic testicular germ cells, when single-copy X-linked genes are transcriptionally silenced. To determine whether palindrome structure is necessary for the expression of palindrome-associated genes in post-meiotic testicular germ cells, I am genetically dissecting two distinct mouse X chromosome palindromes using the CRISPR genome engineering technique to flip or delete single palindrome arms. In mouse lines I have generated that carry either inverted or deleted palindrome arms, I am currently in the process of measuring expression levels of palindrome-associated genes. I will also determine if there are defects in post-meiotic testicular germ cell development.

M5064A Embryonic Spacing in the C3H Mouse: A Model for Abnormal Pregnancies in Mammals? *S. A. Fletcher*[1], J. Dackor[3], D. W. Threadgill[1,2], D. S. Threadgill[1]. 1) Interdisciplinary Program of Genetics, Department of Veterinary Pathobiology, Texas A&M University, College Station, TX; 2) Department of Molecular and Cellular Medicine, Texas A&M University, College Station, TX; 3) 3 Department of Genetics, University of North Carolina, Chapel Hill, NC.

Previous research has indicated tightly regulated, even spacing of embryos during implantation is the conserved phenotype seen not only in the mouse and rat but also in other species such as the rabbit, pig, cow, sheep, and human. This critical event is described as embryos spaced evenly along each of the uterine horns of these species, and is an important factor in the health of both the pregnancy and the embryos. For some species this spacing can result in abortion, and in the case of cattle, a condition can occur in response to shared placentas in which sharing of placental vasculature by male-female twins can result in the masculinization of the female fetus. The importance of the proper spacing of embryos has also been suggested from human studies concerning ectopic pregnancies, placental previa, and cases where human fetuses sharing the same placenta are at increased risk of perinatal mortality and morbidity due to twin-twin transfusion syndrome, selective intrauterine growth restriction, and a higher incidence of congenital heart malformations.

The purpose of this study is to determine the genetic factors responsible for the uneven spacing in the C3H mice and its implications. This study utilizes both wild type C3HeB/FeJ (C3H) and C57BL/6J (B6) mice for the purpose of comparison, control, and matings. Wild type C3H mice display reproducibly uneven spacing of their embryos immediately before implantation while wild type B6 mice display the typical phenotype. The use of C3H/B6 recombinant inbred lines will allow us to look at which crosses are affected by uneven spacing and to identify candidate genes responsible for this phenotype through regional origination analysis of each line affected. Regulated contraction of the uterus is suggested to be critical to proper embryo spacing in placental mammals and knockout mice models for *Lpar3* and *Pla2g4a* demonstrated uneven embryo spacing.

Embryo transfers using the parental strains will allow for the determination of message origin, embryo-uterine cross talk, and give insight into the control of embryo spacing in the mouse. RNAseq on uterine tissue and embryos collected from parental strains before implantation will allow us to look at differing expression levels between strains and at the expression levels of potential candidate genes.

Finally, in our wild-type C3H mouse model, we will address the possibility of freemartins occurring in embryos with fused placentas as a result of the uneven spacing. Tattooing techniques will be utilized for identification, postnatal, of pups that share or have fused placentas *in utero*.

M5065B Role of a 3'UTR-dependent DAZL suppression in mouse postnatal ovary. *K. Fukuda*[1], T. Naka[3], A. Suzuki[3], Y. Saga[1,2], Y. Kato[1,2]. 1) SOKENDAI, Mishima, JP; 2) NIG,Mishima, JP; 3) Yokohama national Univ., Yokohama, JP.

Oogenesis is a crucial process for producing healthy female gamete, oocyte, in ovary. One of essential genes for oogenesis is *Dazl* (deleted in azoospermia-like) that is an RNA-binding protein implicated in translational promotion in mice. Because of its indispensable role in progression of meiosis Ⅰ in embryonic gonads and in progression of meiosis Ⅱ after ovulation, *Dazl* is thought to be required throughout oogenesis.

However, its role in oocyte growth in postnatal ovary remains unknown. To address this question, we analyzed *Dazl* expression by qPCR and western blotting. Surprisingly, we found that DAZL protein was decreased in postnatal ovaries, whereas the higher level of *Dazl* mRNA was maintained from embryo to juvenile ovaries. These results indicate that DAZL is post-transcriptionally suppressed after birth. We asked whether or not *Dazl* is suppressed via its 3'UTR in ovary. To investigate this hypothesis, we asked whether *Dazl* translation is suppressed via its 3'UTR or not by analyzing a bacterial artificial chromosome (BAC) carrying transgenic mouse line in which the *Dazl* 3'-UTR was flanked with Frt sequences followed by rabbit β-globin 3'-UTR. We crossed the BAC transgenic mice to Gt(ROSA)26Sortm2(FLP*)Sor (Rosa-Flp) mice to remove the *Dazl* 3'-UTR and investigated the DAZL expression. The results showed that DAZL expression was increased when the 3'–UTR was removed, suggesting that *Dazl* translation is suppressed in a 3'–UTR–dependent manner. In order to investigate the effect of excess DAZL on female reproduction, we examined the litter size of transgenic females. Interestingly, female mice expressing excess DAZL produced reduced number of offspring. These data indicate that 3'UTR–dependent suppression of DAZL in postnatal oocytes is important for female reproduction. These data also indicate the presence of a mechanism to suppress DAZL expression in the 3'UTR dependent manner.

M5066C SOX9 in developing heart valves and adult valve disease. *P. A. Hoodless*[1,3], V. C. Garside[1], M. Bilenky[2], R. Cullum[1]. 1) Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, CA; 2) Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, CA; 3) Dept. of Medical Genetics, University of British Columbia, Vancouver, BC.

Abnormal heart valve formation leads to a third of all cardiovascular birth defects. Valve defects can have detrimental effects on heart function and can lead to an increase in disease susceptibility. SOX9 is an essential transcription factor required in the development of the embryonic heart valves in mouse. Without its expression in this tissue, embryos have functional heart defects, fail to thrive, and die around E13.5. Previously, we identified the global transcriptional targets of SOX9 in embryonic day (E) 12.5 developing atrioventricular canal (AVC). Unlike many other transcription factors, SOX9 binding is heavily biased to the transcription start site and adjacent promoter regions. we have found that SOX9 has a universal role in regulation of cell cycle and proliferation genes across multiple cell types. Furthermore, analysis of heart ChIP-Seq libraries and transcriptome profiles on *Sox9*-deficient E12.5 heart valves revealed that SOX9 directly controls a critical network of transcription factors known to be involved in heart valve development. We have now generated SOX9 ChIP-seq data from E10.5 embryonic hearts, a time at which SOX9 expression is initiating in the AVC. In addition, we have used histone modification ChIP-Seq data to evaluate the chromatin landscape around the SOX9 binding sites. These histone modifications exhibit asymmetric patterns around the SOX9 site. Integration of data from the earlier time point (E10.5) builds a more complete picture of the regulation by SOX9. Additionally, from motif analysis of this data as well as proteomic analysis, critical factors bound with or to SOX9 in the developing valve have been identified. Our examination of the underlying mechanisms of SOX9 binding, gene expression changes when SOX9 is absent provides insights into the functional role that SOX9 plays in heart development.

M5067A Linear-(de)ubiquitination – a (uro)chordate specific mechanism - regulates Wnt signaling in the mouse. *S. Ivantsiv*[1,2], S. Almeida[1,2], R. Niibori[2], W. Dunham[1,2], A. C. Gingras[1,2], S. Cordes[1,2]. 1) University of Toronto, Department of Molecular Genetics., Toronto, ON, Canada; 2) Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital., Toronto, ON, Canada.

Ubiquitination is a post-translational modification that is crucial for many physiological processes and when disrupted, can lead to human diseases, including autism and neurodegenerative disorders. During ubiquitination, a covalent bond is generated usually between the carboxy-terminal amino acid of ubiquitin and a lysine in the target protein. Ubiquitin itself contains seven lysines that can undergo ubiquitination to form polyubiquitin "chains". An atypical linear ubiquitin chain (called Met1-Ub), which involves fusing the carboxy-terminus of one ubiquitin to the starting methionine of another, emerged in (uro)chordates. Only the linear ubiquitin assembly complex (LUBAC) is known to have linear ubiquitinating activity to date. We identified the first known dedicated linear deubiquitinase *Otulin (Gumby/Fam105b)*. Prior to our work, linear ubiquitination was only known to have postnatal roles in inflammation and adaptive immunity. By analyses of an allelic series of *Otulin* mouse mutants, we showed that linear (de)ubiquitination regulates facial nerve and craniofacial development and angiogenesis.

The *OTULIN* gene resides on human chromosome 5p15.2 (mouse Chromosome 15), deletions of which are associated with craniofacial deficits, intellectual disability and behavioral anomalies seen in Cri du Chat Syndrome (CdCS) patients. By pursuing an interaction between the Wnt signaling component disheveled 2 and OTULIN, we discovered a role for linear ubiquitination in modulating canonical Wnt signaling. In brief, linear ubiquitination inhibits, while deubiquitination (re)activates canonical Wnt signaling. Disruption of Wnt signaling can cause craniofacial anomalies, neural tube defects and neuronal dysfunction, and, thus, may lie at the heart of some CdCS symptoms.

To analyze the roles of linear (de)ubiquitination homeostasis in Wnt signaling and of relevance to CdCS, we have generated a conditional *Otulin* null mutant, which exhibits more severe neurobiological deficits than the extant *Otulin* point mutants. Moreover we have used AP-MS and the linear ubiquitin binding domain (UBAN) of the NEMO protein fused to glutathione-binding protein (UBAN-GST) to recover OTULIN client proteins. Using this approach we show that linear (de)ubiquitination of key Wnt signaling components controls their subcellular fate and thus regulates Wnt signaling.

This work highlights a new chordate-specific level of regulation of WNT signaling. Furthermore OTULIN belongs to a family of pharmaceutically targetable deubiquitinases and thus may present a novel target for modulating WNT dependent processes relevant to human disorders.

M5068B Sperm proteome maturation in the mouse epididymis. *T. Karr*[1], S. Skerget[2], M. Rosenow[3]. 1) Kyoto Institute of Technology, Kyoto, JP; 2) Arizona State University, Tempe, AZ; 3) Caris Life Sciences, Phoenix, AZ.

In mammals, transit through the epididymis, which involves the acquisition, loss and modification of proteins, is required to confer motility and fertilization competency to sperm. The overall dynamics of maturation are poorly understood, and a systems level understanding of the complex maturation process will provide valuable new information about changes occurring during epididymal transport. Using shotgun discovery proteomics, we report the proteomes of sperm collected from the caput, corpus and cauda segments of the mouse epididymis, identifying 1536, 1720 and 1234 proteins respectively. This study identified 765 proteins that are present in sperm obtained from all three segments. We also identified 1766 proteins that are potentially added (732) or removed (1034) from sperm during epididymal transit. Phenotypic analyses of the caput, corpus and cauda sperm proteomes identified 60 proteins that have known sperm phenotypes when mutated, or absent from sperm. Our analysis indicates that as much as one-third of proteins with known sperm phenotypes are added to sperm during epididymal transit. GO analyses revealed that cauda sperm are enriched for specific functions including sperm-egg recognition and motility, consistent with the observation that sperm acquire motility and fertilization competency during transit through the epididymis. In addition, GO analyses revealed that the immunity protein profile of sperm changes during sperm maturation. Finally, we identified components of the 26S proteasome, the immunoproteasome, and a proteasome activator in mature sperm.

M5069C The role of *Robo* genes during development of the intervertebral discs. *Lisa Lawson*, Lance Denes, Brian D. Harfe. University of Florida, Gainesville, FL.

Lower back pain is an often chronic and debilitating condition that poses an economic burden of 100 billion annually in the United States alone. Based on recent projections made by the Global Burden of Disease Study, lower back pain is now predicted to be the leading cause of disability worldwide. Degenerative disc disease is a known contributor to lower back pain. Despite its high lifetime prevalence and economic burden, few effective therapies exist to reverse discogenic back pain. One critical obstacle to disc regenerative therapy, which aims to restore disc function by replacing damaged cells with healthy cells, is that the ontogeny of intervertebral disc cells and the mechanisms by which the discs are formed are not understood. Using the mouse model system we identified potential roles for ROBO-SLIT signaling during development of the intervertebral discs. ROBO-SLIT signaling is known to mediate organogenesis of the brain, kidneys, and foregut through a chemotactic mechanism. The role of ROBO-SLIT signaling during vertebral column and intervertebral disc morphogenesis is unknown. Using molecular approaches, we have determined that *Robo* and *Slit* mRNAs are expressed in complementary patterns during mouse disc development. We found that in the absence of ROBO1 and ROBO2 receptors the vertebral column forms with aberrantly shaped intervertebral discs that contain enlarged annulus fibrosus cells. Lineage tracing analysis using the *Gt(ROSA)26Sor*$^{tm4(ACTB\text{-}tdTomato,\text{-}EGFP)Luo}$ (Rosa-mTmG) dual fluorescence reporter mouse showed that the disc phenotype was not caused by aberrant cell migration and/or cell mixing of nucleus pulposus an annulus fibrosis cells in the disc. Based on the dysregulated presence of *Ihh* and *Col10a1* mRNA in the vertebral growth plates of double *Robo* mutant mice, we propose that the observed disc morphology may result from disruption of ROBO-mediated growth plate dynamics in vertebrae adjacent to the forming discs.

M5070A Study of dendritic cell development *in vitro* and *in vivo* using immortalized hematopoietic stem and progenitor cells. *Chien-Kuo Lee*, Yi-Fang Fan, Ching-Yu Lu. National Taiwan University College of Medicine, Taipei, TW.

Dendritic cells (DCs) are specialized immune cells critical for regulating the innate and adaptive immune responses. While DCs, including conventional DC (cDC) and plasmacytoid DC (pDC) are short-lived and are constantly replenished from their progenitors of myeloid and lymphoid lineages, the study of developmental control of DCs is hampered by limited numbers of the progenitor cells. Here we immortalized hematopoietic stem and progenitor cells (iHSPC) by introducing an inducible *Hoxb8* gene into 5-fluorouracil (5FU)-treated mouse bone marrow. The iHSPCs were able to develop into pDC and cDC in response to Flt3 ligand (FLT3L) treatment. Moreover, the iHSPCs were capable of producing T, B, macrophages and granulocytes in addition to DCs when adaptively transferred into γ-irradiated recipient mice, suggesting that the iHSPCs retained their pluripotency for differentiation. We have previously shown that common lymphoid progenitors (CLPs) exhibited higher pDC potential than did cDCs. TLR-induced inflammation or influenza virus infection remodeled the developmental program by promoting cDC formation but inhibiting pDC generation *in vitro* and *in vivo* through upregulation of, *Id2*, a cDC-specific factor and downregulation of *Tcf4* and *Spib*, two pDC-specific factors. Interestingly, the iHSPCs also phenocopied the developmental features of primary CLPs in response to the same stimulation. Lentivirus-mediated RNA silencing in iHSPC had identified STAT3 and AKT as key signaling molecules controlling pDC development from the progenitors. Knockdown of either molecule resulted in increased cDC but reduced pDC potential from iHSPCs. A similar phenotype was observed in conditional knockout of STAT3 in primary progenitor cells. These results have demonstrated that iHSPC is a powerful tool to systemically investigate signaling events dictating DC homeostasis at steady-state and inflammation. Since malfunction or uncontrolled activation of pDCs is associated with autoimmune diseases, such as systemic lupus erythematosis and psoriasis, this study may provide potential therapeutic strategies to circumvent the diseases.

M5071B A novel hypomorphic smoothened allele results in impaired sonic hedgehog signaling and skeletal defects. *A. B. Long*, J. Ben-Ami, T. Caspary. Emory University, Atlanta, GA.

Smoothened (SMO) plays a key role in vertebrate hedgehog (Hh) signaling, which regulates patterning and proliferation in many tissues through three ligands: sonic (SHH), Indian and desert hedgehog. We have identified an autosomal recessive mutation in mouse *Smo* that causes impaired Hh signaling leading to defects in neural tube patterning and skeletal development. Our mutant, *Smo*^{m1Tc} (AW4), was isolated during a forward genetic *N*-ethyl-*N*-nitrosourea (ENU) screen. Using chromosome mapping and sequencing, we determined the mutation to be a conserved asparagine to lysine change at amino acid 223 of the SMO protein. Through analysis of the mutant embryos, we saw a moderate

dorsalization of the neural tube, consistent with decreased SHH signaling. We isolated fibroblasts from the AW4 embryos and observed decreased levels of target gene transcription after treatment with SHH-conditioned media. Curiously, we saw no SMO enrichment in cilia upon SHH stimulation of AW4 fibroblasts, as occurs in wildtype cells. SMO is a seven-transmembrane G protein-coupled receptor with an extracellular binding pocket. The AW4 mutation, located in the extracellular domain of SMO, is likely to alter the binding pocket of SMO. While *Smo*-null mutants die at E9.0, the AW4 mutant embryos survive until birth – permitting examination of SMO-dependent skeletal development, with a particular focus on craniofacial and limb structure, both of which are abnormal beginning at embryonic day 10.5. The mutant midface and maxilla are both hypomorphic with a moderate collapse toward the midline, and the phalanges are short compared to wildtype embryos. Taken together, our results indicate AW4 is a hypomorphic *Smo* allele and suggest that ciliary SMO enrichment is not absolutely required for pathway activation.

M5072C Mice mutant for *Cecr2*, which codes for a chromatin remodelling protein, show severe male subfertility that significantly improves with age. *Heather E. McDermid*, Chelsey B. Weatherill, Kenji Rowel Lim, Vivian V. Nguyen, Ross C. Humphreys, Kacie A. Norton. University of Alberta, Edmonton, AB, Canada.

Mammalian reproduction requires an exquisitely complex interplay of genes which must be regulated spatially and temporally to ensure both development of the gonad and successful fertilization of gametes. Part of this process depends on chromatin remodellers, which are able to affect nuclear processes such as gene transcription, DNA replication, recombination or repair through modulation of chromatin structure. Mutations in chromatin remodelling gene *Cecr2,* which is expressed in spermatogonial cells, result in an unusual form of male subfertility that is most severe at maturity and improves with age. This improvement is demonstrated by three different measures. First, mutant males (BALB/c-*Cecr2*$^{Gt(pGT1)Hemc}$/*Cecr2*$^{Gt(pGT1)Hemc}$) are least fertile immediately after sexual maturity (42-60 days). Within 2 months, litter sizes improve from 11.7% to 58.3% of the litter sizes of their wildtype brothers. Second, histological analysis reveals severe defects in the seminiferous tubules of newly mature males, including tubules that have very few cell layers and are not completing spermatogenesis. These defects become less prevalent and less severe with age, until testes at 3+ months appear close to normal in structure. Histological analysis of immature testes shows that mutant testes at 14 days are indistinguishable from wild type, and defects are first visible at between 19 and 21 days. Third, analysis of wildtype eggs, fertilized *in vivo* and collected 5 hours later, indicate that subfertility in mutants is due to fewer oocytes being fertilized. Mutants males aged 42-60 days show a fertilization rate of ~5% compared to their wild type brothers. At 60-100 days of age the mutant males show ~35% of the normal fertilization rate, and after 100 days the fertilization rate is not significantly different between mutants and wild types. Intriguingly, this partial rescue of the age dependent phenotype may be due to increasing levels of *Cecr2* transcript within the mutant testis, either from the previously characterized *Cecr2* transcript with a hypomorphic mutation, or from a novel transcript expressed from a newly discovered alternate exon 1. Determining how *Cecr2* causes subfertility in mice and the mechanism by which the phenotype improves with age will advance our understanding of the various roles that chromatin remodeling plays during spermatogenesis and fertilization.

M5073A Regulation of murine coat color by transgenic expression of endothelin 3. *Javier Pino*, Lidia Kos, Alexander Durango. Florida International University, Miami, FL.

The production of pigment involves several signaling molecules essential for the proper development and function of melanocytes. Lethal yellow mice (A^y) have a non-functional MC1R pathway leading to the production of pheomelanin in the hair. Doxycycline (dox) inducible transgenic mice that express *Edn3* under the keratin 5 promoter Tg(KRT5-Edn3)#Kosl showed hyperpigmentation of the skin and coat. Tg(KRT5-Edn3)#Kosl darkened the coat color of A^y mice. We hypothesize that EDN3 compensates for the absence of MC1R signaling by upregulating melanogenic genes. To test if continuous transgenic *Edn3* expression is required to maintain a dark pigmentation phenotype in A^y mice, dox was administered to newborn pups, deactivating transgenic *Edn3* expression. After 6 weeks of dox treatment, the coat color of A^y Tg(KRT5-Edn3)#Kosl mice was similar to those of A^y littermates. The comparative analysis of dorsal hairs from A^y and A^y Tg(KRT5-Edn3)#Kosl mice using high performance liquid chromatography showed that transgenic *Edn3* expression significantly increased both eumelanin and pheomelanin in A^y mice. The number of melanocytes in hair follicles of Tg(KRT5-Edn3)#Kosl mice as evidenced by immunofluorescence with an antibody against tyrosinase related protein 1 was similar to that of non-transgenic littermates. Gene expression analysis of the hair bulbs of A^y Tg(KRT5-Edn3)#Kosl mice showed that EDN3 up regulates the expression of melanogenic genes such as tyrosinase. Our results indicate that the paracrine expression of *Edn3* from keratinocytes is capable of generating and maintaining a dark coat color in the absence of a functional MC1R pathway by the regulation of melanogenic genes.

J. P. was supported by NIH/NIGMS R25 GM061347. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

M5074B Simulation of transient oscillatory *Neurog3* expression during pancreatic duct development. *G. K. H. Przemeck*[1,2], Hendrik B. Tiedemann[1], Elida Schneltzer[1], Johannes Beckers[1,2,3], Martin Hrabe de Angelis[1,2,3]. 1) Helmholtz Zentrum Muenchen, Neuherberg, Germany; 2) German Center for Diabetes Research (DZD), Neuherberg, Germany; 3) Technische Universitaet Muenchen, Freising, Germany.

During pancreas development, *Neurog3* positive endocrine progenitors are specified by Delta/Notch (D/N) mediated lateral inhibition in the growing ducts. During neurogenesis, genes that determine the transition from the proneural state to neuronal or glial lineages are oscillating before their expression is sustained. Although the basic gene regulatory network is very similar, cycling gene expression in pancreatic development was not investigated yet, and simulations of lateral inhibition in pancreas development excluded by design the possibility of oscillations. Here, we developed a dynamic 3D model of a growing duct that results in an oscillatory phase before the determination of endocrine progenitors by lateral inhibition. The basic network (D/N + Hes1 + Neurog3) shows scattered, stable *Neurog3* expression after displaying transient expression. Furthermore, examining network designs that were discussed in neurogenesis we included Hes1 negative

feedback and show the consequences for *Neurog3* expression in pancreatic duct development. A weakened HES1 action on the *Hes1* promoter allows the coexistence of stable patterning and oscillations. In this way, we argue for a unified mode of D/N mediated lateral inhibition in neurogenic and pancreatic progenitor specification.

M5075C Oscillatory Expression of cyclin A2 Requires the E2F Consensus Binding Site. *Jessica B. Rakijas*, Lindsey N. Kent, M. Cecilia Cuitino, Gustavo Leone. The Ohio State University, Columbus, OH.

The Rb-E2F pathway is a critical signaling axis that controls cell cycle transitions. The E2F family of transcription factors comes in two varieties: activators (E2F1-3) and repressors (E2F4-8). The RB tumor suppressor can repress E2F target gene expression through physical interaction with both E2F1-3 activators by inhibiting their transactivation function and E2F4-6 repressors by forming protein complexes that inhibit gene expression. The non-canonical E2F7 and 8 repress gene expression independent of RB. It is generally accepted that modulation of gene expression is a direct result of promoter binding by a transcription factor. As evidence, site-specific transcription factors, such as E2F, appear to require a consensus DNA binding sequence to assert their function. However, it is unclear how a transcription factor family with such varied mechanisms of action can regulate the same genes purportedly through the same DNA binding site. Thus, the purpose of this study is to test the limits of the assumption that all E2Fs require the presence of an intact DNA binding site to regulate target gene expression in a periodic fashion during the cell cycle and development.

We have taken an alternative approach to investigate the requirement of E2F-binding for transcriptional regulation of genes through the cell cycle in both mouse embryo fibroblasts (MEFs) and intact mouse tissues. To this end, we have generated a novel knock-in mouse of a critical cell cycle gene, cyclin A2 (*Ccna2)* by introducing a promoter mutation disrupting the established E2F binding site (STOCK-*Ccna2*^{tm2Gle}). In cycling cells, *Ccna2* expression is dynamic, increasing in late G1, peaks in early G2 and is shut off before the end of mitosis. In the developing liver, *Ccna2* expression is also dynamic, showing high expression during embryogenesis and early postnatal development, but is nearly undetectable by sexual maturity. Using real time PCR and western blot, we demonstrate that in knock-in MEFs and mouse liver, the expression of *Ccna2* RNA and protein is static over the cell cycle and developmental time, respectively. Additionally, we have shown using immunohistochemistry that mutant animals have higher liver macrophage infiltration than their wildtype counterparts, suggesting disruption to E2F binding affects tissue homeostasis in addition to ectopic expression of E2F target genes. We conclude that the E2F binding site in the *Ccna2* promoter is required for cell cycle- and developmentally-dependent oscillatory expression of *Ccna2*, yielding higher levels of inflammation in developing livers.

M5076A The cilia protein ARL13B regulates axon guidance in the mouse hindbrain. *S. K. Suciu*[1,3], L. E. Mariani[2,3], J. Ferent[4], J. Guo[5], E. S. Anton[5], F. Charron[4], T. Caspary[3]. 1) Genetics and Molecular Biology Graduate Program; 2) Neuroscience Graduate Program; 3) Emory University, Atlanta, GA 30322; 4) IRCM, Montreal, Quebec, Canada; 5) University of North Carolina, Chapel Hill, NC 27599.

Sonic hedgehog (SHH) signaling is a critical developmental pathway best known to regulate cell fate specification and cell proliferation. This occurs in the cilium through SHH activation of downstream effector smoothened (SMO) and GLI transcription factors. Additionally, SHH regulates axon guidance in the developing spinal cord and optic chiasm through a distinct, transcription-independent pathway. *Arl13b* encodes a small GTPase enriched in cilia that regulates transcription-dependent SHH signaling at the level of and downstream of SMO. Recessive mutations in *ARL13B* cause the ciliopathy Joubert Syndrome (JS), a disorder defined by intellectual disability, cerebellar hypoplasia, and physical deformities. JS is diagnosed by MRI-evidence of a hindbrain malformation known as the Molar Tooth Sign, which is caused in part by the white matter tracts called superior cerebellar peduncles (SCPs) failing to cross the midline of the brain. JS patients also exhibit midline crossing defects in the optic chiasm and failure of the corticospinal tract to cross the midline. Collectively, these phenotypes indicate JS patients display axon guidance defects; however, no known mechanism connects cilia genes such as *ARL13B* to the regulation of axon guidance. Because transcription-independent SHH signaling can regulate axon guidance, and *ARL13B* mutations lead to axon guidance defects in JS, we hypothesize that ARL13B also regulates transcription-independent SHH signaling to direct axon guidance in the developing brain. To test this hypothesis, we examined SCPs direction and uniformity in mouse brains lacking either SMO or ARL13B in projection neurons by performing diffusion tensor imaging (DTI) MRI. We showed that SCPs lacking SMO display significant midline crossing defects in the hindbrain, indicating axon guidance in these projection neurons is SMO dependent and suggesting SHH as a possible guidance cue. Furthermore, there is a midline crossing defect in SCPs lacking ARL13B, supporting our hypothesis that ARL13B regulates this transcription-independent SHH signaling pathway. Our ongoing work continues to investigate the role of ARL13B and SMO in SCP midline crossing through DTI and fluorescent tract tracing. Through these methods, we aim to define the role of ARL13B in transcription-independent SHH signaling and form a novel connection between ciliary genes and axon guidance.

M5077B Population Variability and The Teratogenic Effects of Exposure to 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin During Pregnancy. *M. R. Warren*[1,2], D. Threadgill[1,2]. 1) Texas A&M Health Science Center, College Station, TX; 2) Texas A&M College of Veterinary Medicine and Biomedical Sciences, College Station, TX.

Dioxin, formally known as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), is a well-known toxin that exhibits carcinogenic and deleterious effects on various tissues and organs. People are exposed to small amounts of this persistent environmental pollutant through everyday products (i.e. styrofoam cups) and common foods. Dioxin exposure during pregnancy is a particular concern as it heightens the risk of fetal malformations. While toxin susceptibility often varies among individuals due to genetic differences, current studies of the teratogenic effects of dioxin do not account for inter-individual variability when evaluating exposure risks. Our study aims to evaluate the effects of dioxin exposure on pregnant females and their embryos in genetically diverse mice to determine how genetic background impacts susceptibility.

According to the National Research Council (NRC), dose-response will linearize with non-cancerous endpoints even when accounting for genetic variability among the population. To test this prediction, we developed an *in vivo* study with a panel of mice that collectively mimic the

amount of genetic variability present in human populations. In this study, 36 strains of pregnant female mice are being exposed to increasing doses of TCDD (0.001, 0.01, 0.1, 1, 10, 50, 100 ng/kg/day) for a period of 10 days following mating. At day (D)10.5 post mating the mice are euthanized and organs (kidney, liver, spleen, brain, uterus, lungs) and embryos collected.

Initial data identified several strains, such as 129S1/SvlmJ and CC019, as "resistant" to all levels of exposure. These strains showed no significant differences in implantation trends or the stage of embryonic development in comparison to untreated controls. High dose exposure led to a significant decrease in implantation rate in non-resistant strains, such as C57BL/6J and CBA/J. High dose exposure of TCDD (50 ng/kg and 100 ng/kg, per day) in these mice caused a trend toward a decrease in the number of viable embryos and delayed development within certain strains.

The data has shown interstrain differences greatly impacts the level of response and the overall outcome of pregnancy during exposure to TCDD. Ultimately the study will determine whether dose responses are linear when analyzed on individual genetic backgrounds, as they are when performed on a combined, genetically mixed population.

M5078C The meiotic functions of aurora kinases during spermatogenesis in mice. *Stephen Wellard*[1], Alexandra L. Nguyen[2], Karen Schindler[2], Philip Jordan[1]. 1) Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; 2) Department of Life Sciences, Rutgers University, New Brunswick, New Jersey, USA.

Co-ordination of the dynamic chromosomal events during meiosis is critical to ensure accurate chromosome segregation for the next generation. Missegregation of chromosomes during meiosis can cause chromosomal aneuploidies, which result in miscarriage and genetic disorders such as Down syndrome. Previous studies conducted in budding yeast have implied that aurora kinases (AURKs) impact a number of critical events during the meiotic prophase I to metaphase I transition (G2/MI), including the disassembly of the synaptonemal complex (SC) and the faithful production of a bipolar spindle. While the events of meiosis are evolutionarily conserved, the mechanisms for controlling these events can differ markedly between distant species, complicating attempts to bridge findings in lower eukaryotes to humans. For instance, budding yeast expresses a single AURK protein, whereas mammals express three AURKs (AURK A, B, and C) with AURKB and AURKC showing structural and functional similarities. To delineate the meiotic roles of these three mammalian AURKs in mice, we have used a combination of chemical and genetic approaches to abrogate AURK activity at various stages of meiotic prophase I. By inducing wild type spermatocytes to undergo the G2/MI transition with okadaic acid (OA) in the presence of specific AURK inhibitors, we have determined a functional role for AURKB and AURKC in initiating desynapsis disassembly of the lateral element component of the SC, SYCP3. Cells treated with AURKB/C inhibitors failed to disassemble SYCP3 stretches and were unable to complete the G2/MI transition. This phenotype was not observed in cells treated with a specific AURKA inhibitor. To gain further insight in the functions of AURKB and AURKC during spermatogenesis, novel STOCK-*Aurkb*$^{tm2.1Mama}$ Tg(Spo11-cre)1Rsw or Tg(Hspa2-cre)1Eddy conditional knockout mice and B6;129S5-*Aurkc*^{tm1Lex} knockout mice were characterized. Despite defects being observed in histological sections of testes as well as aberrancies in spindle morphology of metaphase cells, both AURKB and AURKC mutant mice were fertile. This work supports the meiotic specific function of AURKB and AURKC in the disassembly of the SC during the G2/MI transition, and implies that these two kinases may play functionally compensatory roles in mammalian meiosis. Our current work is focusing on assessing STOCK- *Aurkc*^{tm1Lex} , *Aurkb*$^{tm2.2Mama}$ double knockout mice to further evaluate the meiotic specific functions of the aurora kinases, and improve our understanding of meiotically derived human disorders such as defects that can result in infertility, miscarriage, and developmental abnormalities.

M5079A Epithelial development of pharyngeal arches and intestine requires a member of S100 protein. *S. Xie*[1,2], J. Li[1,2], T. Zhong[1,2]. 1) State Key Laboratory of Genetic Engineering,Fudan University, Shanghai, China; 2) Collaborative Innovation Center of Genetics and Development, Fudan University, Shanghai, China.

Although S100 calcium binding (S100) proteins are unique calcium-binding proteins with time-dependent patterns of expression, their roles in vertebrate development and organogenesis remain unknown. Here we identify that a member of *s100a* subfamily, is necessary for epithelial development of the pharyngeal arches and the gut tube. We generated transgenic zebrafish in which the expression of enhanced green fluorescent protein (EGFP) is under the control of a 4 kb upstream regulatory element of *s100* gene. The EGFP positive cells widely distribute along the surface of the transgenic embryos during early development and then are restricted to the epithelial layer covering pharyngeal arches, olfactory placodes and the gut tube. The EGFP positive cells in the intestinal epithulium display polarized enteroendocrine cell morphology and produce pancreatic peptide. We show that knockdown using morpholinos (MOs) of the *S100a* gene causes curved embryo bodies, reduction of *fgf3* expression and loss of posterior pharyngeal arches. Intestinal endocrine cells expressing *nxk2.2a*, a homeodomain transcriptional factor marking enteroendocrine cells, are absent in the *s100a* gene deficient embryos. Moreover, knockdown of *nkx2.2a* abolishes a subset of the *s100a* subfamily-expressing entroendocrine cells, whereas reducing the *s100a* subfamily in *nkx2.2a*-deficient embryos completely eliminates these enteroendocrine cells, revealing a regulatory complexity during enteroendocrine cell formation. We also established the germline mutant of the *s100a* gene using transcription activator-like effector nuclease (TALEN). All these findings thus establish the first known roles for S100 proteins during vertebrate development, and provide biological insights into the roles of epithelial patterning in development of the pharyngeal arches and the intestine.

M5080B Establishing bipotentiality for gonadal differentiation. *Y. Yang*, S. Sorensen, M. J. Wilson. University of Otago, Dunedin, NZ.

The distinction between sexes is one of the most obvious example of morphological dimorphism in the animal kingdom, that highlights one of the most crucial fate decisions made *in utero*; to become a male or female. In order for sexual development to occur, the formation of gonad anlagen is first required. Mammalian gonads are unique among the animal kingdom as they arise from a bipotential progenitor gonadal tissue called the urogenital ridge (UGR). However, very little is known about how the molecular networks that shape its formation and the molecular

preparations made to allow for two developmental trajectories. The LIM-homeobox gene, *Lhx9* is among only a handful of genes known to be required for UGR formation, but it's regulatory network, and that of the UGR is poorly understood. In order to investigate the molecular underpinnings involved in UGR formation, we took a large-scale approach using chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) to identify genes involved in this process.

ChIP-seq on mouse UGRs was preformed for LHX9 to target regions in the chromatin where LHX9 regulates. In addition ChIP-seq was also performed on two histone modification marks, H3K4me3 and H3K27me3, which highlight regions of active and repressive transcriptional states respectively. Regions in the genome where LHX9 or the histone marks were found were validated using ChIP-qPCR and *in situ* hybridization.

Targets of LHX9 validated by ChIP-qPCR were found to be required for processes such as sex determination, sexual differentiation, cell proliferation, angiogenesis and cell migration. In addition, several other targets whose expression patterns in the UGR were not previously characterized was determined by *in situ* hybridization. Furthermore, looking at both histone mark ChIP-seq datasets, many genes were found to possess a 'bivalent' histone modification dynamic, whereby both H3K4me3 and H3K27me3 were found in the promoter or enhancer regions. This histone dynamic has been characterized as a feature that highlights certain lineage regulatory genes, holding them in a 'poised' transcriptional state. In particular, many genes involved in the *Wnt* signaling pathway were identified to possess bivalent histone marks. Several genes of the *Wnt* family were validated via ChIP-qPCR. Bringing both ChIP-seq datasets together, we provide a wider scope of the transcriptional and epigenetic regulatory network that is necessary for UGR formation, but also the preparation for sexual development.

M5081C Embryonic lethality in mice expressing conditionally-stabilized *Ctnnb1* under control of Tg(Vil-cre)997Gum. *E. Yusi*[1,2], R. Lynch[2], D. Threadgill[2]. 1) University of Surrey, Guildford, United Kingdom; 2) Texas A&M University, College Station, TX.

CTNNB1 (beta-catenin), which is degraded through a ubiquitin-dependent mechanism, is stabilized upon activation of the WNT pathway and functions as a transcriptional co-activator of WNT-responsive genes. Some cancers like colorectal cancers develop dominant acting mutations in *Ctnnb1* that lead to constitutive WNT signaling and cellular transformation. In this study, originally designed to develop a model of CTNNB1-induced colon cancer, we observed that mice carrying both a conditional stabilizing mutation of the *Ctnnb1* gene (*Ctnnb1*^{tm1Mmt}, hereafter called *Ctnnb1*$^{F(Ex3)}$) and a Cre recombinase whose expression is under the control of the murine villin 1 promoter (Tg(Vil-cre)997Gum/J, hereafter called Vil-cre) believed to be specific to the colon and kidney die during embryonic development. To isolate the time point of embryonic death, pregnant dams were euthanized and their embryos dissected at various time points, starting with E10.5. All embryos were inspected for gross abnormalities, and PCR was used to determine the genotype of the embryos. We found that while no double-heterozygous mice were observed neonatally, the litters dissected at E10.5 were developmentally normal and exhibited Mendelian inheritance ratios, suggesting the observed embryonic lethality was not due to preimplantation defects as has been reported for *Ctnnb1* null embryos. However, an increase in reabsorption sites was noted at E13.5 and some remaining embryos exhibited delayed development. These abnormal embryos were found to carry both the *Ctnnb1*$^{F(Ex3)}$ and Vil-cre alleles. These results are suggestive of placental defects. Identifying the molecular mechanisms underlying the cause of embryonic lethality will allow us to better characterize the role of canonical WNT signaling in placental development. Furthermore, the *Vil1* gene has been implicated in gut development and is known to be expressed as early as E9, but has yet to be involved in placental development. Ongoing efforts are focusing on the determining the exact cause of embryonic lethality in *Ctnnb1*$^{F(Ex3)}$), Vil-cre double mutants.

M5082A Post-transcriptional regulation of mouse neurogenesis by pumilio proteins. *M. Zhang*, D. Chen, J. Xia, W. Han, G. Hermes, N. Sestan, H. Lin. Yale University, New Haven, CT.

Mammalian neurogenesis is a key aspect of embryogenesis and persists throughout lifetime. Extensive studies have discovered various regulators of neurogenesis. But little is known about the post-transcriptional regulation that delivers much more rapid and subcellularly localized control of gene expression.

The purpose of this project is to study the post-transcriptional regulation of mouse neurogenesis by two RNA binding proteins, pumilio (PUM) 1 and 2—the two murine members of the evolutionarily conserved PUF protein family. This family is known to mediate post-transcriptional regulation. PUF proteins are required for germline stem cell maintenance in *Drosophila* and *C. elegans* and for spermatogenesis in mice, but their function in neurogenesis has not been well established.

To investigate the function of PUM1 and PUM2 in neurogenesis, we generated double conditional knockout mice by crossing *Pum1* and *Pum2* double loxP mice (with loxP sites flanking certain exons) with a mouse line carrying a Cre-expressing transgene driven by the nestin promoter. PUM1/2 are depleted by the Nes-cre in the entire central nervous system by E15.5. In these mice, we observed severe atrophy of the dentate gyrus (DG) in neonatal and adult brains along with drastically increased apoptosis in neonatal brains. This is very exciting as adult neurogenesis occurs in DG to generate new neurons for the hippocampus--the learning and memory center. Consistent with this phenotype, our behavioral tests revealed that *Pum* mutants are largely impaired in learning and memory. Using markers for different cell types along neurogenesis we found that removal of PUM1 and PUM2 led to increased EOMES (Tbr2)-positive neuronal progenitors but decreased DCX-positive immature neurons. Moreover, the cultured neural stem cells from the double knockout DG are deficient in forming neurospheres and display defects in proliferation and survival. All these lines of evidence suggest that PUM1 and PUM2 together regulate neurogenesis by preventing immature differentiation and apoptosis, which sustains the homeostasis of newborn neurons.

To identify PUM target mRNAs and downstream pathways, we performed iCLIP (cross-linking immunoprecipitation) using PUM1/2 antibody and neonatal brain lysates. The RNA targets and binding sites are identified; they are involved in pathways that are crucial to neurogenesis such as cell proliferation, differentiation, apoptosis, and cytoskeleton.

Overall the results have shown that PUM1 and PUM2 together play a vital role in regulating mouse neurogenesis at post-transcriptional level by regulating their RNA targets in various pathways.

M5083B Wnt/beta-catenin signaling modulates cytoskeleton dynamics to direct mammalian neural tube closure. *C. Zhou*, T. Zhao, Q. Gan, R. Lassiter, Y. Wang, A. Stokes. University of California Davis, School of Medicine, Sacramento, CA.

We have recently demonstrated that neuroepithelial beta-catenin is required for caudal neural tube closure by regulating critical downstream transcription factors *Pax3* and *Cdx2* in the dorsal neural folds. Our unpublished data demonstrate that conditional ablation of a key WNT coreceptor LRP6 in neuroepithelial cells of the dorsal neural folds also causes spina bifida with diminished expression of *Pax3* and *Cdx2*, which are similar as seen in the neuroepithelial beta-catenin mutants. We demonstrate that genetic activation of beta-catenin in the dorsal neural folds can rescue neural tube closure defects in the conditional *Lrp6* mutant mice. These results demonstrate a previously undetermined role of canonical Wnt/beta-catenin signaling pathway in caudal neural tube closure. To further address the roles of Wnt/beta-catenin signaling in neural tube closure and underlying mechanisms, we have generated novel mutants by conditional gene-targeting in the non-neural surface ectodermal cells. Our preliminary results demonstrate that surface ectodermal Wnt/beta-catenin signaling is required for the closure processes of the entire neural tube, which may mainly act through regulation of F-actin-based cytoskeleton dynamics. Because beta-catenin is also a cell adhesion molecule, we have preliminarily addressed the undetermined role of cell adhesion in neural tube closure. Our results reveal novel mechanisms underlying mammalian neural tube closure, which may provide a basis for better understanding and addressing the cause and prevention of neural tube closure defects in humans.

M5084C An inbred *Tp53* rat model exhibits a tumor spectrum similar to human Li-Fraumeni syndrome. *J. Amos-Landgraf*[1], S. Hansen[1], M. Hart[1], S. Busi[1], K. Jones[2], E. Bryda[1]. 1) University of Missouri, Columbia, MO; 2) Huntsman Cancer Institute, Salt Lake City, UT.

Somatic mutations in the *TP53* tumor suppressor gene are one of the most commonly observed genetic alterations in cancer, and germline mutations in *TP53* result in Li-Fraumeni syndrome and predispose individuals to a variety of early-onset cancers. Current *Tp53* mouse and rat models have significant phenotypic and genetic limitations and often do not recapitulate the certain aspects of human disease. The majority of *Tp53* rat mutants are maintained as outbred stocks, potentially contributing to the variability of the phenotypes. We used a marker-assisted speed congenic approach to transfer a well-characterized *Tp53* mutant allele from an outbred Sprague Dawley (SD) rat stock to the genetically inbred Fischer 344 (F344) rat to create the F344-*Tp53*$^{tm1(EGFP\text{-}Pac)Qly}$/Rrrc rat strain. On the F344 genetic background the tumor spectrum shifted, with the primary tumor types being osteosarcomas (32%) and meningeal sarcomas (32%), compared to the hepatic hemangiosarcoma (80% incidence in homozygotes) and lymphoma (55% incidence in heterozygotes) identified in the original outbred stock model. The F344 model is more consistent with the early onset of bone and central nervous system sarcomas found in humans with germline *TP53* mutations. Osteosarcomas represented 36% and 31% of tumors that developed, in homozygous and heterozygous animals, respectively, and were highly representative of the human disease radiographically and histologically, with tumors harbored primarily on long bones with frequent pulmonary metastases.

We crossed the F344-*Tp53*$^{tm1(EGFP\text{-}Pac)Qly}$/Rrrc knockout rat to the F344/NTac-*Apc*^{Pirc} model of familial colon cancer to determine the effect on intestinal and extracolonic cancer development. There was no significant change in tumor multiplicities between any of the genotypic classes; however, there was a reduced number of *Tp53* homozygous mutants than expected and no female homozygous mutants were recovered.

The F344-*Tp53*$^{tm1(EGFP\text{-}Pac)Qly}$/Rrrc knockout rat is a powerful model to investigate compelling questions surrounding the development of osteosarcomas and meningeal sarcomas in the context of an inbred genetic background allowing for easy maintenance of the model and introgression of additional mutations that already exist in other strains on the F344 background. Importantly, the rapid onset of osteosarcomas and the unique lammellar bone structure in the rat compared to mice fills a current void in animal models that recapitulate human pediatric osteosarcomas and may facilitate studies to identify therapeutic targets.

M5085A *Arl13b* is a Novel Target for the Treatment of Medulloblastoma. *S. N. Bay*[1,3,8], B. D. Brown[2,4,8], J. Wen[2,8], R. C. Castellino[2,5,6,7,8], T. Caspary[1,8]. 1) Department of Human Genetics; 2) Department of Pediatrics; 3) Genetics and Molecular Biology Program; 4) Cancer Biology Program; 5) Aflac Cancer and Blood Disorders Center; 6) Children's Healthcare of Atlanta; 7) Winship Cancer Institute; 8) Emory University, Atlanta, GA 30322.

Medulloblastoma is a tumor of the cerebellum and is the most common malignant brain tumor of childhood. Current therapies, while effective, introduce severe negative consequences to patients, and new molecular approaches are needed. Overactivated sonic hedgehog (SHH) signaling causes about 30% of medulloblastomas. In vertebrates, SHH signaling requires the primary cilium, and pathway members – including SHH's receptor patched1 (*Ptch1*), obligate pathway transducer smoothened (*Smo*), and Gli family transcription factors – dynamically traffic in and out of cilia. Mutations in SHH pathway genes (including *Ptch1* and *Smo*) cause medulloblastoma, but Gli repressor has been shown to be protective against medulloblastoma formation. We study a ciliary GTPase called *Arl13b* that uniquely regulates SHH, both at the level of SMO and downstream of SMO, where it regulates Gli activator but not Gli repressor. Loss of *Arl13b* results in ligand-independent constitutive low-level pathway activation but prevents maximal signaling and spares Gli repressor, making ARL13B an attractive target for developing therapies. We hypothesized that the loss of *Arl13b* could reduce the high levels of pathway activation responsible for SHH-derived medulloblastoma formation while leaving Gli repressor intact, without ablating pathway activity completely. Here, we use a conditional allele of *Arl13b* in the mouse to show that ARL13B plays a role in cerebellar development through the regulation of proliferation and that its loss dramatically impacts SHH pathway output in a variety of conditions. Deletion of *Arl13b* effectively reduces signaling levels in the presence of constitutive pathway activation driven by an oncogenic form of SMO, and knockdown of *ARL13B* in human medulloblastoma cell lines reduces their clonogenic potential. Taken together, our *in vivo* and *in vitro* studies demonstrate that ARL13B is a novel target for the treatment of medulloblastoma.

M5086B Determining the significance of space radiation exposures: high resolution genomic mapping to determine overlap in susceptibility loci for HZE-ion induced, γ-ray induced, and spontaneous phenotypes in outbred mice. *E. F. Edmondson*[1], J. Raber[2], O. Iancu[2], D. Gatti[3], E. Garcia[1], N. Kleiman[4], D. Kamstock[1], C. Fallgren[1], M. M. Weil[1]. 1) Colorado State University, Fort Collins, CO; 2) Oregon Health & Science University, Portland, OR; 3) The Jackson Laboratory, Bar Harbor, ME; 4) Columbia University, New York, NY.

Cancer risk from galactic cosmic radiation exposure is considered a potential "showstopper" for a manned mission to Mars. Calculating the actual risks that will be confronted by spaceflight crews is complicated by our limited understanding of the carcinogenic effects of high charge, high energy (HZE) ions, a radiation type for which there are no human epidemiological data. Here, we examine some of the assumptions underpinning the current NASA model used to assess space radiation cancer risk.

To study the effects of HZE ion irradiation in a genetically heterogeneous population, 1850 HS/Npt stock mice of both sexes were genotyped for 77,808 SNPs and exposed to (1) 0.4 Gy of 240 MeV/n ^{28}Si ions or (2) 600 MeV/n ^{56}Fe ions, (3) 3 Gy of ^{137}Cs γ-rays, or (4) sham irradiated. The mice were monitored for cataractogenesis, cognitive deficits, and cancer development until they reached 800 days of age or became moribund. Comprehensive necropsies were performed on each mouse and on all organ systems. Each detected lesion was characterized histologically. Genome reconstructions, which provide the basis for genome-wide SNP imputation, were completed for each mouse using algorithms for probabilistic assembly of founder haplotypes. Polygenic covariance among related individuals was corrected for during quantitative trait loci (QTL) mapping using a kinship term and significance thresholds were determined with permutation tests. To appreciate shared susceptibility loci for multiple neoplastic phenotypes following exposure to HZE ions or γ-rays, LOD scores from each QTL were plotted as heatmaps and hierarchically clustered.

The spectrum of tumors induced by accelerator produced HZE ions is similar to the spectra of spontaneous and gamma-ray-induced tumors, though the proportions of specific tumors differ. Quantitative trait loci (QTL) controlling susceptibilities to spontaneous, γ-ray-induced, and HZE ion-induced tumors are identified, and cluster analyses based on coincident loci point to share susceptibility loci for specific tumors types regardless of their origins (how they were induced). Malignancy, as measured by decreased latency or increased metastatic density, is comparable for radiation-induced and spontaneous tumors. Female mice are at a greater risk for radiogenic tumors than male mice. These findings support the assumptions underlying the current model used by NASA to estimate fatal cancer risks from space radiation exposures.

M5087C Complex genetic regulation of immune cell composition and activity in a genetically variable population. *M. T. Ferris*, A. C. Whitmore, A. P. Morgan, C. R. Morrison, D. R. Miller, F. Pardo-Manuel de Villena, M. T. Heise. Univ North Carolina, Chapel Hill, Chapel Hill, NC.

Immune cell populations represent a first line of defense against a variety of pathogens and immune insults. The composition of these cell populations within a given organ system determines the type and fit of potential responses. Here we utilize a set of approximately 100 F1 crosses between Collaborative Cross recombinant inbred lines to assess both variation in as well as genetic control of immune cell composition and activity levels in the lungs of unperturbed animals. We identified 30 cell populations within the lungs of these animals, and were able to identify 9 significant and 12 suggestive QTL regulating individual cell populations. We conditioned on these QTL loci, and were able to identify additional loci driving the composition of the immune cell population. Furthermore, we identified a population of immune cells who are only present in a subset of strains. Multiple genetic loci contribute to the presence of this population, and C57BL/6J alleles drive the absence of this population, suggesting that this commonly used mouse strain does not have this population of cells. In order to validate the underlying relationships between various cell populations, as well as those genetic loci we have identified, we conducted an analysis of immune cell populations from 100 Diversity Outbred animals. Our results highlight both the strong and complex genetic control of basal immune compositions, as well as the importance of studying immune cell populations across a range of genetically diverse models.

M5088A Evaluation of premetastatic niche formation in a mouse model of spontaneous melanoma lung metastasis. *J. Freitas*, J. Palmer, J. Sportsman, L. Kos. Florida International University, Miami, FL.

The deadliest trait of cancer cells is their capacity to colonize other sites of the body during a complex process called metastasis. Metastasis is the ultimate cause of death in 90% of patients with cancer, and there are still many remaining gaps in our understanding of metastasis formation. A number of studies have proposed the existence of an intricate crosstalk between primary tumors and future sites of metastases in order to transform the environment of these organs into a suitable microenvironment. This microenvironment is called the premetastatic niche, and is established to receive disseminating cancer cells, and support the growth of the metastatic colony. Melanoma is a highly metastatic cancer and preferentially establishes secondary lesions in lungs and brain. We have created a mouse model of melanoma B6(Cg)-Tg(Dct-Grm1)ESzc Tg(KRT5-rtTA)1Glk Tg(tetO-Edn3,-lacZ)Kosl or *(Dct-Grm1/K5-Edn3)* that spontaneously develops melanoma tumors and metastasizes to the lung. In order to describe the premetastatic niche formation in the mouse model of melanoma metastasis we monitored the appearance of bone marrow derived cells (BMDCs) clusters and metastatic cells during tumor progression by immunofluorescence and flow cytometry. We detected both BMDCs and tumors cells in the earliest stage of tumor progression suggesting that the lung melanoma metastasis in the *Dct-Grm1/K5-Edn3* mouse model is independent of premetastatic niche formation. We also found tumorigenic cells staining positive for both melanocytic and hematopoietic markers in higher numbers early during primary tumor formation. The appearance of Melanoma-BMDC hybrids in the lung suggest they might be the metastasis initiating cells, which might explain why the premetastatic niche formation is not required in the *Dct-Grm1/K5-Edn3* mouse model. Furthermore our findings implicate that Melanoma-BMDC hybrids are potential targets for melanoma treatment.

M5089B *GNL3* modulates prostate cancer metastasis susceptibility. *M. Lee*[1], K. A. Williams[1], Y. Hu[2], J. Andreas[1], S. J. Patel[1], S. Zhang[3], N. P. S. Crawford[1]. 1) Genetics and Molecular Biology Branch, NHGRI, NIH, Bethesda, MD 20892, USA; 2) Center for Biomedical Informatics and Information Technology, NCI, NIH, Rockville, MD 20850, USA; 3) Computational and Statistical Genomics Branch, NHGRI, NIH, Bethesda, MD 20892, USA.

Prostate cancer is the second leading cause of cancer mortality in men in developed countries. However, the molecular determinants of prostate cancer metastasis remain unclear. Previously, we reported that germline variation influences metastasis in the C57BL/6-Tg(TRAMP)8247Ng/J (TRAMP) mouse model of prostate cancer. These mice develop prostate tumors similar to a subset of poor outcome, treatment-associated human prostate cancer tumors. Germline variation introduced by PWK/PhJ caused significant suppression of tumor formation and metastases in the (TRAMP x PWK/PhJ) F1 males. *GNL3*, which encodes for a binding partner of p53 and functions in cell cycle regulation, was identified as a novel candidate metastasis susceptibility gene through a systems genetics approach using 201 (TRAMP x PWK/PhJ) F2 males and multiple prostate cancer patient datasets. Quantitative trait locus (QTL) mapping identified a locus on Chromosome 14 (LOD = 4.41; genome-wide *P* = 0.032) encompassing *Gnl3* to be associated with distant metastasis-free survival. In two prostate cancer patient datasets, *GNL3* expression was associated with an increased risk of aggressive disease and poorer disease-free survival. In addition, *GNL3* harbored SNPs associated with aggressive tumorigenesis in the PLCO/CGEMS GWAS of 1,172 prostate cancer patients. Here, we aim to examine the molecular mechanisms of GNL3 as a metastasis susceptibility modifier using human prostate cancer cell lines PC-3 and LNCaP stably over-expressing *GNL3.* Over-expression of *GNL3* in PC-3 caused an increase in cell proliferation and decreased *in vitro* cell migration and invasion compared to control cells. In soft agar assays, over-expression of *GNL3* in both PC-3 and LNCaP cells increased colony count. Subcutaneous injections into the flanks of NU/J male mice of *GNL3* over-expressing PC-3 cells, but not LNCaP cells, caused a significant increase in tumor burden compared to control. Pathway analysis of microarray data from cells over-expressing *GNL3* demonstrated that over-expression of *GNL3* modulates cell cycle regulators. These results collectively demonstrate a role for GNL3 in modulating metastasis susceptibility possibly by regulating cell cycle-related gene expression. This study, in addition to our previous studies, continues to exemplify how mouse models can be used to identify metastasis susceptibility genes through a novel systems genetics approach, and gives new insight into the molecular mechanisms of fatal prostate cancer.

M5090C Molecular analysis of epidermal growth factor receptor (EGFR)-independent colorectal cancers. *C. MANTILLA ROJAS*[1], M. YU[2], D. THREADGILL[1]. 1) Texas A&M University, College Station, TX; 2) 2University of North Carolina, Chapel Hill, NC.

According to the American Cancer Society, colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States. Early detection efforts have reduced mortality, yet approximately 50,000 deaths are expected this year. Much of the etiology underlying CRC remains unclear, which hampers efforts to design more efficacious therapies. As such, a strong need exists to better understand the molecular mechanisms governing CRC progression. One of the first targets for molecular targeted therapies was the tyrosine kinase receptor epidermal growth factor receptor (EGFR). Preclinical studies in our lab and others showed that targeting EGFR greatly reduced incidence of CRC in mouse models. However, subsequent clinical trials using EGFR inhibitors were less efficacious, suggesting that targeting EGFR alone does not result in a significant benefit in most CRC cases. Mutations in *Kras* can explain some non-responding CRCs, but even in cancers lacking *Kras* mutations, little is known about which cancers are likely to respond. In this study, we used intestine-specific genetic ablation of *Egfr* in the $Apc^{Min/+}$ (genetic) and axozymethane (carcinogenic) CRC mouse models. We discovered that 10% of colonic tumors arise independent of EGFR activity. By molecular analysis we confirmed the absence of *Egfr* in these tumors, and conclusively demonstrated the existence of an EGFR-independent mechanism by which CRC can arise and progress. Residual tumors lacking EGFR were larger in size than those developing under normal EGFR activity, suggesting these cancers may be a more aggressive form of CRC. We also have evidence that ERBB3, a related EGF receptor, mediates compensatory and alternative pathways, suggesting an important role of ERBB3 may be in EGFR-independent CRC progression. Furthermore, we have generated an additional model that conditionally inactivates ERBB3 ($Erbb3^{tm1Dwt}/Erbb3^{tm1Dwt}$), and when combined with $Egfr^{tm1Dwt}/Egfr^{tm1Dwt}$>, these mice will have EGFR and ERBB3 combined deficiency in the intestinal epithelia. The innovative models we developed will provide powerful tools to genetically dissect molecular pathways contributing to CRC, leading to novel targets and associated biomarkers for therapeutic intervention. The study will advance our understanding of EGFR biology during colonic tumorigenesis, ultimately contributing to better therapies for CRC.

M5091A Host genetic and gut microbiota variability within the C57BL/6-Apc^{Min} mouse affects the intestinal tumor phenotype. *J. Moskowitz*, S. Busi, M. Hart, C. Franklin, J. Amos-Landgraf. University of Missouri, Columbia, MO.

Colorectal cancer (CRC) is a multifactorial disease that develops as a result of well-established genetic factors such as mutation of the tumor suppressor *Adenomatous Polyposis Coli (APC)* gene. However, other poorly characterized factors such as the gut microbiota (GM) likely play an important role in tumor development and progression. Our laboratory has found significant differences in both small intestinal (SI) and colonic tumor numbers between two Apc^{Min} mouse colonies; the C57BL/6J-Apc^{Min} (B6-*Min*/J) from the Jackson Laboratories, and the C57BL/6JD-Apc^{Min} (B6-*Min/*D) closed colony that has been maintained at the University of Wisconsin. It is unclear whether underlying host genetic factors or differences in the GM are responsible for these phenotypic differences. To determine the potential impact of GM on disease phenotype, we used complex microbiota targeted rederivation (CMTR) to rederive isogenic embryos of the two *Apc* mutant colonies onto CD1 surrogate dams that possessed GM from either The Jackson Laboratory (Crl:$CD1_{GMJAX}$) or Envigo (Hsd:$CD1_{GMHSD}$). We generated a total of four Apc^{Min} groups: B6-*Min*/J_{GMJAX}, B6-*Min*/J_{GMHSD}, B6-*Min*/D_{GMJAX}, and B6-*Min*/D_{GMHSD}. At three months of age, all animals were sacrificed to determine both SI and colonic tumor multiplicity. We observed a significant increase in both SI (p=0.03) and colonic tumors (p=0.006) in the B6-*Min*/J_{GMHSD} group as compared to the B6-*Min*/J_{GMJAX} group. Additionally, we observed trending increases in SI and colonic tumor numbers in B6-*Min*/D_{GMHSD} compared with B6-*Min*/D_{GMJAX} mice. These data suggest that the GM has a critical role in the SI and colonic tumor phenotype. We also noted

that B6-*Min*/D animals had more SI tumors than B6-*Min*/J animals within each GM group. To ensure that no known C57BL/6J modifiers existed in the population, founder animals were genotyped and found negative for the B6-*Atp5a1*^{Mom2} resistance allele, indicating that the *Mom2* mutation is not responsible for the phenotypic differences between the two colonies. Importantly, there was no difference in colonic tumor numbers between groups with the same GM. Together, these data suggest that host genetic differences play a role in the SI phenotype, but not the colonic phenotype. We conclude that both the GM and host genetic factors within the C57BL/6J-*Apc*^{Min} population contribute to the SI tumor phenotype. However, the GM primarily defines the colonic tumor phenotype. In addition to revealing highly important information regarding tumorigenesis in the *Apc*^{Min} mouse, these results will have a critical impact on reproducibility of studies using this model.

M5092B Nuclear to cytoplasmic relocalization of cyclin C directs stress-induced mitochondrial fission and promotes apoptosis in yeast and mouse cell lines. *R. S. Strich*[1], J. Jazek[1], V. Ganesan[1], A. Joshi[1], A. Di Cristofano[2]. 1) Rowan University-SOM, Stratford, NJ; 2) Albert Einstein University, NY USA.

In response to cellular damage, the mitochondria undergo extensive fragmentation, exhibit mitochondrial outer membrane permeability (MOMP), and release pro-apoptotic factors. Although the basic fission machinery is required for this hyper-fission, the molecular switch that induces this process remained elusive. In response to oxidative stress, the yeast[1] and mammalian[2] transcription factor cyclin C translocate from the nucleus to the cytoplasm where they associate with the GTPase Drp1/DNM1 at the mitochondria. In both systems, cyclin C is both necessary and sufficient to induce extensive mitochondrial fragmentation. The conservation of cyclin C activity is remarkable as treating permeabilized mouse embryonic fibroblast cultures with purified yeast cyclin C rapidly induced complete mitochondrial fission without an added stress signal. Further analysis revealed that the mouse cyclin C is also required for stress-induced MOMP and apoptosis. Using a CCNC knockout MEF culture system, we found that cyclin C is required for efficient mitochondrial recruitment of the pro-apoptotic BH-3 protein BAX. This activity appears direct as cyclin C co-immunoprecipitates with activated BAX following oxidative stress. Consistent with the role in stress-induced apoptosis, we found that cyclin C suppresses hyperplasia and adenoma formation in a PTEN phosphatase thyroid tumor model. While either single mutant presents with a modest phenotype, combining *Ccnc* and *Pten* null alleles dramatically accelerates thyroid hyperplasia resulting in premature death. These results indicate that cyclin C is a previously undescribed solid tumor suppressor. These results suggest that cyclin C cytoplasmic localization alters mitochondrial dynamics and influences apoptotic sensitivity in mammals.

[1]Cooper et al., (2014) Dev. Cell. 28:161. [2]Wang et al. (2015) Mol. Biol. Cell 26:1030.

M5093C Combinatorial regulation of BATF and BATF2 in LPS-stimulated and Mycobacterium-infected inflammatory responses. *H. Suzuki*[1], S. Roy[1], R. Guler[2,3], S. Schmeier[4], S. Parihar[2,3], M. Ozturk[2,3], F. Brombacher[2,3]. 1) RIKEN CLST, Yokohama, JP; 2) ICGEB, Cape Town, SA; 3) Cape Town Univ., Cape Town, SA; 4) Massey Univ., North Shore, NZ.

Basic leucine zipper transcription factor *Batf* belongs to the activator protein (AP-1) family of transcription factors, consisting of *Batf*, *Batf2* and *Batf3*, and is a positive regulator of various cellular processes in DCs, T and B cells. During our recent finding that BATF2 induces inflammatory responses in classically activated macrophages, lipopolysaccharide (LPS) or *Mycobacterium tuberculosis* (Mtb) infection, we found that *Batf* expression was also induced in LPS-stimulated and Mtb-infected macrophages, but not in IFNγ-activated classical activation. In order to explore BATF function in macrophages, we assessed the effect of *Batf* knockdown in LPS-stimulated mouse bone marrow-derived macrophages and compared it with that of *Batf2* knockdown. We found that BATF and BATF2 regulate different set of genes; the gene ontology analysis revealed that BATF suppresses negative regulators of inflammatory response. Further, *Batf2* knockdown up-regulated *Batf* expression, suggesting that BATF2 is a negative regulator of *Batf* expression. Taken together with the BATF2 function, our results suggest that BATF plays an important role of augmentation of the inflammatory response in LPS-stimulated macrophages.

M5094A Susceptibility to diethylstilbestrol exposure in mice. *D. L. Aylor*, N. E. Allard, T. I. Konneker. North Carolina State University, Raleigh, NC.

Diethylstilbestrol (DES) is a drug that caused infertility and cancer in some adults who were exposed prenatally before its use was ended in 1971. Some DES-exposed mouse strains also display reproductive defects (male and female) and cancer; yet other mouse strains are unaffected. This indicates a strong genetic component to susceptibility. Our goal is to identify the gene x environment interactions (GxE) associated with clinical traits and molecular profiles in DES-exposed inbred mouse strains. To accomplish this goal, we are screening a large panel of inbred mouse strains that includes 50 lines from the Collaborative Cross (CC) genetic reference panel. We observe novel strain-specific reproductive defects in DES-exposed males, including some that lead to azoospermia and infertility. In females, we have measured the acute effects of DES exposure on uterine gene expression for a known susceptible strain (FVB/NJ) and a known resistant strain (C57BL/6J). A substantial number of genes are differentially expressed between DES-exposed and control mice in both strains, but only 61% of these genes are shared. This illustrates the large effect genetic background can have on DES-induced changes in uterine gene expression. These strain-specific responses to DES involve coordinate regulation of hundreds of genes and can help narrow down sets of genes associated with disease susceptibility. We have identified gene modules that characterize strain-specific DES response and we relate these to clinical data derived from uterine histopathology. We conclude that the combination of genetic variation with functional genomics is a powerful tool for identifying genes involved in differential susceptibility to toxicants, and we propose that this approach is broadly relevant to other toxicological studies in rodents.

M5095B Systemic metabolic effects exerted by a point mutation in the RED subdomain of PAX6. *N. F. Chhabra*[1,2], M. Wu[1,2], M. Fütterer[1,2], I. Irmler[1,2], J. Beckers[1,2,3], M. Götz[4,5], J. Rozman[1,2], G. Przemeck[1,2], M. Hrabe de Angelis[1,2,3]. 1) Institute of Experimental Genetics, Helmholtz Zentrum München, Neuherberg, Germany; 2) German Center for Diabetes Research (DZD e.V.), Neuherberg, Germany; 3) Chair of Experimental Genetics, Centre of Life and Food Sciences, Weihenstephan, Technische Universität München, Freising, Germany; 4) Institute of Stem Cell Research, Helmholtz Zentrum München, Neuherberg, Germany; 5) Physiological Genomics, Institute of Physiology, Munich University, Munich, Germany.

The paired box protein 6 (PAX6) is a major transcription factor involved in eye development. Additionally, its role in the development of the pancreas has previously been documented, although the underlying mechanisms in the homeostasis of the adult pancreas remain largely unknown.

The *ENU*-generated C3;CAnN-*Pax6*^{Leca2} mouse line with a point mutation (R128C) in the RED subdomain of the PAX6 protein, recapitulates a human mutation causing foveal hypoplasia. In addition to the retinal defects, the mutation translates into numerous observable abnormalities in the pancreas. Progressive islet distortion was discernable from the age of 4 weeks including various dysregulated genes. More specifically, up-regulation of the endocrinal progenitor marker *Neurog3* and down-regulation of several β-cell specific markers in addition to increased proliferation within the islet suggested the prevailing phenotype to be most consistent with β-cell dedifferentiation.

Moreover, glucose stimulation of isolated islets *in vitro* and an intraperitoneal glucose challenge *in vivo* indicated reduced insulin content and secretion. However, this reduction was accompanied by a decreased fasting blood glucose level and a normal glucose clearance, possibly explained through the evident increased insulin sensitivity and decreased hepatic glucose production. Additionally, increase in locomotor activity and energy expenditure indicated an effect mediated via the hypothalamus, as an effect of the mutation. Taken together, the data suggests, as yet, unknown systemic function of PAX6 affecting the overall metabolism of the organism.

M5096C Congenic localization of the *Moo1* obesity QTL to 319 kb. *S. M. Clee*, C. L. K. Leung, S. Karunakaran, J. Dong, C. S. Yan, Z. J. Wu, A. Manji, S. Mahmoodi. University of British Columbia, Vancouver, Canada.

The BTBR T+ *Itpr3*^{tf}/J (BTBR) inbred strain has increased susceptibility to obesity and diabetes compared to the C57BL/6J (B6) strain. These effects are exaggerated in the presence of an obesogenic stimulus, e.g. homozygosity for the *obese* allele of the leptin gene(*Lep*^{ob}). Prior genetic studies mapped the loci affecting body weight and metabolic traits (fasting glucose and insulin) segregating between these strains. These studies identified the modifier of obesity 1 (*Moo1*) locus on mouse Chromosome 2 as the major locus controlling the difference in adiposity between these strains in *Lep*$^{ob/ob}$ mice. Congenic strains initially identified a ~6 Mb region of Chromosome 2 where replacement of BTBR alleles with those from B6 reduces body weight ~10% (the Moo1-C strain). Work by my laboratory has shown that this locus also affects obesity induced by high fat feeding, and is associated with alterations in body fat, food intake and glucose tolerance. We have also detected an interaction between this locus and stress that affects body weight. To localize the causative genetic variation we created a panel of 10 sub-congenic strains from Moo1-C strain recombinants. Analysis of these strains revealed *Moo1* is comprised of at least 2 independent QTLs. The proximal of these, *Moo1a*, is encompassed within a strain (Moo1-V) in which B6 alleles replace only 319 kb of BTBR genome (rs27970625 – D2Mit328). Homozygosity for B6 alleles in this region reduces body weight of high fat fed BTBR mice by ~8 % (P<0.001). This region spans 2 known genes: *Itga6* and *Pdk1*. We identified numerous (non-coding) sequence changes within and near these genes and each gene has a single coding variation between the strains, although these are not predicted to affect protein function. We assessed expression of these genes in metabolically relevant tissues and found an ~50% reduction in expression of both *Itga6* and *Pdk1* in many tissues. *Pdk1* regulates the entry of glucose-derived metabolites into the Krebs cycle for oxidation. This also regulates substrate availability for the synthesis of fat, making this an obvious candidate. We obtained mice deficient in PDK1 from the KOMP repository, but found no evidence of alterations in obesity in high fat-fed heterozygous or homozygous knockouts compared to their wildtype littermates. *Itga6*, although the less obvious candidate, is an integrin that could affect obesity in several ways. Analysis of mice with reduced ITGA6 is ongoing. These data highlight the power of mouse genetics to discover novel molecular pathways affecting complex metabolic phenotypes that are highly influenced by environmental factors and to identify specific gene-environment interactions.

M5097A *Dll1*- and *Dll4*-mediated Notch signaling in adult pancreatic β-cells is essential for the structural integrity of the islets of Langerhans and maintenance of glucose homeostasis. *M. Fuetterer*[1,2], N. Chhabra[1,2], M. Irmler[1,2], J. Beckers[1,2,3], G. Przemeck[1,2], M. Hrabě de Angelis[1,2,3]. 1) Institute of Experimental Genetics, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany; 2) German Center for Diabetes Research (DZD e.V.), Neuherberg, Germany; 3) Chair of Experimental Genetics, Center of Life and Food Sciences, Weihenstephan, Technische Universität München, Freising, Germany.

The Notch signaling pathway is a short-range communication transducer involved in the regulation of many cellular processes. Genes of the pathway are expressed in different cell types and organs at different time points during embryonic development and adulthood. For example, the Notch ligand delta-like 1 (DLL1) controls the decision between endocrine and exocrine fates of progenitor stem cells in the developing pancreas; and loss of *Dll1* function leads to premature differentiation of the pancreatic endocrine cell lineage. The ligands DLL1 and DLL4 as well as other members of the pathway are also expressed in the adult pancreas. However, the role of Notch signaling in adult tissue homeostasis is not well understood. Here, we describe cell-type specific, distinct expression of Notch pathway members in the adult murine pancreas. Using ligand-specific conditional loss- and gain-of-function mouse models we demonstrate alterations in islet morphology and effects on blood-glucose regulation as well as other metabolic parameters. Hence, we provide a first impression on functional aspects of Notch signaling in the adult islet and its importance for maintenance of tissue homeostasis.

M5098B Quantitative Genetic Analysis of MUC5AC and MUC5B in a Mouse Model of Asthma. *Samir N. P. Kelada*, Alessandra Livraghi-Butrico, Lauren Donoghue, Joseph P. Thomas, Kathryn McFadden, Gang Chen, Lucas T. Laudermilk, Wanda K. O'Neal, Richard C. Boucher. UNC, Chapel Hill, NC.

Rationale Mucus hyper-secretion is a hallmark feature of asthma and other obstructive airway diseases. Two mucins, MUC5AC and MUC5B, represent the major glycoprotein components of mucus. Little is known about the genetic determinants of MUC5AC and MUC5B protein expression. We aim to identify genetic regulators of MUC5AC and MUC5B protein concentrations in a mouse model of asthma. **Methods** We applied a house dust mite allergen model of asthma to both founder lines (n=8) and incipient lines (n=154) of the Collaborative Cross (CC). We collected whole lung lavage samples 72 hours after allergen challenge and quantified MUC5AC and MUC5B by agarose gel electrophoresis followed by western blotting. Each CC mouse was genotyped on a high density Affymetrix array. Protein expression in lungs was examined using immunohistochemistry. Gene knockdown *in vitro* was accomplished using lentiviral constructs expressing shRNAs. **Results** CC founder lines sensitized and challenged with allergen exhibited statistically significant differences in both MUC5AC and MUC5B, providing evidence of heritability. Incipient CC lines exhibited a broad range of MUC5AC and MUC5B secretion, consistent with a polygenic architecture for these two phenotypes. Quantitative trait loci (QTL) for MUC5AC and MUC5B were identified on Chromosomes 13 (at 75 Mb) and 2 (at 154 Mb), respectively. We focused on the MUC5B QTL because of the large effect size and found that *musculus*-derived alleles were associated with lower MUC5B compared to alleles from *domesticus* and *castaneus*. We validated the difference in MUC5B concentrations due to QTL region genotype by performing a second set of experiments using independent CC lines of contrasting haplotypes (*musculus* vs. *domesticus*). Using additional gene expression and SNP datasets, we identified *Bpifb1* as a candidate gene. We show that BPIFB1 expression colocalizes with MUC5B in airway epithelia and is upregulated by allergen treatment. Finally, we show that knockdown of *BPIFB1* affects MUC5B secretion in primary human airway epithelial cells. **Conclusions** Concentrations of MUC5AC and MUC5B in the allergen-challenged lung are controlled by distinct genetic loci, and these loci are distinct from those that regulate mucin gene expression. Our data indicate that variation in *Bpifb1* is strongly associated with MUC5B concentration after allergen challenge.

M5099C GeneLab: A systems biology platform for spaceflight omics data. *S. S. Reinsch*. NASA-Ames Research Center, Moffett Field, CA.

NASA's GeneLab project is maximizing the science output from spaceflight experiments conducted aboard the International Space Station (ISS) by: (1) developing a unique public bioinformatics database that includes space bioscience relevant "omics" data (genomics, transcriptomics, proteomics, and metabolomics) and experimental metadata; (2) partnering with NASA-funded flight experiments through bio-sample sharing or sample augmentation to expedite omics data input to the GeneLab database; and (3) developing community-driven reference flight experiments.

The data hosted in the GeneLab data system to date represents omics data from numerous model organisms including microbes, yeast, *C. elegans*, *Drosophila*, *Arabadopsis*, rodents and human cell lines. In this presentation we will show an overview of omics data in the context of existing physiological data from spaceflight.

In 2015 GeneLab partnered with two Biological Research in Canisters experiments (BRIC-19 and BRIC-20), which examined the proteomic and transcriptomic responses of *Arabidopsis thaliana* to spaceflight. GeneLab also partnered with Rodent Research-1 (RR-1), the maiden flight to test the recently developed rodent habitat, and is generating transcriptomic, proteomic and epigenomic data from various tissues including liver, muscle, eye, kidney etc. GeneLab is establishing partnerships with other planned flights for 2016 including a comparative study of two bacterial species. GeneLab is also establishing sample sharing collaborations with multiple future rodent missions.

Overall, GeneLab will facilitate the generation and query of parallel multi-omics data, and deep curation of metadata for integrative analysis, allowing researchers to uncover cellular networks as observed in systems biology platforms. Consequently, the scientific community will have access to a more complete picture of functional and regulatory networks responsive to the spaceflight environment. Analysis of GeneLab data will contribute fundamental knowledge of how the space environment affects biological systems, and enable emerging terrestrial benefits resulting from mitigation strategies to prevent effects observed during exposure to space. As a result, open access to the data will foster new hypothesis-driven research for future spaceflight studies spanning basic science to translational science.

GeneLab is funded through NASA's Space Life and Physical Sciences Research and Applications Division (SLPSRA) and the International Space Station Research Integration Office (ISSRIO).

M5100A Rat Resource and Research Center. *E. C. Bryda*, H. Men, A. C. Ericsson, J. M. Amos-Landgraf, Y. Agca, C. L. Franklin, R. S. Prather. University of Missouri, Columbia, MO.

The Rat Resource and Research Center (RRRC) was established in 2001 with funding from the National Institutes of Health (NIH) with the goals of 1) shifting the burden for maintaining and distributing rat models from individual investigators to a centralized repository, and 2) providing the biomedical community with ready access to valuable rat strains/stocks and other related services that enhance the use of rats in research. Currently, the RRRC has close to 400 donated rat lines in its inventory. Upon importation of strains/stocks into the RRRC, sperm and embryos are cryopreserved to ensure against future loss of the model. The RRRC distributes live animals, cryopreserved sperm and embryos as well as rat embryonic stem (ES) cell lines. Quality control measures for all materials include extensive genetic validation and health monitoring. The RRRC has expertise in rat reproductive biology, colony management, health monitoring, genetic assay development/optimization, and isolation of germline competent ES cell lines from transgenic rats; our staff and researchers are readily available for consultation and collaborations and a number of services are available on a fee-for-service basis. Our website (www.rrrc.us) allows user-friendly navigation and provides information about all strains/stocks, cell lines, model donation procedures, on-line ordering, lists of services, and protocols. Current research efforts include refinement of models, characterization of the rat microbiota and its influence on model phenotypes, and generation of new rat models, including new models of Inflammatory Bowel Disease (IBD) generated using

CRISPR/Cas9 technology. In addition to the RRRC, the University of Missouri is home to two other NIH-funded animal resources: the MU Mutant Mouse Resource and Research Center (MMRRC) and the National Swine Resource and Research Center (NSRRC) as well as the newly established MU Metagenomics Center (MUMC). Together, these highly collaborative groups provide a variety of animal model-related services across species to facilitate biomedical research.

M5101B Using the web-based genome browser *gEVAL*, to evaluate and improve the draft assemblies of 18 strains for the Mouse Genomes Project. *W. Chow*, K. Howe. Wellcome Trust Sanger Institute, Cambridge, UK.

The Mouse Genomes Project used next generation sequencing technologies to sequence and assemble 16 key laboratory mouse strains and 2 wild derived mouse strains to produce a comprehensive picture of variation amongst mouse genomes. However, like many *de novo* genome projects, the initial draft assemblies revealed regions of discordance and misassemblies.

To aid in the improvement and curation of these genomes, and to incorporate new long-range datasets, the assemblies of these 18 strains were recently added to the web-based genome evaluation browser gEVAL (BioRxiv, 2016). gEVAL has been successfully used to curate the human mouse and zebrafish reference genomes, which has later led to other reference assemblies such as rat, pig, chicken, and tasmanian devil to be included in the browser.

gEVAL provides a one-stop solution to assess the compliance of a given assembly with a multitude of available data such as the correct pairing and suitable distance of mapped clone ends, the placement of markers and cDNAs, inter-comparative alignments between assemblies, long range genome/optical mapping datasets and many more.

Having these assemblies publically accessible in gEVAL, along with a whole range of supporting datasets, allows the wider mouse research community an early, easy way of accessing and evaluating the contiguity of a region of interest and view the already available gene sets.

Furthermore, gEVAL provides a platform to assist our curation groups that are tasked with improving these assemblies and the annotation of features such as genes, in hopes that these efforts will contribute to yielding full genome sequences of quality comparable to the reference standard .

Website: http://geval.sanger.ac.uk/

BioRxiv: http://dx.doi.org/10.1101/038638 (accepted in Bioinformatics, March 2016).

M5102C Phylogenetically based Gene Ontology (GO) Annotations using the Phylogenetic Annotation and INference Tool (PAINT). *K. R. Christie*[1], M. Feuermann[2], P. Gaudet[2], S. E. Lewis[3], D. Li[4], H. Mi[5], M. C. Munoz-Torres[3], P. D. Thomas[5], J. A. Blake[1], The Gene Ontology Consortium. 1) The Jackson Laboratory, Bar Harbor, ME; 2) Swiss Institute for Bioinformatics, Geneva, Switzerland; 3) Lawrence Berkeley National Laboratory, Berkeley, CA; 4) Phoenix Bioinformatics, Redwood City, CA; 5) University of Southern California, Los Angeles, CA.

A major goal of the Gene Ontology (GO) project is to describe the functions of genes from all kingdoms of life in a consistent way. For a limited set of model organisms, annotation of the functions of at least some of the genes can be done based on direct experimental data. However, for many organisms, little or no experimental data exists. Even for model organisms, there are many genes that are not directly characterized. In these situations, other methods of capturing functional data are necessary. One method utilized by the GO community uses phylogenetic trees of related sequences (via Panther) that are overlaid with experimental GO annotations from which evolutionarily based functional annotations can be inferred. The PAINT (Phylogenetic Annotation and INference Tool) curation tool has been developed to support this effort. The curator can view the distribution of GO terms that are based on direct experimental evidence for each species to infer the likely evolutionary history of functions and thus which functions can be propagated to which sequences within the tree. This phylogenetic method helps provide more complete annotations of genomes for use in term enrichment and genomic analyses, sometimes more detailed that what is provided using domain analysis, e.g. via InterPRO domains. For example, the small subunit (SSU) processome involved in biogenesis of the small ribosomal subunit is well characterized in *S. cerevisiae*, but not in the laboratory mouse. In the case of the mouse *Wdr3* gene, which is homologous to *S. cerevisiae DIP2* (aka *UTP12*), curation using phylogenetic analysis provided detailed annotations for mouse *Wdr3*. In contrast, the InterPRO domains for this gene did not provide useful annotations, and an annotation transferred by sequence similarity comparison provided a somewhat misleading annotation to an overly specific RNA binding term. Thus annotations from this phylogenetic annotation method can provide detailed GO annotations for genes based on experimental characterization of their homologs in other organisms.

This work is funded by HG 002273 to the Gene Ontology Consortium.

M5103A Catalogue of identified mutations in RIKEN ENU Mutant Mouse Library: a new approach for the studies on polygenic traits. *R. Fukumura*[1], H. Kotaki[1], S. Makino[1], Y. Ishitsuka[1], Y. Nakai[1], Y. Minakuchi[2], A. Toyoda[2], A. Fujiyama[2], Y. Gondo[1]. 1) RIKEN BRC, Tsukuba, Ibaraki, JP; 2) NIG, Mishima, Shizuoka, JP.

A model mouse for any human disease helps to elucidate the pathogenic mechanism and to develop a therapeutic application(s). Therefore, many model mice have been developed. IMPC have been in progress to phenotype KO mouse strains established by IKMC. In this international efforts, the genetic background was unified in C57BL/6N focusing on monogenic traits and diseases. Recently, the genome editing technologies, in particular, the CRISPR/Cas9 system, have made it possible to quickly introduce mutations to a few target loci in a single strain. The genetic studies of polygenic traits and disorders such as mental diseases, metabolic syndromes and life-style related diseases are, however, still limited. We have made RIKEN ENU Mutant Mouse Library of 10,000 G1 males open to public since 2002 and many users have already used and reported their outcomes. Each G1 mouse has ~5,000 mutations; thus, it is plausible to exhibit some polygenic traits due to the interaction among the thousands of mutations. Two such cases have been indeed suggested by users. In order to assess what a part of thousands of mutations interact each other in each G1 strain, we are now conducting systematic QTL analysis focusing on the body weight. Firstly, two outlier G1 strains, G1DB012260 and G1DB008748, for body weight were chosen from the 10,000 G1 Library and revived them by IVF/ET

method. We have obtained 81 and 51 G2 progenies and their intercross have given rise to 72 and 128 G3 mice so far from G1DB012260 and G1DB012260, respectively. The 8-weeks-old body weight of the G3 populations showed an increase of genetic variance comparing to the control population, which indicates the existence of some genetic interaction among induced mutations. At the same time, Whole Exome Sequencing (WES) and SNV calls of the two G1 genomes identified 159 and 182 ENU-induced mutations and the genotyping of identified mutations in G3 mice were conducted. The preliminary results of QTL analysis based on the body weight and genotyping of the G3 populations also showed several interactive peaks. We thus encourage users to apply the RIKEN Mutant Mouse Library not only for the monogenic analyses but also multigenic/polygenic studies to establish models for various complex traits and diseases. We have so far identified 944 ENU-induced mutations in 428 target regions based on user's requests. In addition, WES has catalogued 4801 ENU-induced mutations in 53 G1 strains. A total of the 5,745 mutations are also open to public.

M5104B Utilizing NCBI's Mouse Genome Resources. *Tripti Gupta*, Kelly McGarvey, Terence Murphy, Kim Pruitt. National Center for Biotechnology Information, National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894.

Complete and accurate genome annotation is essential to researchers using genomic data. A major focus of the Reference Sequence (RefSeq) database at the National Center for Biotechnology Information (NCBI) is to provide an accurate and comprehensive annotation of the mouse genome through computational and manual curation. The RefSeq database contains annotated genomic, transcript, and protein sequence records derived from data in public sequence databases and from computation, curation, and collaboration. This combinatorial approach results in a high-quality annotation that focuses on representation of full-length, non-redundant sequence data and that is regularly updated through re-annotations every 12 to 18 months. RefSeq provides whole genome annotation of the reference strain C57BL/6J genome assembly, including variation on alternate locus scaffolds, maintained by the Genome Reference Consortium (GRC) and annotation of the mixed strain Celera assembly. In addition, complete annotations of 15 other rodents, including Peromyscus maniculatus bairdii and Rattus norvegicus, are available and can be downloaded from the NCBI RefSeq Genomes FTP site (ftp://ftp.ncbi.nlm.nih.gov/genomes/refseq/vertebrate_mammalian/). In order to provide the most consistent and comprehensive annotation possible, RefSeq scientists manually curate genes in close collaboration with the Mouse Genome Informatics database, the Consensus Coding Sequence project, and the GRC. Manual curation methods continually incorporate new data sets such as genome wide promoter-associated epigenetic data and PolyA-Seq data to improve annotation and define manually annotated features on transcript and protein sequences. RefSeq curation efforts have traditionally focused on representing full-length transcripts of protein coding genes, primarily using transcript data, protein alignments, and published data as evidence; however, in recent years, we have incorporated additional data into our computational and manual curation methods, allowing for more complete and accurate annotation. For example, changes to NCBI's eukaryotic annotation pipeline allowed the incorporation of RNA-Seq data, resulting in significant increases in the numbers of predicted protein-coding variants and non-coding transcripts. Incorporation of the RNA-Seq data has been particularly valuable to our recent efforts to expand the representation of long non-coding RNAs. The current annotation of the GRCm38 assembly includes 31,500 predicted non-coding RNAs, a 26% increase relative to the previous annotation, which did not utilize RNA-Seq data. This poster provides an overview of NCBI's mouse genome resources and highlights recent curation efforts by the RefSeq group.

M5105C Analysis of the Collaborative Cross founder strains at the German Mouse Clinic identify new and known phenotypes. *H. Kollmus*[1], M. Horsch[2], M. Gegenfurtner[2], German Mouse Clinic Consortium[2], H. Fuchs[2], V. Gailus-Durner[2], R. Balling[3], K. Schughart[1,4,5], M. Hrabe de Angelis[2,6]. 1) Helmholtz Centre for Infection Research, Braunschweig, Germany; 2) Helmholtz Zentrum München, Munich, Germany; 3) Luxembourg Centre for Systems Biomedicine, University Luxembourg, Luxembourg; 4) University of Veterinary Medicine, Hannover, Germany; 5) University of Tennessee Health Science Center, Memphis, Tennessee, USA; 6) Technische Universität München, Freising-Weihenstephan, Germany.

We performed a comprehensive and comparative phenotyping screening of the CC founder strains carried out at the German Mouse Clinic (GMC, www.mouseclinic.de). The Collaborative Cross (CC) is a large panel of mouse-inbred lines derived from eight founder strains, including three wild-derived strains (A/J, C57BL/6J, 129S1/SvlmJ, NOD/ShiLtJ, NZO/HILtJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ). The phenotyping analyses cover phenotypes related to human diseases such as behavioural and cardiovascular parameters, clinical chemistry, dysmorphology, bone and cartilage, energy metabolism, eye and vision, immunology, lung function, neurology, nociception, followed by a detailed pathological examination. For each strain, at least sixteen females and males were phenotyped leading to a highly robust data set. Metadata analysis showed that the three wild-derived strains are most different from the laboratory strains. For some parameters the wild-derived strains are more similar to each other than to the laboratory strains. NZO/HILtJ, A/J and 129S1/SvlmJ were most different from all other strains. The analysis of screen-specific parameter sets recapitulated the global tendency of phenotypic similarities between laboratory strains, but also detected phenotypes that distinguished single strain from all others. Our results confirmed already known strain characteristics but also identified new phenotypes. For instance reduced hearing sensitivity was described in A/J and NOD/ShiLtJ before but not for NZO/HILtJ. This data set will be a valuable baseline for understanding phenotype effects in CC strains.

M5106A Mouse SNPs and polymorphisms data on Mouse Genome Informatics. *MeiYee Law*, Janan Eppig, Carol Bult, Mouse Genome Informatics. The Jackson Laboratory, Bar Harbor, ME.

Mouse Genome Informatics (MGI, http://www.informatics.jax.org) provides free access to mouse genetic, genomic and biological data in support of mouse as a model for human biology and disease. Here we describe MGI's newly implemented SNP searching and analysis tools that provide greater flexibility for researchers to explore and extract SNP data. The new SNPs query form includes data from 88 inbred strains from dbSNP. Using search options such as multiple genes search, genome coordinates search, strains of interest search, and comparison reference

strain, results returned highlight allele variation at each available genomic location, dbSNP, links to MGI and other genome browsers, etc. SNP results also can be filtered by additional criteria such as restricting results to coding or non-coding sequences. The application of mouse SNPs data is extensive. Due to the high sequence similarity between human and mouse at the nucleotide and amino-acid level, a knock-in of human SNPs can also be used to study the disease in mouse models. Information from SNPs data is crucial for the understanding of novel or existing mouse strains, QTL mapping for susceptibility to diseases of interest, evaluating copy number variants, applying SNP profiles to drug choices, etc. Our poster will demonstrate the SNPs search tool and function on MGI database. SNP data in MGI is supported by NIH grant HG000330.

M5107B Mouse Genome Nomenclature at MGI, Improved by Collaboration. *M. McAndrews*, D. Reed, J. Recla, C. Bult, J. Eppig. The Jackson Laboratory, Bar Harbor, ME.

The Mouse Genome Informatics (MGI, www.informatics.jax.org) group implements the rules and guidelines established by the International Committee on Standardized Genetic Nomenclature for Mice and maintains the international authoritative resource for the identity and names of mouse genes, genetic markers, alleles, chromosome aberrations, genomic features, and mouse strains. Nomenclature follows the established guidelines, but there is input from others to develop accurate and informative names and symbols that will be useful to and used by the scientific community.

Each gene and genome feature is given a genome feature type using the Sequence Ontology (SO, http://www.sequenceontology.org/browser/obob.cgi). Feature types provide information that cannot always be included in nomenclature. Example SO feature terms include protein-coding, miRNA, long noncoding RNA, and pseudogene. MGI petitions the SO to create additional feature types when new ones are necessary. Every mouse strain, allele, gene and genome feature receives a unique MGI_ID. Some, depending on the object, receive a list of synonyms including common 'lab' names, published alternate names, and previous official names. These aid researchers in identifying an object of interest in as many ways as possible. Designations have evolved for spontaneous, induced, targeted, endonuclease-mediated, and gene-trapped alleles of endogenous genes. Transgenic alleles have their own symbols distinct from the others. The different nomenclature rules for allele symbols help investigators distinguish among these types.

The representation of Quantitative Trait Loci or QTL is an area of focus undergoing refinement and expansion. QTL identified from literature need official nomenclature and mapping coordinates.

Robust homology information is crucial for translational researchers. MGI collaborates with human and rat nomenclature committees and subject matter experts to unify the nomenclature of homologs. MGI participates in the Consensus CDS (CCDS) project, a collaborative effort to identify a core set of human and mouse protein-coding regions that are consistently annotated and of high quality, hosted at NCBI https://www.ncbi.nlm.nih.gov/CCDS/CcdsBrowse.cgi. The Consensus CDS (CCDS) project is working toward convergence on a standard set of gene annotations. Updated nomenclature makes homology relationships clear.

This work is supported by NIH grant HG000330.

M5108C Informing the Genetic Basis of Disease: Informatics for The International Mouse Phenotyping Consortium. *T. Meehan*[1], A. Mallon[2], D. Smedley[3], H. Parkinson[1], on behalf of the MPI2 consortium. 1) EMBL-EBI, Hinxton, Cambridge, UK; 2) MRC Mammalian Genetics Unit, MRC Harwell, Harwell, UK; 3) Queen Mary University London, London, UK.

Attempts at correlating phenotypic aspects of disease with causal genetic variants are confounded by the lack of knowledge for most genes. The International Mouse Phenotyping Consortium (IMPC) is building the first truly comprehensive functional catalog of a mammalian genome by producing and characterizing a knockout mouse strain for every protein-coding gene. Data from a standardized, broad-based phenotyping pipeline are collected and archived for both male and female mice by the IMPC-Data Coordinating Center. Dedicated 'data wranglers' coordinate with each phenotyping center to ensure proper transfer and quality control of data. A sophisticated statistical analysis pipeline applies the best statistical test to the data and identifies knockout strains with significant changes while accounting for bias from confounding effects. In addition, hundreds of new disease models are computationally identified by analysis of phenotype overlap and automated 3-D embryo dysmorphology detection using software developed or adapted by the IMPC. With phenotype data now available for over 2600 genes at mousephenotype.org, this talk will focus on the new insights the IMPC is providing into development, fertility, disease and the wide-prevalence of sexual dimorphism.

M5109A The Systems Genetics Core Facility at UNC. *D. R. Miller*, T. A. Bell, S. E. Cates, C.-P. Fu, J. M. Holt, C.-Y. Kao, K. F. Manly, A. P. Morgan, G. D. Shaw, B. Wanstrath, L. McMillan, F. Pardo-Manuel de Villena. Univ of North Carolina at Chapel Hill, Chapel Hill, NC.

The Systems Genetics Core Facility at UNC provides Collaborative Cross mice; genotyping services using the MUGA arrays and tools to utilize both the mice and the genotypes.

The Collaborative Cross is a genetic reference population derived from eight inbred strains by an international consortium of researchers. The Systems Genetics Core Facility (SGCF; http://csbio.unc.edu/CCstatus/index.py?run=AvailableLines) at UNC distributes CC lines that have reached a defined minimum level of inbreeding. The SGCF has also reconstructed the founder mosaic of each CC line. We have combined these reconstructions with the whole genome sequence published by the Sanger Institute to generate pseudogenomes from each CC line that incorporate all high quality SNP and indel variants while retaining the extensive annotation of the mouse reference genome (http://csbio.unc.edu/CCstatus/index.py?run=Pseudo). We are currently sequencing mice from each of the distributable lines and expect to make those genomes available before the end of 2016.

The SGCF has distributed CC mice to >40 laboratories. CC projects fall into four categories: Strain surveys to determine the genetics of a wide variety of traits; Follow up experiments in smaller sets of CC strains; Development of models of human disease and Identification and genetic and molecular dissection of novel biological phenomena. Manuscripts using the CC have been published recently including a strain survey for

susceptibility of Ebola virus infection, a new mouse model for spontaneous colitis, the discovery of a meiotic drive system and a new parent of origin effect on gene expression. We will present summaries on the status of the CC population (number of lines, inbreeding and breeding performance); use of the CC and publications.

Genotyping arrays using iterations of the Mouse Universal Genotyping Array (MUGA) have been designed at UNC and implemented through GeneSeek (Neogen) in Nebraska. MUGA (7000 SNPs), MegaMUGA (70,000 SNPs) and GigaMUGA (150,000 SNPs) have each required separate and improved tools which are all available at http://csbio.unc.edu/CCstatus/index.py?run. The reissuance of the MUGA array can also be very useful for F2 analysis.

M5110B What's New in Mouse Genome Informatics (MGI)? *J. Richardson*, J. Kadin, M. Ringwald, J. Blake, C. Bult, J. T. Eppig, MGI Team. The Jackson Laboratory, Bar Harbor, ME.

Newly implemented in MGI in the last year: (1) MGI Gene Pages, the most frequently viewed pages in MGI are re-designed, including a new human disease table showing OMIM diseases with the human causitive gene, and human diseases modeled in mice with mutations in the gene being viewed, and Grid displays providing overviews of phenotypes, functional annotations, and embryonic gene expression in tissues [see poster Blake et al]; (2) Improvements to the Recombinase search, allowing users more specific parameter searching and providing autocomplete fields. The results summary also allows filtering and sorting of the data returned; (3) GO tables are enhanced to include a Category column from a GO slim subset of terms and a Context column providing details of the conditions used in the experiment [see poster Christie et al]; (4) MGI's Mouse dbSNP Query is re-designed to vastly improve performance. Users can now filter results by funtional class and re-order columns to reposition strains of interest. [see poster Law et al]; (5) A Batch Search utility has been added to the Gene Expression (GXD) Query to facilitate searches with lists of gene symbols or IDs. GXD searches return a tissue-by-gene matrix view as part of its multi-tabbed data summary [see poster Smith et al & Shaw et al].

MGI also is working with model organisms on collaborative projects: (1) A project to port individual organism InterMine instances (in MGI's case, MouseMine) to a shared Cloud environment to make accessibility across model organisms more transparent for users. (2) A project including MGI, RGD (Rat Genome Database) and DO (Disease Ontology) to enhance and improve DO depth and structure so that it can support disease annotations from mouse and rat. (3) A project including MGI and Wormbase to test and improve Textpresso as a literature triage tool that could save significant curator time in screening biomedical literature.

MGI also recently completed a major upgrade to its software infrastructure, porting nearly 200 tables in the database to Postgres and rewriting data loads, software for curation, and scripts supporting the web interface. Most MGI software now resides on the Jackson Laboratory server cloud. As a result we have improved the stability and speed of MGI and simplified future maintenance. Supported by NIH grants HG000330, HG002273, HD064299.

M5111C Mouse Genome Informatics tools for batch data searches and retrieval. *D. R. Shaw*, J. A. Blake, C. J. Bult, J. A. Kadin, J. E. Richardson, M. Ringwald, J. T. Eppig. Jackson Laboratory, Bar Harbor, ME.

Mouse Genome Informatics (MGI, http://www.informatics.jax.org) web resources provide free access to detailed information on the biology of the laboratory mouse. Searches of MGI are aided by the use of standardized nomenclatures for genes, alleles, and strains and by defined, hierarchical vocabularies: the Mammalian Phenotype (MP) Ontology, the Gene Ontologies (GO) and Mouse Developmental Anatomy. Data include over 291,000 MP annotations, 310,000 GO annotations and 1.5 million expression assay results.

MGI provides several tools for generating tab-delimited files of search results and for querying with lists of genes, alleles and IDs. Dozens of weekly database reports are available for download as well as full database dumps for MySQL and PostgreSQL. Data integration allows complex queries across multiple data sets and most web searches provide tab-delimited data export options. An easy to use Batch Query supports a variety of queries using IDs and symbols. An even more powerful tool, MouseMine, offers iterative querying, built-in enrichment analysis, and API support. A Batch Search for gene expression data returns a tissue-by-gene matrix view that facilitates a comparison of expression patterns between genes.

Live demonstrations of MGI resources are also available at the MGI Booth exhibit: 433. MGI's dedicated User Support group is available at mgi-help@jax.org.

This work is supported by NIH grants HG000330 and HD062499.

M5112A The Gene Expression Database (GXD): mouse developmental expression information at your fingertips. *C. M. Smith*, J. H. Finger, T. F. Hayamizu, I. J. McCright, J. Xu, J. T. Eppig, J. A. Kadin, J. E. Richardson, M. Ringwald. The Jackson Laboratory, Bar Harbor, ME.

The Gene Expression Database (GXD) is an easily searchable, freely available database of mouse developmental gene expression information (www.informatics.jax.org/expression.shtml). It provides researchers with critical insights into the function of genes and the molecular mechanisms of development, differentiation, and disease processes. GXD captures expression data from wild type and mutant mice. It integrates data from different assay types, including RNA *in situ* hybridization, immunohistochemistry, knock-in *in situ* reporter, RT-PCR, as well as Northern and Western blot experiments. Data are acquired from the literature and from groups engaged in large-scale expression studies. Currently GXD contains over 1.5 million expression results for over 14,000 genes. GXD contains detailed metadata recorded in standardized ways, including: time and tissue of expression (or non-expression); pattern and strength of expression; numbers and sizes of detected bands (for blots); genetic background of the samples; and a description of the probe/antibody used in the assay. These data are accompanied by over 275,000 images, allowing users to view the primary data and interpret it themselves. As an integral part of the Mouse Genome Informatics (MGI) resource, GXD integrates its expression data with other genetic, functional, phenotypic, and disease-oriented data, thus allowing researchers to evaluate expression data in the larger context; search by a wide variety of biologically- and biomedically-relevant parameters;

and discover new data connections to help in the design of new experiments. Recently developed features provide quick and intuitive access to the data. These include: interactive tissue-by-developmental stage and tissue-by-gene matrix views; image summaries defined by user search criteria; and data filters that can be used to iteratively refine search results. The expression section of MGI gene detail pages now features a graphical gene expression overview, as well as links to expression data from chicken, *Xenopus*, and zebrafish, enabling a gene-based comparison of expression information between mouse and these species. GXD is funded by NIH grant HD062499.

Population, Evolutionary & Quantitative Genetics Meeting

Poster Session Abstracts

P2001A Identifying population differentiation in the clam shrimp Eulimnadia texana through genome assembly and pooled sequencing. *J. G. Baldwin-Brown*[1], A. D. Long[1], S. C. Weeks[2]. 1) University of California, Irvine, Irvine, CA; 2) University of Akron, Akron, OH.

Identifying population differentiation at specific sites across the genome in non-model organisms has been difficult historically. Here, I present the results of a combination of approaches to convert the non-model organism Eulimnadia texana (the clam shrimp) into a practical system for genomics, and show evidence of differentiation of genomic sites apparently due to adaptive evolution. Clam shrimp are attractive for genetics because of their small genome size (150Mb), large populations, short generations (~3 weeks), and their ability to lay eggs that can remain in diapause for decades without loss of viability. I generated a whole genome, hybrid de novo assembly of the clam shrimp genome (assembly N50: 18Mb) using a combination of Illumina short read genomic data and PacBio long read data. I used Illumina RNA sequencing of both male and hermaphrodite clam shrimp in order to annotate the genome, then compared the genome to those of Drosophila melanogaster and the closest sequenced relative of the clam shrimp, the water flea Daphnia pulex, and used differential gene expression analysis to identify genes that significantly differ in expression between males and hermaphrodites. I generated allele frequency estimates from pooled sequencing data from 11 separate wild populations of clam shrimp and estimated average linkage disequilibrium, FST, and other classic population genetics statistics. Both bayesian methods and FST analysis of populations revealed candidate selection sites. I correlated these with a variety of environmental factors, including presence of predators, vernal pool dimensions, and ratio of clam shrimp males to hermaphrodites. I identified multiple sites that appear significantly diverged and that have annotated genes that may be under adaptive evolution in these populations.

P2002B Genetic characterization of populations of the African Jewelfish (*Hemichromis letourneuxi*) introduced to the waterways of Florida. *N. M. Belfiore*[1], P. J. Schofield[2]. 1) University of Tampa, Tampa, FL; 2) US Geological Survey, Gainesville FL.

The African jewelfish, *Hemichromis letourneuxi*, is an invasive, predatory cichlid that has been introduced at least once in the 1960s to Florida. Its native range is in freshwater bodies west of the Red Sea, spanning Egypt, North Sudan, and Eritrea in northeastern Africa, and thus it is likely adapted to a variety of tropical and subtropical habitats. It was first encountered, introduced by unknown means, in the Miami area in the 1960s. In subsequent decades, the fish has been encountered in waterways spreading west and north rapidly. It is tolerant of a wide range of aquatic conditions, freshwater and brackish, including shallow, vegetated or rocky areas of canals, tidal creeks, culverts, rivers, and marshes. This invasive species is a threat to other aquatic species, including native fishes, shrimp and snails because of its predatory behavior. We compare mitochondrial and nuclear DNA sequence data from jewelfish sampled in six Florida populations, spread throughout the current range of this introduced species. Using gene and species tree reconstruction methods, we are hypothesizing the historical relationships among jewelfish populations using Bayesian phylogenetic reconstruction and concordance methods. In conjunction with geographic information and the timing of arrival of this species in different drainages, we estimate the number of introductions of jewelfish from unique backgrounds, the times of introduction(s), the mode of spread, and where the initial introduction(s) occurred. Principles learned through understanding the history of this species could help to prevent future introductions of invasive species.

P2003C *Speckled feathers and bladder eyes: pleiotropic effects of the Almond mutation in pigeon*. *R. L. Bruders*[1], E. J. Osborne[2], Z. Kronenberg[2], M. Yandell[2], M. D. Shapiro[1,2]. 1) Department of Biology,University of Utah, Salt Lake City, UT; 2) Department of Human Genetics,University of Utah, Salt Lake City, UT.

The molecular basis of phenotypic diversity in wild and domesticated animals is poorly understood. My project addresses how diversity arises by identifying genetic and developmental origins of unique traits. The domestic rock pigeon (*Columba livia*) is a compelling model to understand molecular mechanisms of diversity because this species consists of over 300 different breeds with spectacularly variable phenotypes. One derived trait, “Almond,” is characterized by seemingly random sprinkling of pigmented and apigmented regions within and between feathers throughout the body. Classical genetic studies suggest Almond feather pigmentation is caused by a dominant sex-linked mutation located near the major color locus, recently identified by our lab as Tyrp1. Additionally, these studies found that homozygous Almond males (ZZ sex chromosomes) develop severe eye defects and completely lack pigmentation whereas hemizygous Almond females (ZW), which lack a wild-type copy of the Almond allele, do not develop these defects. This suggests that dosage of the mutant allele, rather than absence of the wild-type allele, is responsible for these eye phenotypes. We compared the genomes of 10 Almond pigeons to 76 non-almond pigeons of various colors using pFst in order to identify a candidate region on scaffold 6 a Z chromosome scaffold. Further investigation showed a substantial increase in coverage in this region in Almond birds, indicative of copy number variation in this region. There are 5 genes in this region including MLANA, a melanosome maturation gene. Expression analysis using Q-RT-PCR on regenerating feathers revealed a suprising decrease in MLANA expression in the light feathers of Almond birds despite a increase in copy number. Currently no coding changes have been found in the genes in this region, indicating that substantial increase in copy number of MLANA leads to the sprinkling feathers and eye defects seen in Almond pigeons.

P2004A *CYP2D6*: Detecting New Structures for Clinical Practice. *B. Carvalho Henriques*[1*], Y. Wang[1], R. Whitford[1], CJ Slomp[1], D. Rossolatos[1], J. Paya-Cano[2], S. Curran[2], P. Santosh[2], IW Craig[2], KJ Aitchison[1,2]. 1) University of Alberta Edmonton, Canada; 2) SGDP Centre London, UK.

Background

A gene that has been the focus of extensive pharmacogenomic research is the cytochrome P450 enzyme 2D6 (CYP2D6), which is highly polymorphic. Identifying how different alleles impact phenotype in terms of metabolism is desirable because CYP2D6 is involved in the metabolic pathway of up to 50 different drugs currently used in medicine. This project continues work that identified individuals with

anomalous copy number calls[1], that might indicate combinations of *CYP2D6* with its adjacent gene (*CYP2D7*), known as hybrid alleles. Our objective is to identify precisely which such hybrid variants are present. In this manner, the improvement in technology gained will enable correct identification of a wider range of variants of this enzyme than was previously possible, for translation into clinical practice in the form of more accurate pharmacogenetics testing.

Methods

The methodology applied was a long-PCR approach to identify hybrid alleles of *CYP2D6* using the technique described by Kramer *et al*. (2009)[2] and Black *et al*. (2012)[3], with fragment delineation by both agarose gel and Agilent 2100 Bioanalyzer (Agilent Technologies, Canada) electrophoresis.

Results

Results showed some successful amplification for the conditions established. Comparison made between different runs point to effective changes to the initial method. The technique employed is a modified version of that described by Kramer *et al*., using a buffer that is specific for GC-rich regions. Preliminary results have confirmed the method application in gene characterization, having successfully amplified structures inherent to *5-like genotype

Acknowledgements

BCH held an Undergraduate Research Initiative (URI) Summer Studentship and is now funded by an Alberta Centennial Addiction and Mental Health Research Chair fund held by KJA. A sample processed was collected as part of Workpackage 3 of the STOP study (www.stop-study.com), funded by the European Commission's 7th Framework Programme, grant agreement 261411.

P2005B Evolutionary implications of recombination rate variation among populations of *Drosophila melanogaster* . *J. Cruz Corchado*[1], J. Comeron[1,2]. 1) Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA; 2) Department of Biology, University of Iowa, Iowa City, IA.

Recombination is a crucial biological process and one of the most fundamental parameters in evolution. Under most conditions, meiotic recombination is essential for ensuring that organisms adapt to ever changing biotic and abiotic conditions and, as such, it shapes evolutionary change within and between species. Yet, and despite its importance, recombination rates are fast evolving and vary between closely related species. In this study, we estimate and compare recombination rates in five *Drosophila melanogaster* populations (Zambia, Rwanda, Cameroon, France and USA). We observe that recombination rates not only change in total magnitude but also in their relative distribution within chromosomes (recombination landscapes). We also show that differences in recombination landscapes among populations play a significant role explaining population-specific differences in nucleotide diversity without requiring adaptive events (local adaptation). Our results suggest that inter-population differences in local recombination rates and the corresponding differences in local Background Selection (BGS) need to be considered as a possible explanation for population-specific differences in nucleotide diversity at specific genomic regions.

P2006C The Effects of Demographic History on the Detection of Recombination Hotspots. *A. L. Dapper*, B. A. Payseur. University of Wisconsin - Madison, Madison, WI.

In many species, meiotic recombination is concentrated in small genomic regions. These "recombination hotspots" leave signatures in fine-scale patterns of linkage disequilibrium, raising the prospect that the genomic landscape of hotspots can be characterized from sequence variation. This approach has led to the inference that recombination hotspots evolve rapidly in some species, but are conserved in others. Historic demographic events, such as population bottlenecks, are known to affect patterns of linkage disequilibrium across the genome, violating population genetic assumptions of this approach. Such events are prevalent, yet demographic history is generally unaccounted for when making inferences about the evolution of recombination hotspots. To determine the effect of demography on the detection of recombination hotspots, we use the coalescent to simulate haplotypes with a known recombination landscape. We measure the ability of popular linkage disequilibrium-based programs to detect recombination hotspots under different demographic histories, including population bottlenecks, hidden population structure, population expansions and population contractions. We find that demographic events, and in particular, population bottlenecks and exponential population growth, have the potential to greatly reduce the power to discover recombination hotspots, in some cases by up to 90%. Furthermore, demographic events also have the potential to increase the false positive rate of hotspot discovery. In the worst-case scenario, long, slow population contractions quadruple the frequency of false positives, even under stringent significance cutoffs. We tested whether simple genomic parameters, such as nucleotide diversity or heterozygosity, could be used to predict the reduction in power due to non-equilibrium demographic histories. We found that neither the power, nor the false positive rate, of hotspot detection could be predicted without also knowing the demographic history of the sample. Our results suggest that ignoring demographic history likely overestimates the power to detect recombination hotspots and underestimates the degree to which recombination hotspots are shared between closely related species. We make specific recommendations for how demographic inference can be incorporated into population genetic inferences about recombination hotspots.

P2007A Exogenous RNA in the serum of healthy persons. *Supriyo De*[1], Douglas Dluzen[2], Alan B. Zonderman[3], Toshiko Tanaka[4], William H. Wood[1], Luigi Ferrucci[4], Michele K. Evans[2], Kevin G. Becker[1], Nicole Noren Hooten[2]. 1) Gene Expression and Genomics Unit, Laboratory of Genetics, National Institute on Aging, NIH, Baltimore, MD; 2) Health Disparities Research Section, Laboratory of Epidemiology and Population Science, National Institute on Aging, NIH, Baltimore, MD; 3) Behavioral Epidemiology Section, Laboratory of Epidemiology and Population Science, National Institute on Aging, NIH, Baltimore, MD; 4) Longitudinal Studies Section, Translational Gerontology Branch, National Institute

on Aging, NIH, Baltimore, MD.

The discovery of circulating cell-free DNA (exDNA) and RNA (exRNA) in human body fluids, including serum, has sparked great interest in using these nucleic acids as markers of disease, as diagnostic tools or as therapeutic molecules. Here, we examined the exRNA/exDNA profiles in community-dwelling individuals. Surprisingly, we found a considerable percentage of sequenced extracellular DNA/RNA remains unaligned to the human genome/transcriptome compared to DNA/RNA extracted from cells. In this project, we have tried to identify the nature of the unaligned reads after filtering out the sequencing adapters and primers. Total RNA was extracted from the serum of 10+ healthy young individuals and 10+ healthy old individuals and total RNA-seq on the Ion Torrent Proton sequencer was performed using a modified protocol to obtain both small and large sized RNAs. The reads were first aligned to the human genome (hg19) using a two-step process with Tophat2 and Bowie2 (not presented here) and unaligned reads were aligned to all known organisms in the Refseq v70 database (54,118 organisms) encompassing prokaryotes (archaea and bacteria) and eukaryotes (fungi, plants and vertebrate & invertebrate animals). Kraken software was used to align against 618.8 billion DNA sequences and 24.2 billion RNA sequences in a sequential manner of the different kingdoms of life. The results surprisingly show that different individuals have different percentages of fragments of DNA and/or RNA from numerous organisms. Some of the organisms identified are bacteria (including Clostridium sp. and Pseudomonas sp.), fungi (including Malassezia sp., Saccharomyces), plants (such as corn, tobacco, poplar etc.), insects (including mites, deer tick and body lice), vertebrates (such as turkey and cow). This suggests that the species we are identifying in serum may give important information about diet and behavior among the individuals studied. Many of the sequenced reads from the serum of different individuals align to specific regions of the genome of an organism (e.g. Pseudomonas aeruginosa), indicating that perhaps these fragments (kilobases in size) are particularly more stable, which allows them to withstand degradation in body fluids. Thus, far we have identified these organisms through computational methods, but further experimental validation will be performed.

Acknowledgement: This research was supported by Intramural Research Program of the National Institute on Aging, NIH.

P2008B Genetic variation, population structure, and genome assembly of the threatened Neosho madtom catfish (*Noturus placidus*). *J. E. Decker*[1,2], L. K. Whitacre[1,2], M. L. Wildhaber[3], G. S. Johnson[4], J. M. Downs[5], T. Mhlanga-Mutangadura[4], V. M. Tabor[6], D. Fenner[7]. 1) Informatics Institute, University of Missouri, Columbia, MO; 2) Division of Animal Sciences, University of Missouri, Columbia, MO; 3) U.S. Geological Survey, Columbia Environmental Research Center, Columbia, MO; 4) Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, MO; 5) The Peoria Tribe of Indians of Oklahoma, Miami, OK; 6) U.S. Fish and Wildlife Service, Kansas Ecological Services Field Office, Manhattan, KS; 7) U.S. Fish and Wildlife Service, Oklahoma Ecological Services Field Office, Tulsa, OK.

The Neosho madtom (*Noturus placidus*) is a small catfish, generally less than 3 inches in length, unique to the Neosho-Spring River system within the Arkansas River Basin. It was federally listed as threatened in 1990, largely due to habitat loss. As part of conservation efforts, we generated whole genome Illumina paired-end sequence data from ten Neosho madtom (average 39X coverage) originating from three geographically separated subpopulations to evaluate genetic diversity and population structure. One slender madtom (*Noturus exilis*) was also sequenced as an outgroup. Lack of a reference genome necessitated variants be discovered using De Bruijn graphs implemented in CORTEX v1.0.5.21. Approximately 1.64 million high confidence single nucleotide polymorphisms (SNPs) were observed. Only 86,155 SNPs were variable across the Neosho madtoms sequenced, indicating overall low level genetic diversity. While principal component analysis based on these genotypes accurately clustered individuals from the same location together, insignificant eigenvalues indicated weak population structure, suggesting these subpopulations are genetically compatible for reintroduction among these three locations. We also completed a draft *de novo* assembly of the Neosho madtom genome from 120X of sequences pooled across 3 individuals. We assembled the ~1 Gb genome into 149,885 contigs with a N50 of 12,261 bp. Using only 50X coverage of paired-end and mate pair data from a single fish, we were able to assemble the genome into 68,147 scaffolds with an scaffold N50 of 120 kb, demonstrating the value in assembling a genome from a population that is closely related to a species of economic interest (i.e., channel catfish, *Ictalurus punctatus*) but has lower genetic diversity and is easier to assemble. Ongoing efforts aim to improve the assembly by using sequences from DISCOVAR recipe libraries (250 bp paired-end reads), assembled with DISCOVAR de novo and MaSuRCA. The ability to generate a reference genome allows us to use demographic models, such as PSMC, to compare the effective population sizes between the Neosho madtom and the slender madtom. With genomic tools developed, we can now better manage this endangered species and investigate the basic biology of why such a low-diversity species can subsist.

P2010A Population Genetic Analysis of Autophagy and Phagocytosis genes in *Drosophila melanogaster*. *J. Im*, B. Lazzaro. Cornell University, Ithaca, NY.

Phagocytosis and autophagy are cellular mechanisms that recognize and eliminate pathogens from the extracellular space and cellular interior, respectively. These mechanisms, as a part of the immune system, defend the host from a wide range of ever-changing pathogens that evolve to escape, resist or compromise host immunity. Some bacteria use effector proteins that manipulate host autophagy and phagocytosis to either inhibit immune defense or to promote bacterial invasion into the interior of the cell. In response, the host may experience selective pressure to adapt. This dynamic conflict may result in coevolution, leading to recurrent positive directional selection on host genes. Therefore, we hypothesize that host phagocytosis and autophagy genes may have experienced more positive selection than other genes in the genome. We specifically hypothesize that genes that are known to interact with bacterial factors are most likely to have undergone positive selection.

To test this hypothesis, we perform population genetic analyses on a curated set of phagocytosis and autophagy genes, as well as control genes that are matched with case genes by gene length and genome location, using 197 *Drosophila melanogaster* sequences from the Drosophila Genome Nexus Project. Initial analyses suggest strong, recent selection on a subset of autophagy genes, including Atg8a and Atg13.

Atg8a is known to be involved in the expansion of autophagosome, while Atg13 is involved in autophagosome induction. Previous work has shown that an effector protein from the intracellular pathogen *Legionella pneumophila* inhibits host autophagy by blocking the proper conjugation of Atg8a with Atg3 and Atg7, supporting our hypothesis that Atg8a may be a target of host-pathogen coevolution. This is the first study to examine the evolution of non-receptor phagocytosis and autophagy genes involved in immune defense and will provide a novel insight into the evolution of the innate immune responses.

P2011B Insertion polymorphisms of mobile elements in sexual and asexual populations of *Daphnia pulex*. *x. jiang*, H. Tang, M. Lynch. Indiana University, Bloomington, IN.

Transposable elements (TEs) constitute a substantial portion of many eukaryotic genomes, and can in principle contribute to evolutionary innovation as well as genomic deterioration. *Daphnia pulex* serves as a useful model for studying TE dynamics as a potential cause and/or consequence of asexuality. We analyzed insertion polymorphisms of TEs in 8 sexual and 8 asexual isolates of *D. pulex* from whole-genome sequencing data. Our results show that the total fraction of the derived sequences of TEs is not substantially different between asexual and sexual *D. pulex* isolates. However, in general, sexual clones contain fewer common (fixed) TE insertions, and in the mean time, demonstrate higher insertion polymorphisms than in asexual clones, supporting the hypothesis that sexual reproduction facilitates the spread of TEs while limiting the deleterious load imposed by TE insertions. We identified a subset of asexual-specific TE insertions, including the insertions of 20 LTR retrotransposons, two non-LTR retrotransposons and five DNA transposons. By comparison, no sexual-specific TE insertions were observed in our analysis. Furthermore, 24 out of the 27 asexual-specific insertion sites are located in contigs from chromosomes VIII, IX, and X that are known to be associated with obligate asexuality in *D. pulex* and partially derived from *D. pulicaria.* We found that 18 out of the 27 asexual-specific TE insertions can also be detected in some *D. pulicaria* isolates, indicating only a small number of TE insertions have likely accumulated after the origin of obligate asexuals.

P2012C The Relationship between Host Genetic Architecture and Pathogen Susceptibility in Caenorhabditis elegans. *L. M. Johnson*, C. F. Baer, J. M. Ponciano, S. A. Rouse. University of Florida, Gainesville, FL.

The association between host genotype and the susceptibility to and clinical manifestation of particular pathogens is well-documented, but the particulars of this relationship are rarely understood. A vital first step in better understanding the genetic basis of pathogen susceptibility is analyzing the role of mutation. This study aims to quantify the per-generation input of genetic variance by mutation for susceptibility of *Caenorhabditis elegans* to *Pseudomonas aeruginosa*. A set of mutation accumulation (MA) lines, which have undergone 250 generations of single hermaphrodite decent, along with their ancestral parent, were infected with *P. aeruginosa*. A chain binomial model was then used to estimate the average survival age of all infected individuals. A significant difference was seen in survival probability between ancestral worms and those which had undergone 250 generations of mutation accumulation, with MA worms showing a decreased time until death. We will also report line mean and variance for MA and control lines tested.

P2013A Characterization of Genic Microsatellite Markers (EST-SSRs) in the Endangered Tree *Quercus georgiana*. *PRIYANKA KADAV*. MICHIGAN TECHNOLOGICAL UNIVERSITY, HOUGHTON, MI, USA.

Characterization of Genic Microsatellite Markers (EST-SSRs) in the Endangered Tree *Quercus georgiana*. PRIYANKA KADAV[1], MURPHY WESTWOOD[2], ANDREA KRAMER[3], JEREMIE FANT[3], ANDREW HIPP[2], RAAKEL TOPPILA[2], SEAN HOBAN[2], OLIVER GAILING[1], [1]Michigan Technological University, [2]The Morton Arboretum, [3] Chicago Botanic Garden

Quercus georgiana is an endangered endemic oak species that exists only in a few scattered populations in the southeastern US. The aim of the project is to characterize genic microsatellite markers (EST-SSRs) in this endangered tree species in four populations from Georgia. A total of 27 EST-SSRs have been tested for locus-specific amplification in eight *Q. georgiana* samples from the four different populations, out of which 12 have been selected based on the amplification of a single polymorphic gene locus for the analysis of all 300 samples. Samples were amplified in a GeneAmp PCR system 2700 using the EST-SSRs. PCR amplicons were separated on an ABI 3730 Genetic Analyzer with the internal size standard GS-LIZ-500 and scored with GeneMarker V.2. 6.7. An initial assessment of the genetic variation parameters expected heterozygosity (H_e), observed heterozygosity (H_e) and number of alleles per locus (N_a) was done in GENAlEx6 using EST-SSRs. In addition, the inbreeding coefficient F was calculated for each marker in GENAlEx. Observed heterozygosity (Ho) and expected heterozygosity (He) for individual markers ranged from 0.273 to 1.000 and from 0.247 to 0.867, respectively. The number of alleles per locus (Na) ranged from 2 to 11. Genetic differentiation among populations (F_{ST}=0.067) was comparatively high with pairwise F_{ST} values ranging from 0.024 to 0.119. The mean genetic variation for the four populations fragments was 0.677 (H_o) and 0.655 (H_e). High genetic differentiation between neighboring populations could indicate genetic drift.

P2014B Population genomics of *Fusarium graminearum* head blight pathogens in North America. *A. Kelly*, T. Ward. USDA-ARS, Peoria, IL.

In this study we utilized comparative genomics to identify candidate adaptive alleles in the fungus *Fusarium graminearum,* the primary pathogen of Fusarium head blight (FHB) in cereal crops. Recent epidemics of FHB have been economically devastating to agriculture, as *F. graminearum* reduces cereal yield and contaminates grain with harmful mycotoxins. We performed Illumina whole-genome sequencing (50X coverage) on 60 *F. graminearum* strains from the US and Canada, and then utilized a combination of reference-based mapping and *de novo* sequence assembly to assess genomic diversity and differences in gene content. Bayesian clustering and phylogenetic analyses based on 505,748 SNPs revealed three major *F. graminearum* populations. Isolates with the recently discovered NX-2 toxin type were genetically distinct

from the resident and emergent populations that are typically associated with FHB in North America. However, 8 out of 20 NX-2 isolates shared a substantial proportion of their ancestry (63% on average) with resident isolates that have the 15ADON toxin type. High-resolution genome scans of diversity revealed multiple loci that exhibited genetic signatures consistent with adaptive divergence, in that these regions were highly differentiated among populations, had an excess of rare variants and showed reduced intrapopulation diversity. Candidate selected regions included the trichothecene toxin gene cluster encoding the core metabolic enzymes responsible for structural differences among toxin types. A second locus encoded a protein kinase (PKS3) involved in fruiting body pigmentation and a type of RNA helicase that has proven critical for cold adaptation in other fungal species. A third locus encoded four uncharacterized proteins with homology to primary metabolic enzymes. *De novo* assembly of unmapped reads identified 22,788 contigs (average length 618 bp) that were missing or highly diverged from the *F. graminearum* reference genome. Though most unmapped contigs (87%) were found in a single isolate, we detected 162 genes that were widely distributed and differentially maintained across populations. These population-enriched genes showed homology to proteins in other phytopathogenic fungi with predicted functions in secondary metabolism, fungal self/non-self recognition and fungal cell-wall degradation. Our findings suggest that cold tolerance and trichothecene toxin diversity may be driving local adaptation in *F. graminearum* populations. Furthermore, differences in gene content suggest that competitive interactions with other fungi and secondary metabolite diversification have shaped the population genome of *F. graminearum*, and may be contributing to differences in how these pathogens exploit the agricultural landscapes of North America.

P2015C The effects of linked selection on *Capsella grandiflora*. *Tyler Kent*, Stephen Wright. University of Toronto, Toronto, Ontario, Canada.

The pervasiveness of the effects of selection on neutral and total genetic diversity in species is a major question in evolutionary genetics. It is becoming increasingly understood that selection can greatly alter the local coalescent histories of genomic regions linked to selected loci, which are otherwise considered to evolve neutrally. This can have major impacts on estimates of summary statistics and demography and can bias population genetic models. Additionally, in plants, linked selection studies to date have mainly focused on species-wide datasets, which may lead to underestimation of the impact of linked selection on local populations. Here we present a model fitting of linked selection to a sample of 189 individuals from a single population of the obligate outcrossing plant model species Capsella grandiflora. We show that linked selection has wide-ranging effects on genetic diversity, and compare our results to similar findings in the highly self-fertilizing Arabidopsis thaliana. Improved knowledge of local coalescent histories and landscapes of genetic diversity in this model species will improve the quality of future population genetic analyses as well as guide future studies on the relative impacts of positive and negative selection and genetic drift in a widely applicable plant family..

P2016A Exact calculation of the joint site frequency spectrum for generalized isolation with migration models. *Andrew Kern*. Rutgers University, Piscataway, NJ.

Population genomic datasets collected over the past decade have spurred interest in developing methods that can utilize massive numbers of loci for inference of demographic and selective histories of populations. The site frequency spectrum (SFS) provides a convenient framework for such analysis and accordingly much attention has been paid to predicting theoretical expectations of the SFS under a number of different models. However, to date, exact solutions for the joint SFS of two or more population under models of migration and divergence have not been found. Here we present a novel Markov chain representation of the coalescent on the state space of the joint SFS that allows for rapid, exact calculation of the joint SFS under generalized isolation with migration (IM) models. In turn, we show how our Markov chain method, in the context of composite likelihood estimation, can be used for accurate inference of parameters of the IM model using SNP data. Lastly, we apply our method to recent whole genome datasets from *Drosophila melanogaster* and human populations.

P2017B The evolution of herbicide resistance in an agricultural weed, *Capsella bursa-pastoris*. *Julia Kreiner*, John Stinchcombe, Stephen Wright. University of Toronto, Toronto, Ontario, CA.

How much is adaptive evolution driven by local *de novo* mutation, selection on standing variation, or gene flow from other populations? Weed populations regularly sprayed with herbicides generally experience stronger and more predictable selection than natural populations, and have well-characterized target loci subject to selection, making them an interesting model for understanding the population genomics of adaptation. Here we investigate the population genomics of resistance to acetolactate synthase (ALS) inhibitor herbicides in the agricultural weed *Capsella bursa-pastoris* across a broad geographic range in Alberta and Saskatchewan, Canada. We genotyped 96 individuals via genotype-by-sequencing (GBS) and sequenced whole genomes from 192 individuals distributed among two pools collected from low and high resistance geographic regions. We compared the number of independent resistance mutations and the frequency of resistance mutations from our pooled-seq data to population structure estimates from our GBS data. We infer the extent to which herbicide resistance adaptation was driven by local vs. global selective sweeps, and/or from selection on standing genetic variation. The evolution of resistant agricultural weeds in response to single target herbicides is becoming increasingly common since reports were first made in 1968 (Jasieniuk et al., 1996) and underlines the importance of understanding how the evolutionary rescue of populations proceeds under increasing levels of stress. The origins and spread of herbicide resistance alleles is important for contemporary weed management, food security, and conservation.

P2018C ***Saccharomyces eubayanus*** **population dynamics in nature and industry.** *Quinn Langdon*[1], David Peris[1], Ryan V. Moriarty[1], Kayla Sylvester[1], Guillaume Charron[2], Jean-Baptiste Leducq[2], Christian R. Landry[2], Diego Libkind[3], Chris Todd Hittinger[1]. 1) University of Wisconsin-Madison, Madison, WI; 2) Université Laval, Québec City, Canada; 3) CONICET-UNComahue, Bariloche, Argentina.

Members of the *Saccharomyces* genus have played a key role in genetic research and are widely utilized in industrial applications. However, much is sill unknown about their wild population structure, genetic diversity, and domestication for fermentation applications. One species in this genus, *Saccharomyces eubayanus*, is a parent of the hybrid lager yeasts and, despite its important role in brewing, in the wild, it has never been isolated in Europe and is mainly isolated in Asia and South America. *S. eubayanus'* role in industry and its global distribution allows us to ask evolutionary questions of domestication and wild population structure across different time scales from ancient to modern. Utilizing whole genome sequencing data and population genomic techniques, we have begun to disentangle *S. eubayanus*' current demographics and elucidate the genetic diversity at the time of hybridization to form lager yeasts. Analysis of all genomes currently available shows two major populations of *S. eubayanus;* with evidence of an ancient population split, followed by subsequent secondary contact and gene flow primarily into only one of the populations. Within one of the major populations there are several subpopulations whose genetic diversity and geography inform our understanding of its complex past and present demography. One subpopulation has only been found in the Holarctic ecozone and includes the lager lineages. From this subpopulation, we can infer the diversity of the ancient subpopulation that gave rise to lager yeasts despite having few modern representatives of this subpopulation. Another subpopulation contains closely related strains largely from Patagonia, but also Oceania and North America suggesting the potential for migration and niche expansion. Finally, adding to our understanding of recent population dynamics is enhanced by a lineage that is the result very recent admixture of the two the major populations. Interestingly this lineage is found in two locations in North America, suggesting that this admixed group can thrive outside of Patagonia and raising questions of the migratory possibilities of this yeast. *S. eubayanus'* dynamic population structure has allowed us survey ancient to modern gene flow and work back in time to illuminate our knowledge of the subpopulation that gave rise to lager yeasts. Understating the modern population structure and diversity of industrially important yeasts informs our understating of both the wild dynamics of yeasts and the different paths taken to domesticate this important genus.

P2019A A Haplotype Method Detects Diverse Scenarios of Local Adaptation from Genomic Sequence Variation. *Jeremy Lange*, John Pool. University of Wisconsin-Madison, Madison, WI.

Identifying genomic targets of population-specific positive selection is a major goal in several areas of basic and applied biology. However, it is unclear how often such selection should act on new mutations versus standing genetic variation or recurrent mutation, and furthermore, favored alleles may either become fixed or remain variable in the population. Very few population genetic statistics are sensitive to all of these modes of selection. Here we introduce and evaluate the Comparative Haplotype Identity statistic (χ_{MD}), which assesses whether pairwise haplotype sharing at a locus in one population is unusually large compared with another population, relative to genome-wide trends. Using simulations that emulate human and *Drosophila* genetic variation, we find that χ_{MD} is sensitive to a wide range of selection scenarios, and for some very challenging cases (*e.g.* partial soft sweeps), it outperforms other two population statistics. We also find that, as with F_{ST}, our haplotype approach has the ability to detect surprisingly ancient selective sweeps. Particularly for the scenarios resembling human variation, we find that χ_{MD} outperforms other frequency and haplotype-based statistics for soft and/or partial selective sweeps. Applying χ_{MD} and other between-population statistics to published population genomic data from *D. melanogaster*, we find both shared and unique genes and functional categories identified by each statistic. The broad utility and computational simplicity of χ_{MD} will make it an especially valuable tool in the search for genes targeted by local adaptation.

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P2020B How a Framework for Evolutionary Systems Biology Can Accelerate Reproducible Modeling of Mechanistic Fitness Landscapes. *Laurence Loewe*. Laboratory of Genetics and Wisconsin Institute of Discovery, University of Wisconsin-Madison, Madison, WI.

Systems approaches are becoming increasingly important, as population genomics aims to understand factors shaping the reproduction and survival of genotypes based on the phenotype they encode and their environment. Current interest in personalized medicine, disease prediction and understanding GxPxE interactions can easily be frustrated by the computational work required and by the limitations of current tools, which often excel at the specialized tasks they were written for, but are of little use for closely related tasks that often only differ in minor details. Considering the computational challenges of mechanistically predicting phenotypes, diseases or fitness in non-trivial systems raises the question, how often it will be possible to extract reliable biology from simulations in which signals can be hard to separate form the noise of mishandling data, numerical errors, bugs, logic errors and more. Sound programming and modeling advice emphasize the importance of good questions to solve specific problems. This often leads to results or tools that are highly specific to some type of question, ignoring other closely related questions. Asking such questions often triggers so much code-wrestling, data shoveling, version wrangling and other silicon digging, that little time remains for considering big picture questions, such as how the relentless New Evolutionary Synthesis that started in the 1920s can be advanced to the point where fitness landscapes become useful in the real world.

To facilitate the development of computational tools for ultimately enabling interactive "flight simulators for mechanistic fitness landscapes", it is pivotal to understand the corresponding requirements and to provide a sound and usable conceptual framework that is stable enough to serve as a solid basis for writing long-term backwards compatible code that is reproducible enough to enable efficient model-reuse, so researchers can focus again on the biology instead of resolving tedious computational problems.

Earlier work, discussions at a series of EvoSysBio meetings, and newer advances enabled the development of such a framework for

Evolutionary Systems Biology that combines the rigor and expertise of molecular biology and other Intra-Organism Biology results with the rigor of models in Population-Genetics Biology and other Trans-Organism Biology. The result is a redefinition of EvoSysBio that lays out in much improved clarity and precision the long road to enabling fully automated mechanistic predictions of phenotypes and diseases from genotypic information. Cancer cell evolution will used as an example.

P2021C Program number not assigned.

P2022A Genotype calling from population-genomic sequencing data. *T. Maruki*, M. Lynch. Indiana University, Bloomington, IN.

Genotype calling plays important roles in population-genomic analyses. Although many statistical methods have been developed, the performance of the widely used genotype-calling methods is not understood well, especially when the population deviates from Hardy-Weinberg equilibrium (HWE). In this study, we develop a maximum-likelihood (ML) method for calling genotypes that incorporates population-level prior estimates of genotype frequencies and error rates to improve the accuracy of genotypes called from low-coverage sequencing data. We compare the performance of the proposed method with that of GATK and Samtools using computer simulations under genetic conditions where the population may deviate from HWE. The results show the proposed method yields more accurate called genotypes than the currently widely used methods.

In addition to the method for low-coverage sequencing data, we develop another ML method for calling genotypes from high-coverage sequencing data, which does not require prior population-level estimates and enables identification of polymorphisms with arbitrary number of alleles. Using computer simulations, we examine when the coverage is high enough to accurately characterize polymorphisms using the proposed method. Taking the results of the performance evaluation into account, we apply the proposed method to high-coverage (mean 18×) whole-genome sequencing data of 83 clones from a population of *Daphnia pulex*. Our results using multiple procedures for minimizing analyzing sites with mismapped reads indicate that a nonnegligible fraction of polymorphisms in this species is triallelic, demonstrating the importance of relaxing the assumption of biallelic polymorphisms. Because of the efficiency and flexibility, the proposed method can in principle be extended to population-genomic analyses of polyploid data. As an example, we extend it to analyses of triploid sequencing data. Using computer simulations, we examine the performance of the proposed method. Our results show that calling accurate genotypes from triploid sequencing data requires much higher coverage than that from diploid sequencing data, which will help researchers to design sequencing strategies for population-genomic studies in polyploid organisms.

P2023B Whole animal genetics-by-sequencing approaches to investigate starvation resistance. *B. T. Moore*[1], J. M. Jordan[1], E. A. Bowman[2], R. Chitrakar[1], A. Hung[1], R. E. W. Kaplan[1], J. D. Hibshman[1], L. R. Baugh[1]. 1) Duke University, Durham, NC; 2) Vanderbilt University, Nashville, TN.

The advent of high coverage and low cost sequencing technologies has allowed for newer and more powerful approaches in molecular and population genetics. Transposon sequencing, where genome-saturated mutant populations' allele frequencies are measured before and after selection, functionally characterizes each and every gene in the genome in a single experiment. The approach has been successfully applied to a variety of phenotypes in a variety of unicellular systems: growth and motility in *E. coli*, synthetic genetic interactions in yeast, and pathogen-resistance in mammalian cell lines. However, transposon insertion typically produces null alleles, which can be valuable to identify gene function, but evolutionary insight relies on identification of naturally occurring polymorphisms affecting the trait of interest. Genome-wide association studies (GWAS) can be used to study the effect of natural genetic variation on a trait, but they grow prohibitively expensive if the number of individuals to genotype and phenotype becomes large. Techniques such as pool-GWAS, where individuals are pooled prior to genotyping, and restriction site-associated DNA sequencing (RAD-seq), where a subset of the genome is targeted for sequencing, have lowered the cost and increased the power of GWAS.

Here we describe the application of transposon sequencing and pool-GWAS (with RAD-seq genotyped natural variants) in the whole metazoan model, *C. elegans*. Transposon sequencing has not been previously implemented in an animal model. Specific challenges we have solved include the lack of high-throughput transformation (in contrast to unicellular models) and creation of a representative transposon-insertion sequencing library. We are using our new transposon-sequencing and pool-GWAS methods to study the genetic underpinnings of starvation survival in the nematode. When a *C. elegans* larva hatches in the absence of food its development must be halted and its metabolism changed, requiring precise coordination within cells and between tissues. We present preliminary data of a transposon sequencing screen for starvation survival. Furthermore, we present preliminary data of a pool-GWAS experiment of 96 *C. elegans* wild isolates for starvation survival.

P2024C Two locus allele frequency statistics with demography and selection using a diffusion approach. *Aaron Ragsdale*, Ryan Gutenkunst. University of Arizona, Tucson, AZ, USA.

Patterns of polymorphism within and between populations are determined by the demographic and evolutionary history of those populations. By measuring the present genetic diversity and patterns of linkage disequilibrium within a population, we may infer its demographic history and patterns of selection. The allele frequency spectrum (AFS) is an informative summary statistic of observed genetic variation within a sample, and has been widely used in demographic inferences for single and multiple populations, as well as to infer the effects of selection on that observed variation. AFS-based methods assume independence between sites, and the AFS does not describe levels of linkage disequilibrium between nearby sites. However, frequencies of neighboring alleles are correlated, and selection on linked sites affect patterns of observed variation, so observed levels of linkage disequilibrium are informative about the demographic and evolutionary history of

the population. We present a novel numerical approach to the forward-in-time diffusion model for the joint distribution of two-locus allele frequencies and linkage disequilibrium, which can model the simultaneous effects of demography and selection on one or both loci. This allows for improved inferences on single-population demography, and describing the expected patterns of allele frequencies subject to natural selection at linked sites, including the effects of background selection and hitchhiking events.

P2026B Frequency, variance and power: how genetic model and demography impact association studies. *J. S. Sanjak*. UC Irvine, Irvine, CA.

To understand the genetic architecture of complex traits we need theoretical models that make useful predictions that are consistent with empirical observation. Simulations of complex traits are widely used for inference of population parameters and in-silico testing of new experimental or analytical methods. Despite this, the approaches in the field of complex trait simulation are very heterogeneous. One common thread is that classic models consider particular mutations ("SNPS") as separate loci. However, we wish to model a genomic region as a functional unit or gene. As such, there are important implications of the structure of the relationship between mutations in the region and it's functional output in a diploid individual. Also, it is well known that demography plays an important role in shaping patterns of DNA sequence variation, but the specifics of how demography interacts with underlying genetic model are unknown. We use forward-time simulation to explore the properties of a co-dominant model and two different recessive models, in constant-sized and recently expanded populations, in the context of heritability estimation and genetic association studies. In particular, we find that the population frequency by effect-size distribution and statistical properties of association studies are both impacted by genetic model. Consequently, when explicitly modeling DNA sequence variation underlying a complex trait it is critical to differentiate between sites within a functional unit (gene) and those in distinct functional units. Comparing the effect of population growth across multiple genetic models suggests that, perhaps, the genetic model is more important than the demographic model.

P2027C Robust identification of hard and soft sweeps in humans via machine learning. *Daniel R. Schrider*, Andrew D. Kern. Rutgers University, Piscataway, NJ.

Detecting the targets of adaptive natural selection from whole genome sequencing data is a central problem for population genetics. Numerous approaches have been devised to detect the population genetic signature of a *de novo* beneficial mutation sweeping rapidly to fixation (a hard selective sweep). To date most of these methods to detect sweeps show poor performance under realistic demographic scenarios. Moreover, over the past decade there has been a renewed interest in determining the importance of selection from standing variation (soft sweeps) in adaptation of natural populations, yet few methods are sensitive to this mode of selection. Here we introduce a new tool, S/HIC, which uses supervised machine learning to precisely infer the location of both hard and soft selective sweeps. We show that S/HIC has unrivaled accuracy for detecting sweeps under demographic histories that are relevant to natural populations, and distinguishing sweeps from linked as well as neutrally evolving regions. Moreover we show that S/HIC is uniquely robust among its competitors to demographic misspecification: even if the true demographic model of a population differs catastrophically from that specified by the user, S/HIC still retains impressive discriminatory power. Next, we apply S/HIC to resequencing data from human European and African population samples from the 1000 Genomes Project. S/HIC reliably recovers selective sweeps that have been identified earlier using less specific and sensitive methods, and identifies several compelling novel candidates, including a tumor suppressor gene that is often mutated or deleted in breast tumors. Lastly we perform the first genome-wide examination of the prominence of hard versus soft sweeps in human populations, finding a much greater frequency of soft sweeps in Africa. This result confirms theoretical predictions that larger populations will more often respond to adaptive challenges by selecting on previously standing polymorphisms.

P2028A Comprehensive genome-wide disease characterization (URSA(HD)) and tissue-specific networks (GIANT) guide discovery and functional elucidation of novel predicted disease-associated genes. *Chandra Theesfeld*[1], Young-suk Lee[1], Casey Greene[3], Arjun Krishnan[1], Aaron Wong[1,2], Emanuela Ricciotti[3], Rene A. Zelaya[4], Daniel S. Himelstein[5], Ran Zhang[1], Boris M. Hartmann[6], Elena Zaslavsky[6], Stuart C. Sealfon[6], Daniel I. Chasman[7], Garrett A. FitzGerald[3], Kara Dolinski[1], Tilo Grosser[3], Olga Troyanskaya[1,2]. 1) Princeton University, Princeton, NJ; 2) Simons Foundation, New York, NY; 3) University of Pennsylvania, Philadelphia, PA; 4) Geisel School of Medicine at Dartmouth, Hanover, NH; 5) University of California, San Francisco, San Francisco, CA; 6) Icahn School of Medicine at Mount Sinai, New York, NY; 7) Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Complex diseases are driven by multiple genetic changes and characterized by genome-wide perturbations of cellular pathways and functions. Gene expression profiling experiments comparing normal to disease samples while useful in uncovering molecular pathology of diseases, are limited in that they cannot discern similarities between related diseases. Discovery of truly-disease specific attributes requires a comprehensive approach comparing many diseases and many normal samples. We have developed URSA(HD), a unified probabilistic framework to identify and quantify distinctive disease signals for 309 human diseases based on gene expression profiles of clinical samples. URSA(HD) can uncover subtle differences between similar diseases and highlights identifiable aspects of rare diseases. URSA(HD) outperforms other approaches of using individual disease genes or the typical normal/disease differential expression analyses. URSA(HD) can also be used by researchers to make sample predictions(both cancerous and non-cancerous) for a given clinical gene expression profile (ursa.princeton.edu).

URSA's resulting biological models constitute feature sets specific to each disease: different from those for all other diseases (including similar diseases), and different from all normal tissue. In the biological model for neuroblastoma, a pediatric cancer, 16 of the top 20 genes are documented causal or biomarker genes, and the remaining four genes are uncharacterized. We tested the relevance of these four genes and

found clear growth or cell migration phenotypes for three genes in one or two different human neurobastoma cell lines. Since literature is limited or non-existent for these four genes, further characterization of their roles will require consideration of potential roles in cell and tissue physiology. URSA(HD) models retain only the tissue signatures that are relevant to the disease over and above normal tissue signal. Genome-wide functional studies and access inclusive tissue information and predictions, we have developed human genome-wide tissue-specific networks: GIANT.princeton.edu. With these networks, known and predicted tissue-specific roles played by proteins, including widely expressed proteins, are accessible. Associations within these networks are directly usable by scientists for hypothesis generation and testing and will be useful**.**

P2029B Polygenic adaptation to an optimum shift. *K. R. Thornton*. Univ California, Irvine, CA.

The design and interpretation of genome scans for selection have been largely influenced by models that assume either continuous directional selection on unconditionally beneficial new mutations ("hard" or "classic" sweeps) or fluctuating selection on a previously neutral or weakly-deleterious mutation ("soft" sweeps). However, many examples of adaptive phenotypes in natural population involve complex traits, and the above models may not be adequate descriptions of how such complex polygenic traits evolve. Here, I use forward-time simulation to examine the dynamics of adaptation to a sudden environmental shift, which is modeled as a shift in the optimum value of a complex trait with a broad-sense heritability less than one. The model integrates the existing concepts of "soft" sweeps from standing variation and "classic/hard" sweeps from new mutations, but the strength of selection on individual mutations changes over time as the mean trait value approaches the new optimum value. Adaptation initially proceeds via soft sweeps, fixing mutations of relatively large effect that arose prior to the optimum shift. Subsequent evolution involves hard sweeps of mutations whose effect sizes decrease as time goes on. The site frequency spectrum (SFS) and linkage disequilibrium (LD) show time-dependent deviations from equilibrium values, providing a means of inferring the magnitude of the optimum shift. Finally, the genetic variation for fitness takes a long time to return to equilibrium, and there is continued directional selection after the new optimum phenotype is reached as the population is loses mutations that increase variance in fitness.

P2030C Genome wide association in presence of high density marker panels and genotyped causal variants. *S. Toghiani*[1], L. Y. Chang[1], S. Aggrey[2,3], R. Rekaya[1,3]. 1) Department of Animal and Dairy Science, The University of Georgia, Athens, GA, USA; 2) Department of Poultry Science, The University of Georgia, Athens, GA, USA; 3) Institution of Bioinformatics, The University of Georgia, Athens, GA, USA.

Genome wide association studies (GWAS) rely on estimating the association between phenotypic variation and a large number of SNPs often within the framework of a linear regression (LR) or mixed linear (ML) models. Although both models have a certain level of statistical equivalency, they differ in the manner of associating phenotypic variation to genetic polymorphisms. Linear regression models directly associate the phenotype with SNP genotypes allowing for a direct dissection of the trait especially if causal variants are included on the panel. However, these models suffer from the excessive high dimensionality of the parameter space and co-linearity between SNPs. ML models remove some of the issues associated with LR methods and allow for straightforward accommodation of confounding due to population structure and family relationships. ML models rely on the estimation of the realized genetic similarity matrix (GM) between individuals using all available SNP genotypes. It is well known that the GM matrix change very little with the increase in the number of markers in the panel once a certain density threshold is reached even when causal variant genotypes are available. As the density of genotyping panels continuous to increase in density so does the probability of causal variants being genotyped. In order to investigate the robustness of ML models in the era of next generation sequencing, a simulation study was carried out for a trait with heritability of 0.4. One chromosome with 75,000 SNPs and 35 casual variants explaining 5% of total genetic variance was simulated mimicking a 2 million SNP marker panel and 1000 casual variants at the genome level. Simulated data was analyzed using LR and ML models either including or excluding causal variants. Using ML models, prediction accuracy defined as the correlation between true and estimated genetic values was the same independently of panel density, exclusion (0.293) or inclusion (0.294) of causal variants. Genetic variance was under estimated (0.013 vs. 0.02). Prediction accuracy increased by around 3% when a LR model was used and causal variants were included. Genetic variance was more accurately estimated (0.017 vs. 0.02). LR model used all available SNPs and its performance is expected to increase if a model averaging (BayesB) or SNPs prioritizing approaches were applied. As density increases and more causal variants are include in the marker panels, ML models will need some improvements mainly in the calculation of GM matrix.

P2031A Detecting patterns of microgeographical adaptation to a patchy saline environment of a single popupation of *Medicago truncatula*. *w. vu*[1], P. Chang[1], K. Moriuchi[4], E. von-Wettberg[2], M. Friesen[3], S. Nuzhdin[1]. 1) Univ Southern California, University Park Campus, Los Angeles, CA 90089; 2) Florida International university 11200 SW 8th St, Miami, FL 33199; 3) Michigan State University 220 Trowbridge Rd, East Lansing, MI 48824; 4) University of California, Davis 1 Shields Ave, Davis, CA 95616.

Divergent selective pressures across a heterogeneous environment can result in the maintenance of genetic variation and adaptation to local environmental conditions. While this process has been well documented at larger geographical scales, it remains unknown whether micro-environmental variation can result in the maintenance of genetic variation. Therefore we aim to understand the genetic basis of plant response to micro-environmental variation. Our study examines the patterns of micro-scale adaptation to a saline habitat within a single population of *Medicago trucatula*—an annual selfing legume that is native to Mediterranean regions.

Early flowering is a predictor of increased plant performance and reproductive output in saline conditions. We observe that flowering time is

negatively correlated with soil salinity levels in the field, indicating that the variability in soil patches can maintain phenotypic variation and potentially genetic variation. This suggests that variable selective pressure between soil patches is likely driving divergent flowering time responses within a single selfing population. Furthermore, SNP frequency is strongly associated with latitude, longitude, elevation and soil salinity levels. Some of the polymorphisms correlated with environmental variability cause functional changes in genes associated with flowering time, disease resistance, salt and osmotic stress. Overall, these results suggest that micro-scale environmental variation can potentially shape patterns of adaptation to heterogeneous environments.

P2032B Genome-wide divergence among microhabitats in *Fundulus heteroclitus*. *D. N. Wagner*, T. Baris, D. Dayan, X. Du, M. Oleksiak, D. Crawford. University of Miami, Miami, FL.

Selective differences among environments rely on isolation or selection coefficients that exceed migration, thus in a highly connect environment there is little expectation of genetic divergence among habitats. The teleost fish *Fundulus heteroclitus* lives in *Spartina* saltmarshes along the eastern coast of North America where the tide flushes and then drains these saltmarshes, typical of a well mixed population. The natural history supports this supposition: these fish have small home range of 36m and they reproduce in a common part of the saltmarsh, laying their eggs at the highest tides in the upper tidal regions in mussel shells or among leaves of *Spartina alterniflora*. Yet, within a single marsh are three microhabitats: 1) tidal basins, 2) intertidal creeks, and 3) tidal ponds, that have meaningful environmental differences in their daily maximum temperatures and oxygen concentration. Based on mark-recapture studies, individuals are often associated with a single habitat. These environmental differences and site fidelity could select for different genotypes. To examine any microhabitat differences in genotypes, individuals from the 3 different microhabitat types from 3 replicate saltmarsh populations along the New Jersey Coast (Mantoloking, Rutgers Field Station, and Stone Harbor) were genotyped at more than 4,000 SNPs using genotyping-by-sequencing (GBS). GBS identified between 2.2-4.4% of single nucleotide polymorphisms (SNPs) with significant outlier F_{ST} values between microhabitats of resident fish (p-value < 0.01, FDR = 1%). These SNPs may be adaptively important, and suggest that selection is surprisingly effective in altering allele frequencies over very small geographical distances.

P2033C Essential proteins evolve slower than non-essential ones during evolution experiments. *D. Alvarez-Ponce*[1], B. Sabater-Muñoz[2], C. Toft[3,4], M. X. Ruiz-González[2,5], M. Fares[2,5]. 1) University of Nevada, Reno, Reno, NV; 2) Department of Genetics, Smurfit Institute of Genetics, University of Dublin, Trinity College Dublin, Dublin, Ireland; 3) Department of Genetics, University of Valencia, Valencia, Spain; 4) Departamento de Biotecnología, Instituto de Agroquímica y Tecnología de los Alimentos (CSIC), Valencia, Spain; 5) Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Valencia, Spain.

The Neutral Theory of Molecular Evolution is considered the most powerful theory to predict the evolutionary behavior of proteins. One of the main predictions of this theory is that essential proteins should evolve slower than dispensable ones owing to increased selective constraints. Comparison of genomes of different species, however, have revealed only small differences between the rates of evolution of essential and non-essential proteins. It has been argued that comparing relatively distant genomes may entail a number of limitations. For instance, many of the genes that are dispensable in controlled lab conditions may be essential in some of the conditions faced in nature. Moreover, essentiality can change during evolution, and rates of protein evolution are simultaneously shaped by a variety of factors, whose individual effects are difficult to isolate. Here, we conducted two parallel mutation accumulation experiments in *Escherichia coli*, during 5500–5750 generations, and compared the genomes at different points of the experiments. Our approach (a short-term experiment, under highly controlled conditions) enabled us to overcome many of the limitations of previous studies. We observed that essential proteins evolved significantly slower than non-essential ones during our experiments. Strikingly, rates of protein evolution were not affected by expression level, protein abundance, or protein length.

P2034A Subpopulation structure in long-term cultures of *Escherichia coli* K-12. *M. Behringer*, B. Choi, S. Miller, T. Doak, M. Lynch. Indiana University, Bloomington, IN.

Inexpensive whole genome sequencing now permits us to look at genome-wide patterns of adaptation within populations, but this approach is only starting to be used to examine the extensive population structure that arises under long-term evolution studies. To determine the effects that effective population size and variable mutation rates have on genome evolution, we conducted a long-term evolution experiment using *Escherichia coli* populations with six different genetic backgrounds, carried through 3 years of continuous culture. After whole-population sequencing of these lines, we identified recurring mutations across populations—specifically genes associated with biofilm formation, global regulation, and peptidoglycan recycling.

To further investigate this phenomenon, we isolated eight random clones from twelve long-term evolution populations polymorphic for mutations in *fimE* (biofilm formation), *hns* (global regulation), and *nlpD/mppA* (peptidoglycan recycling). These twelve populations also represent three of the genetic backgrounds used: wild-type (WT), Δ*mutL*, and a *mutL*+ line preloaded with mutations for ~4000 generations of mutation accumulation. Genomic sequence of the clones, finds genetic evidence for subpopulation structure in the long-term evolution populations. Further, we find phenotypic differences within populations for lag-time, maximum growth rate, diauxic growth, auxotrophy, and biofilm formation.

For our wild-type focus population—where mutation rate is low—we identified three major haplotypes among the eight clones. Associated with these haplotypes, we identified genetic and phenotypic evidence of auxotrophy for thiamine in five clones. Additionally, these five clones displayed slower maximum growth rates, but shorter lag times and diauxic growth curves (indicating resource prioritization), presenting one

possible adaption to coexistence in a fluctuating environment.

In our Δ*mutL* focus population, with mutation rates ~150 times greater than wild-type, we identified six major haplotypes. Furthermore, because of the increased mutation rate, we were able to conduct pathway analyses shedding light on the specialization of each clone. Interestingly, because of the potential for non-competing niches, independent evolutionary changes within identical pathways occurred in clones belonging to different haplotypes. We conclude that our methods gives us the mutational resolution to describe evolutionary changes within hetrogenous, intraspecies communities, an important step toward understanding intraspecies communities in both nature and disease.

P2035B Phenotypic variation in individuals isolated from *Escherichia coli* long-term evolution populations. *Brian Choi*, Megan Behringer, Samuel Miller, Thomas Doak, Michael Lynch. Indiana University, Bloomington, IN.

How organisms evolve and/or adapt is one of the central questions in population genetics. While evolution can be driven by four major forces: mutation, recombination, natural selection and random genetic drift, when investigating experimentally evolved cultures it can be difficult to determine if shared genotypes occur due to mutational hotspots in the genome or parallel adaptive evolution. Previous long-term evolution experiments conducted by Richard Lenski and collaborators have demonstrated multiple avenues of adaptation for *Escherichia coli* strain B in liquid culture (Elena and Lenski, 2003). Using a similar long-term investigation, we aimed to explain how ancestral genetic background, starvation, and an environment where subpopulations can inhabit their own niche affects population dynamics and long-term evolution. As such, we characterized 96 clones isolated from 12 out of 200 populations involved in a long-term evolution experiment. Our 12 focus populations were propagated from one of three possible *E. coli* K-12 ancestors, and transferred every 24 - 48h for 2.5 years (~4000 generations) in liquid LB medium. Among clones, auxotrophic, behavioral, morphological, and growth variations were identified. We additionally evaluated each of the clones for improved or worsened fitness compared to the wild-type ancestor through relative growth curve assays. Lastly, we applied a bioinformatics approach for lineage and mutation analysis to identify patterns and commonalities between the varied phenotypes and their respective genotypes. Here, we are able to trace homologous changes in bacterial evolution to suggesting parallel response to pressures due to their respective conditions, background, or ecological niche. The complex structuring of ecological niches among the long-term populations is reflective of the shifts in gene polymorphisms.

P2036C Quantifying host genome response to gene drive using experimental evolution. *K. Fisher*, G. Lang. Lehigh University, Bethlehem, PA.

Gene drives—selfish alleles that bias inheritance in their favor—have great potential for solving epidemiological and agricultural problems. Seeding populations with transgenic individuals containing drive elements linked to a trait of interest, such as resistance, has been proposed as strategy to control disease vectors and agricultural pests. The most predictable, and consequently useful, of these elements are targeted endonucleases that recognize and insert themselves into homologous alleles in which they are absent by cleaving a target sequence and allowing homologous recombination to result in gene conversion. Homing endonucleases and CRISPR endonucleases have been demonstrated to be effective knockout drivers with reliable activity and specificity in laboratory populations (Windbichler et al. 2011; DiCarlo et al, 2015; Gantz & Bier, 2015), and thus are possible tools for this type of population level genetic manipulation in the field.

Models of gene drive suggest that the gains from distorting inheritance can overcome selection such that even strongly deleterious alleles can spread through drive (Deredec et al. 2008; Unckless et al. 2015). These studies, however, do not consider coevolutionary dynamics between drive elements and host genomes. Homing endonucleases rarely exert deleterious effects in their native hosts, suggesting that coevolution readily occurs in response to drive to lessen intragenomic antagonism. Changes in response to selection on the part of either drive elements or host genomes could potential derail the intended effects of synthetic gene drives. To date, no studies have assessed the efficacy and reproducibility of gene drive in evolving populations or to the predictability of host genome response.

Here we propose to directly test the efficacy of gene drive in evolving populations of the budding yeast, *Saccharomyces cerevisiae.* We have successfully constructed several drive elements. Alleles have been designed so that we can vary the fitness cost of drive across experimental replicates. Drive and target alleles will be fluorescently tagged to quantify their frequencies over time. Whole genome sequencing will be used to characterize reproducibility of host genome responses. We predict that host genomes will evolve in response to drive and that fates of drive elements will be predictable as a function of increasing selection, but unpredictable between replicate populations of the same selective regime due to the stochastic nature of molecular adaptation.

P2037A Leveraging haplotype-aware inference for evolve-and-resequence studies. *S. Greenblum*[1], S. Tilk[1], A. Bergland[1,2], P. Schmidt[3], D. Petrov[1]. 1) Stanford University, Stanford, CA; 2) University of Virginia, Charlottesville, VA; 3) University of Pennsylvania, Philadelphia, PA.

In the face of sharp environmental fluctuations, massive adaptive change has been observed in metazoan populations on timescales of just tens of generations. Despite the importance of rapid adaptation for predicting the dynamics of critical health threats such as host-pathogen co-evolution and viral outbreak, the genetic basis of this phenomenon remains unclear. Large-scale evolve-and-resequence (E+R) experiments, entailing serial sequencing of pooled samples from populations subject to selection, hold enormous promise for studying the dynamics of rapid adaptation in a controlled yet realistic setting. However, more work is needed to devise experimental and analytic frameworks that maximize the power and precision of these studies, given available technologies, resources, and informatics tools.

Here we leverage both data from the experimental evolution of *Drosophila melanogaster* populations, as well as simulated data, to demonstrate how various experimental designs incorporating fully-sequenced founder lineages influence the detection resolution of alleles under strong short-term selection. In particular, we focus on the tradeoff between experimental schemes that minimize linkage disequilibrium

among founder haplotypes to uncouple the trajectories of strongly adaptive alleles and nearby sites, and schemes that incorporate new computational tools that *make use* of founder linkage to increase the precision of individual allele frequency measurements, thereby reducing sequencing costs and facilitating higher levels of replication.

We demonstrate how this tradeoff informs best practices for E+R, and quantify the increased power afforded by linkage-informed analyses in detecting the genomic targets of rapid adaptation. Improved frameworks building on these results will immediately benefit future studies of experimental evolution in many model organisms, and will help clarify the true extent of the *Drosophila* genome that is subject to short-term seasonal selection as well as the broader impact of rapid adaptation on genome architecture.

P2038B Genome-wide Analysis of Starvation-selected *Drosophila melanogaster*- a Genetic Model of Obesity. *Christopher Hardy*[1], Molly Burke[2], Mira Han[1], Logan Everett[3], Kathryn Lantz[1], Allen Gibbs[1]. 1) University of Nevada Las Vegas, Las Vegas, NV; 2) Oregon State University, Corvallis, OR; 3) North Carolina State State University, Raleigh, NC.

Experimental evolution affords the opportunity to investigate adaptation to stressful environments. Combining experimental evolution with recent developments in whole-genome sequencing has elevated the field by providing insight into the dynamics of evolution as well as a new tool to discriminate genes involved in polygenic traits. Here, we performed an "Evolve and Resequence" (E&R) study where we selected for starvation-resistance in populations of *Drosophila melanogaster* for over 80 generations. In response to selection, the starvation-selected lines have developed an obese condition, storing nearly twice the level of fats than their unselected controls. While the excess fats provide a ~3-fold increase in survival time upon starvation, the imbalance in lipid homeostasis incurs evolutionary cost. Some of these tradeoffs resemble obesity-associated pathologies in mammals including metabolic depression, low activity levels, dilated cardiomyopathy and disrupted sleeping patterns. To determine the genetic basis of these traits we sequenced genomic DNA from the selected lines and their controls. We found 1,046,373 polymorphic sites, many of which diverged between selection treatments. In addition, we found a wide range of genetic heterogeneity between the replicates of the selected lines, suggesting multiple mechanisms of adaptation. Genome-wide levels of heterozygosity in the selected lines were low, with many large blocks of SNPs nearing fixation. To find loci under selection, we generated a new algorithm to control for the effects of genetic drift. We mapped these loci to a set of 1,453 genes, which were enriched for genes related to morphogenesis, development, tissue differentiation and regulation of metabolism. The results of our study speak to the evolutionary origins of obesity and provide new targets to understand the polygenic nature of obesity in a unique model system.

P2039C Investigation of the prevalence of antagonistic pleiotropy. *L. Herissant*[1], D. Yuan[2], P. Humphrey[3], M. Johnson[3], A. Agarwala[4], D. Fisher[4], M. Desai[3], D. Petrov[2], G. Sherlock[1]. 1) Department of Genetics, Stanford University, Stanford, CA; 2) Department of Biology, Stanford University, Stanford, CA; 3) Department of Organismic and Evolutionary Biology and of Physics, and FAS Center for Systems Biology, Harvard University, Cambridge, MA; 4) Department of Applied Physics, Stanford University, Stanford,CA.

Pathogenicity, drug resistance and cancer progression are examples of mutation-driven processes, where increased selective advantage is conferred upon cells carrying new mutations. While these mutations may be beneficial in one specific condition, they may be deleterious in other conditions, a phenomenon known as Antagonistic Pleiotropy (AP). AP is thought to lead to evolutionary trade-offs and the persistence of deleterious alleles. But what is the prevalence of AP? Which genes or pathways are more likely to be involved in AP, and under which conditions? Is there any specific type of mutation that results in AP? One study investigated AP and observed ~14% of non-essential gene deletions display AP (Qian et al., 2012) but the prevalence and nature of AP for beneficial mutations is largely unexplored.

To answer these questions, we are using an experimental system that allows us to track and measure the fitness values of ~500,000 separate lineages within an evolving yeast population via DNA barcodes (Levy et al 2015). We performed evolution on haploid population as well as on diploid population. One preliminary observation is that, diploids arise in the haploid population and have some fitness advantage depending on the growth conditions.

With this barcoding system, we are able to identify lineages that gain a beneficial mutation, based on how their frequencies increase over time. We will select clones that harbor beneficial mutations in order to measure their fitness in alternate conditions. We will infer AP for any clones that carry beneficial mutations in the first environment that now show a fitness lower than wild-type in at least one alternate environment. On clones of interest, we will perform whole genome sequencing to determine the mutation that induces AP in those clones.

These data will result in the largest set of fitness measurements for adaptive mutations ever collected across multiple environments. It will allow us to determine, for example, the extent of AP among new beneficial mutations and any correlations between the magnitude of the beneficial effect a mutation confers in one environment and whether it exhibits AP in other environments. The results will indicate whether beneficial mutations in certain pathways are more likely to exhibit AP and will provide the first insight of how mutation-driven processes can confer selective evolution or why deleterious alleles can be conserved upon evolution.

P2040A Analyses of Breast Cancer Type 1(BRCA 1) Gene Of Different Mammalian Species. *E.VictorI. Ikpeme*, O.Ogbu Udensi, H.Edim Etta, U.Benjaimin Ekaluo, M. Ozoje, B.Bendiwhobel Ushie, E. Echea, M.Edem Kooffreh, E.Archiebong Okon. University of Calabar, Calabar, Cross River, Nigeria.

Breast cancer type 1 (BRCA1) gene also known as breast cancer type 1 susceptibility protein homologue plays important role in DNA double-strand break repair, homologous recombination, chromatin remodeling, cell cycle regulation and transcription regulation. Considering the importance of this protein, the present study was undertaken to analyse BRCA1 gene of different mammalian species by assessing the identity and similarity, phylogenetic relationship, physicochemical properties, predict the motifs, secondary and the tertiary structures. Seventeen

nucleotide and protein sequences of BRCA1 gene of different mammalian species were retrieved from National Centre for Biotechnology Information (NCBI). Multiple sequence alignment was done using CLUSTAL W software, while identity and similarity was determined by constructing a pairwise comparison. Results obtained at the end of the experiment showed that there was no percentage Identity or similarity that was less than 70%. The phylogenetic relationship of BRCA1 gene of the mammalian species clustered into aquatic, herbivores, carnivores and omnivores, respectively. The highest time of divergence (95MYA) of the BRCA1 gene was observed between the BRCA1 gene of killer whale and human, while the least time of divergence (4.6MYA) was observed between the BRCA1 gene of cattle and American buffalo. Physicochemical properties of BRCA1 proteins in the five mammalian species (cattle, sheep, pig, American buffalo and human) were found to be unstable, hydrophilic and intracellular in nature. The following motifs were present at various positions in the BRCA1 gene of the five mammalian species; zinc finger RING, BRCT domain, N-myristoylation site, N-glycosylation, cAMP and cGMP dependent protein kinase phosphorylation site, Tyrosine kinase phosphorylation and cell attachment sequence, but leucin zipper pattern and microbodies c-terminal motifs were found in only human BRCA1 gene. The BRCA1 secondary structure contained the alpha helix, extended strand and random coil. Based on the results obtained, it can be deduced that BRCA1 gene has identical homologue, functional similarity and highly conserved in these mammalian species.

P2041B Experimental evolution of drift robustness in digital organisms. *T. LaBar*, C. Adami. Michigan State University, East Lansing, MI.

Both recurring deleterious mutations and mutation accumulation due to genetic drift can reduce a population's average fitness. To counteract these effects, selection should favor genetic architectures that are robust and limit these deleterious effects. For instance, populations can evolve mutational robustness by reducing the average deleterious effect of a mutation so as to minimize the mutational load. Here, we test whether populations can evolve to limit the reduction in fitness caused by deleterious mutation accumulation and genetic drift in small populations (i.e., the drift load) using experimental evolution with digital organisms. In other words, we ask whether populations can evolve robustness to genetic drift ("drift robustness") when adapting to survive in small populations, similar to populations evolving mutational robustness to survive high mutation rates. We found that small populations do evolve drift robustness, while large populations evolve drift fragility. Drift robustness emerges from the evolution of genetic architectures that reduce the likelihood of deleterious mutations with small effect sizes. We discuss specifically the role selection plays in the spread of drift robust genotypes, and show that drift robustness can arise from mutations that drastically decrease the likelihood of deleterious mutations while significantly increasing the likelihood of lethal mutations. These findings may have implications for genome evolution of organisms with small population sizes, such as bacterial endosymbionts and eukaryotic organelles.

P2042C A gene's view of a long-term evolution experiment with *Escherichia coli*. *Rohan Maddamsetti*[1], Philip Hatcher[2], Barry Williams[1], Jeffrey Barrick[3], Richard Lenski[1]. 1) Michigan State University, East Lansing, MI; 2) University of New Hampshire, Durham, NH; 3) University of Texas at Austin, Austin, TX.

In my dissertation research, I used genomic data from a long-term evolution experiment with *Escherichia coli* (LTEE) to answer two questions, among others. First, what do the evolutionary dynamics in the LTEE look like? Second, what kinds of genes evolve in the LTEE? These studies share the same cast of characters: *pykF, iclR, spoT, nadR, topA,* and *malT*. These genes make the strongest contribution to adaptive evolution in the LTEE. I use these genes to show that the evolutionary dynamics that play out in the LTEE involves two processes that are often difficult to tell apart: clonal interference and negative frequency-dependent selection. I then show that even though these genes evolve rapidly and repeatedly in the LTEE, they are well-conserved across *E. coli* isolates and have diverged slowly since the *Salmonella–Escherichia* split. In fact, adaptive evolution in the LTEE largely involves mutations at conserved protein residues within conserved core genes such as *pykF*. The apparent simplicity of LTEE belies considerable complexity, making the LTEE a powerful model system for evolutionary genomics.

P2043A The fitness spectrum in adaptation of diploid yeast. *David Yuan*[1], Lucas Herissant[2], Atish Agarwala[3], Daniel Fisher[3], Gavin Sherlock[2], Dmitri Petrov[1]. 1) Department of Biology, Stanford University, Stanford, CA; 2) Department of Genetics, Stanford University, Stanford, CA; 3) Department of Applied Physics, Stanford University, Stanford, CA.

Adaptation is one of the central processes in evolution. Not only does adaptation drive phenotypic change in organisms, it also underlies many human diseases, such as cancer and the emergence of drug resistance in their treatment. Yet our current understanding of adaptation suffers from a major limitation.

While many organisms of interest to biology and medicine are diploid, many studies characterize adaptive mutations—the agent of evolutionary change—only in haploids. Mutations driving adaptation likely have very different properties depending on whether they arose in diploids or haploids. For instance, diploid adaptive mutations cannot be fully recessive and may be gain-of-function more often than haploid ones. This can lead to profoundly different adaptation dynamics between diploids and haploids. We previously investigated this difference using a theoretical model [Sellis et al. 2011], though little empirical data on adaptation in diploids exist to date. Thus, our understanding of adaptation in diploid populations often requires extrapolating parameters from haploid experiments. This is particularly troubling in studies of cancer—an adaptive process in diploids.

To overcome this limitation, we are isolating and characterizing a statistically representative number of adaptive mutations in both diploids and haploids. To achieve this, we have used experimental evolution in yeast coupled with a high-resolution molecular barcoding system that enables us to measure and track the fitness of ~5x105 lineages over time [Levy et al. 2015]. We carried out experimental evolution in barcoded S. cerevisiae diploid and haploid populations in multiple evolutionary conditions. Results from haploids provide a basis of comparison, while

multiple conditions take us towards generalizability of results.

As a first step towards understanding how adaptation differs between diploids and haploids, we will identify lineages that acquire adaptive mutation by increases in the frequencies of their barcodes. We will then estimate the distribution of fitness among thousands of adaptive clones for each evolution experiment. These adaptive clones will be taken from time point(s) in which most have one causal adaptive mutation. This data will be the largest and most precise to date on the dynamics of adaptation in diploid populations. The results will inform us on how diploids differ from haploids in terms of speed of adaption and effect size of adaptive change. In the future, we will identify the causal adaptive mutations to deeply characterize how diploids differ from haploids in terms of target and pathway of adaptation, dominance, and environmental pleiotropy.

P2044B Genomic Basis of Craniofacial Diversity in Lake Malawi Cichlids. *K. Abdilleh*, C. Patil, J. T. Streelman. Georgia Institute of Technology, Atlanta , GA.

Craniofacial disorders represent some of the most common birth defects affecting ¼ of the human population worldwide. Many causal mutations implicated in craniofacial malformations are also responsible for generating the natural craniofacial trait variation observed between species. The advancement of next generation sequencing technologies has allowed for rapid acquisition and identification of genome-wide variants mediating adaptive craniofacial differences between species. Rock dwelling and sand dwelling cichlid fishes of Lake Malawi (LM) exhibit extensive variations in craniofacial morphologies, specifically in jaw size and length making them an outstanding model for the study of craniofacial morphological evolution. Here, we performed whole-genome re-sequencing of 16 rock and sand dwelling LM cichlids and identified regions of genomic differentiation between the groups. We show evidence that (1) regions of genomic differentiation are enriched for genes involved in pathways and processes relating to cranial neural crest cell differentiation and development (2) alternately fixed SNPs between rock and sand species are within and around crucial genes of the cranial neural crest cell gene regulatory network and (3) alternately fixed SNPs between rock and sand species are also within enhancer elements regulating craniofacial and neural crest development. Taken together, our comparative genomic analysis provides a global picture of the key genomic changes likely modulating species-specific craniofacial differences between rock dwelling and sand dwelling LM cichlids.

P2045C Repeated horizontal transfer of a fused gene encoding adjacent metabolic enzymes. *N. M. Anderson*[1], S. W. Roy[1,2]. 1) University of California, Merced, Merced, CA; 2) San Francisco State University, San Francisco, CA.

We report a case of gene fusion and horizontal gene transfer (HGT), two noncanonical molecular evolutionary processes whose functional significance remains obscure. Diffusion of products between enzymes can be a limiting step in metabolic pathways, a problem that can be mitigated in part by the physical association of enzymes that act in adjacent steps of the pathway. A manual search of the 1000 Fungal Genomes Project revealed 10 species within the large Pezizomycotina (Ascomycota) containing a single gene that encodes both aconitate hydratase (aconitase family) and isocitrate/isopropylmalate dehydrogenase (IsoDH), two enzymes that are adjacent in the citric acid cycle and other pathways. Surprisingly, the species containing this fusion were found to be distantly related, a finding that could be due to either: a) repeated independent fusion of these two genes; or b) a single gene fusion event and repeated horizontal transfer of the fused gene.

To distinguish between these possibilities, we reconstructed phylogenetic trees for each of the enzymes separately and compared these gene trees to the consensus species tree. Our results strongly support a grouping of the fused genes, implying multiple cases of HGT. Expanding this search to other fungal groups revealed additional species harboring aconitase-IsoDH fusions in a pattern that suggested additional HGT events, but also suggested a fusion event independent from those within Pezizomycotina. The striking propensity for HGT of these fused genes suggests that they impart a fitness advantage, likely through increased efficiency of metabolic pathways, revealing the ways that HGT can disseminate useful molecular innovations throughout the tree of life.

P2046A Mapping the Origins of Inter-Population Skin Color Variation with Admixed Indigenous Populations . *K. C. Ang*, V. A. Canfield, T. C. Foster, K. C. Cheng. Penn State College of Medicine, Hershey, PA.

The genetic basis of population-specific differences in human skin color is a fascinating, socially relevant, and enduring mystery of human biology. For Europeans, we now know about two genes that together account for much of their lighter skin color as compared with Africans, and other genes that affect intra-population variation in skin, eye and hair color. However, the genes responsible for the lighter skin color of East Asians and Amerindians (as compared with West Africans) remain unknown. To map those genes, we searched for populations admixed for either East Asian or Amerindian ancestry and a darker-skinned ancestry. The only populations with such an admixture that also lack significant European admixture that would confound our analysis were two indigenous populations, the Orang Asli of Malaysia and the Kalinago of Dominica. Both groups exhibit large variation in skin pigmentation. The Orang Asli of Peninsular Malaysia consist of three tribes, Senoi, Proto-Malay, and Negrito, who have a complex ancestry. The Kalinago have primarily Amerindian and West African ancestry. DNA samples and skin reflectance measurements were collected from a total of >1000 individuals. Skin pigmentation, expressed as Melanin Index, ranged from 20 to 80 units, averaging 45.5 and 45.8 for the Senoi and Kalinago, respectively (as compared with 25 and 21 for East Asian and European and 55, and 56 for Negrito and West African, respectively). Samples containing European *SLC24A5*^{A111T} or *SLC45A2*^{L374F} alleles will be excluded from our downstream analysis to minimize interference with East Asian and Amerindian pigmentation alleles. For the same reason, we identified and are excluding individuals with an *OCA2* albinism allele found in the Kalinago population. SNP genotyping of subsamples of 92 Kalinago and 78 Orang Asli samples revealed that the Negritos are distinct, that the six Senoi tribes show significant diversity, and that a highland Senoi subtribe is distinct from the rest of the Senoi. Analysis of a Kalinago subsample showed 61% Amerindian, 31% African, and 8% European ancestry, which

is considerably higher Amerindian ancestry than has been reported for other Caribbean populations. Progress will be reported towards screening of whole-genome sequences from phenotypic extremes from these samples for polymorphisms that are nearly fixed in East Asians but nearly absent in Africans. CRISPR-mediated zebrafish knockouts, morphants, and RNA rescue are being used to validate candidate genes.

P2047B Genomic deletion and silencing on the Y chromosomes of Rumex hastatulus. *F. Beaudry*, S. Wright. University of Toronto, Toronto, ONTARIO, CA.

Our support of evolutionary theory depends greatly on the empirical ubiquity of its effects. Although old sex chromosomes in animal systems are well characterized, the young sex chromosomes of plants are sparsely investigated. Here, we determine the amount of chromosomal degeneration observed in the XYY chromosomal system of the dioecious annual plant Rumex hastatulus. Using both RNAseq and genomic Paired-End data, we observe both deletion and silencing of single copy hemizygous genes on the Y chromosomes, where more genes are deleted than silenced. Genes in high copy number are enriched for copies of genes from the chloroplast genome. We conclude that using transcriptome and genome data together allow for the ascertainment of the most complete set of sex-linked and hemizygous genes.

P2048C Horizontal transfer can drive a greater transposable element load in large populations. *J. P. Blumenstiel*, S. B. Groth. University of Kansas, Lawrence, KS.

Genomes are comprised of contrasting domains of euchromatin and heterochromatin and transposable elements play an important role in defining these genomic regions. Therefore, understanding the forces that control transposable element abundance can help us understand the chromatin landscape of the genome. What determines the burden of transposable elements in populations? The standard model suggests that drift plays a determining role. In small populations, mildly deleterious transposable element insertion alleles are allowed to fix, leading to increased copy number. However, it is not clear how the rate of exposure to new transposable element families, via horizontal transfer, can contribute to broader patterns of genomic TE abundance. Here, using simulation and analytical approaches, we show that when the effects of drift are weak, exposure rate to new transposable element families via horizontal transfer can be a critical determinant of genomic copy number. Since larger populations are expected to have a higher rate of exposure to rare horizontal transfer events, this leads to the counter-intuitive prediction that larger populations may carry a higher transposable element load. This work has implications for our understanding of the evolution of chromatin landscapes, genome defense by RNA silencing and recombination rates. It may also have implications for devising methods for pest control by gene drive.

P2049A Defining microRNA molecular origins to facilitate target prediction. *Glen Borchert*. University of South Alabama, Mobile, AL.

MicroRNAs (miRs) are small noncoding RNAs that typically act as regulators of gene expression by base pairing with the 3' UTR of messenger RNAs (mRNAs) and either repressing their translation or initiating degradation. Descriptions of the molecular origins of independent miR molecules currently support the hypothesis that many miR hairpins were generated by the adjacent insertions of two related transposable elements (TEs) at one genomic locus. Thus transcription across such TE interfaces establishes many, if not the majority of functional miRs. We have recently completed an in depth analysis of the genomic origins of annotated miR loci, detailing the formations of 3,605 distinct miR loci primarily from TEs. Interestingly, our analyses also identify evidence for a second, novel mechanism of miR locus generation through describing the formation of 273 miR loci from mutations to other forms of noncoding RNAs. The implications of these findings are substantial for understanding how (in particular) TEs confer increased genomic fitness, describing miR transcriptional regulations, and making accurate miR target predictions. As such, hypothesizing that a miR and its mRNA target sites might actually be formed in parallel by the ongoing colonization of a common ancestral transposable element, led us to develop a novel miR target prediction strategy in which we limit miR target searches to mRNAs containing the TE (or noncoding RNA) initially giving rise to a miR: OrbId (Origin-based Identification of microRNA targets). In stark contrast to the principal miR target algorithms (which rely heavily on target site conservation across species and are therefore most effective at predicting targets for older miRs), we find OrbId is particularly efficacious at predicting the mRNA targets of miRs formed more recently in evolutionary time.

P2050B *Cis*-regulatory enhancers of social insects share ultraconserved core elements flanked by taxa specific modifications. *Thomas Brody*, Ward Odenwald, Neural Cell-Fate Determinants Section. NINDS/NIH, Bethesda, MD.

Ultra-conserved sequence elements in vertebrate enhancers suggest that fundamental gene regulatory mechanisms are shared across phylogenetically diverse species. Our previous studies took advantage of the availability of the assembled genomic sequences of the Mediterranean fruit fly *Ceratitis capitata*, the domestic housefly *Musca domestica*, and *Drosophila* to explore the integrity of regulatory sequences across the ~100 million years of divergence between these dipterans. We have shown that many of the conserved sequence blocks (CSBs) that constitute *Drosophila* enhancers, identified using the multispecies alignment algorithm EvoPrinter, are often nearly completely conserved in both *Ceratitis* and *Musca*. Analysis of the linear positioning of these CSBs with respect to the associated structural genes is also conserved. Availability of the genomic sequence the bumble bee *Bombus impatiens*, honey bee *Apis mellifera* and the European bee *Megachile rotundata* reveals conserved sequence elements bearing strong structural resemblance to those found in flies. Surprisingly, alignment of regulatory sequences from bee species with those of 10 ant species, representing two families of social insects of the order *Hymenoptera*, revealed widespread alignment of the bee sequences with orthologous ant sequences. These alignments also revealed bee- and ant-specific

sequences intermingled with CSBs common to both taxa. We conclude that comparison across ant and bee species reveals ultra-conserved enhancers that bear taxa specific sequence modifications. These studies allow for a greater understanding of enhancer evolution and constitute a first step in the comparative analysis of gene regulation in these species.

P2051C Single molecule real time sequencing reveals the detailed structure of a Y-autosome fusion in *Drosophila pseudoobscura*. *C. Chang*, A. Larracuente. U of Rochester, Rochester, NY.

Fusion events between sex chromosomes and autosomes are an important part of karyotype evolution in animals. In some cases, ancestral sex chromosomes revert back into autosomes, for example the dot chromosome of *Drosophila* is an ancient X chromosome. However, there are few documented cases of Y chromosome reversals to autosomes. *D. pseudooscura* is well-suited to study Y chromosome reversal: a fusion between the ancestral Y chromosome and an autosome (Y-to-dot) occurred ~15 MYA making the ancestral Y chromosome now autosomal. To gain insight into the evolutionary forces shaping this Y chromosome reversal, we used single molecule real time sequencing reads generated with Pacbio to assemble the dot chromosome of *D. pseudoobscura,* including the Y-to-dot region. This contig has all identified ancestral Y-linked genes, ~100 conserved dot-linked genes and telomere sequences. Surprisingly, the ancestral Y and the conserved part of the dot chromosome are only ~70kb apart, suggesting centromere is not located in the fusion region. Notably, the Y-to-dot does not contain many tandem repeats or satellite DNAs in both its intergenic and intron regions and is only 340 kb—one tenth of the size of the Y chromosome in *D. melanogaster*. Our analysis of transcriptomic data revealed that Y-to-dot genes expression remain testis-specific, even after becoming autosomal for ~15 MY. In addition, both Y-to-dot and conserved dot regions showed lower nucleotide diversity, consistent with the low recombination rates on dot chromosome in *D. pseudoobscura* and *D. miranda*. Because Y chromosomes lack recombination and have a reduced efficacy of natural selection, they tend to accumulate repetitive elements and other deleterious mutations through Muller's ratchet. As a consequence, Y chromosomes tend to have large introns including some that are megabases long. The Y-to-dot region does have longer introns than other parts of the dot chromosome and other autosomes. However, except for the absence of Y-linked mega-introns, there are no significant reductions in intron sizes on Y-to-dot of *D. pseudoobscura* compared to the Y chromosome of *D. melanogaster*. While previous studies suggest that recurrent selective sweeps favoring shorter introns shaped the Y-to-dot region, an alternative hypothesis is that the ancestor of the *obscura* group had a much smaller Y chromosome than *D. melanogaster*. Consistent with this hypothesis, our cytological survey of Y chromosomes in *obscura* group suggests that most species in this group have small Y chromosomes. Therefore, the smaller size of the Y-to-dot region may be at least in part due to a small Y chromosome in the ancestor of the *obscura* group.

P2053B Transposase genes are actively expressed in vespertilionid bat somatic tissues. *Rachel Cosby*, Ellen Pritham. University of Utah, Salt Lake City, UT.

Transposition is often deleterious, and is generally repressed by the host in both germline and somatic tissues. Despite this, transposition does occur, and studies of various elements from fly (P element, *hobo*) indicate that transposition is most common in the germline. Recent studies have challenged this idea in mammals, demonstrating that transposition occurs in somatic tissues, such as brain and tumor tissue. Most of these studies were done in human, where only LINE-1 retrotransposons remain active. Thus, the extent of somatic transposable element (TE) activity in mammals is unexplored. DNA TE activity in mammals is understood even less, due to their recent extinction in most lineages. DNA transposons are active in many other vertebrates, indicating that mammals are the exception, rather than the rule. Notably, the Vespertilionid (vesper) bat family, has experienced numerous, recent waves of DNA TE activity in conjunction with retrotransposon activity. Here we identify expressed, full-length, putatively active transposase genes in vesper bat tissues. We performed RNA-sequencing and *de-novo* transcriptome assembly on liver, kidney, and testis tissues and fibroblast cell lines obtained from four vesper bat species, *Myotis lucifugus, M. occultus, M. velifer,* and *Eptesicus fuscus.* We also incorporated publicly available datasets for kidney, liver, and brain from another vesper bat, *M. brandtii*. We then compared existing transposase protein coding sequence to predicted open reading frames (ORFs) in our transcriptome dataset in order to identify transcripts with intact transposase ORFs. We also searched for intact transposase domains using HMMER. In doing so, we identified several expressed transposase transcripts, including both DNA TEs *(hAT, PiggyBac,* others) and retrotransposons (endogenous retroviral *pol*, *gag*, and *env* genes, and LINE-1 transposase), across all vesper bats examined. Many of these elements have been predicted to be active in vesper bats previously, but often no full-length elements were found, indicating that utilizing *de-novo* transcriptome data may identify low frequency events in the population as well as overcome difficulties due to poor genome assemblies. Overall, our data strongly implies that somatic transposase expression is common in bats, and that these TEs may be actively transposing in somatic tissue, perhaps due to a lack of regulation in somatic tissues. Thus, the contribution of TE expression and transposition to the somatic transcriptome and genome of mammals may be greater than initially anticipated. Further, expression in somatic tissues may favor horizontal transfer, granting TEs another avenue to propagate outside the germline.

P2054C Cytogenetics in the post-genomic era: *Standing chromosomal variation* associated with rapid divergence in a young species pair. *A.-M. Dion-Côté*[1], R. Symonová[2], F. C. Lamaze[3], S. Pelikánová[4], P. Ráb[4], L. Bernatchez[1]. 1) Université Laval, Québec, Canada; 2) University of Innsbruck, Mondsee, Austria; 3) Ontario Institute for Cancer Research, Toronto, Ontario; 4) Institute of Animal Physiology and Genetics, Liběchov, Czech Republic.

The role of chromosome rearrangements in speciation and reproductive isolation remains a debated topic, although certain conditions associated with divergence should promote their emergence. We examined chromosome changes in two Lake Whitefish (*Coregonus clupeaformis*) lineages that recently diverged after the colonization of post-glacial lakes following allopatry. A dwarf limnetic form evolved

repeatedly from the normal benthic form, becoming reproductively isolated. We applied conventional and molecular cytogenetic methods to three such pairs of sympatric Lake Whitefish populations, in order to i) test the null hypothesis of no difference in chromosomal rearrangements between pure lineages and ii) test the hypothesis of chromosomal instability in healthy and unfit backcross embryos.

While the dwarf and normal karyotypes were highly similar, more detailed cytogenetic analyses revealed an extensive polymorphism partly shared by the three species pairs. Multivariate analyses on cytogenetic markers revealed ongoing genomic rearrangements between these incipient species, consistent with chromosomal divergence initiated in allopatry. Our results also support the hypothesis of mitotic instability in healthy backcross embryos. We found extensive aneuploidy in unfit backcross embryos, indicating meiotic breakdown in their F1 parent.

Thus, intra-chromosomal differentiation in the Lake Whitefish system may contribute to divergence by destabilizing mitotic and meiotic chromosome segregation in hybrids. Importantly, the fine chromosome structures detected here, such as centromeres, repetitive elements and heterochromatin remain difficult to sequence and assemble. Therefore, cytogenetics is highly complementary to genomics, and should be integrated with population and quantitative genetic studies towards a better understanding of speciation.

P2055A Catching *de novo* genes as they arise in natural populations. *E. Durand*, I. Gagnon-Arsenault, L. Nielly-Thibault, G. Charron, C. R. Landry. Institut de Biologie Intégrative et des Systèmes, Département de Biologie, PROTEO, Pavillon Charles-Eugène-Marchand, 1030 avenue de la Médecine - Université Laval - Québec (QC) G1V 0A6, Canada.

The emergence of new genes is a driving engine for the acquisition of adaptive innovations. New genes may arise *de novo* from previously non-genic regions or from pre-existing gene structures such as gene duplication. The *de novo* origination is the initial source of genetic novelties and comparative studies between and within species brought to light the potential contribution of intergenic regions in this process. D*e novo* gene origination involves the acquisition of an intergenic ORF by mutations conferring a gain of in frame start and stop codons, and regulatory sites to allow its transcription and translation. Here we explore the dynamics of recently emerging *de novo* genes in natural *Saccharomyces paradoxus* populations. We first characterized intergenic ORF diversity in 24 *S. paradoxus* strains, sampled in North America, that are structured in 3 lineages. These lineages are used to investigate an on ongoing speciation event, making them a perfect model in the context of recently emerging genes and their role in adaptation and potentially speciation [1]. We annotated intergenic ORFs and classified them according to the conservation of their positions. In total, 73,657 ORF families were identified: some (2%) are conserved between *S. cerevisiae* and *S. paradoxus* while 28 and 8% are fixed and specific to *S. cerevisiae* or *S. paradoxus* respectively. Interestingly, the remaining 62% of intergenic ORFs still segregate within and among the *S. paradoxus* lineages. This diversity illustrates the power of using population data to investigate the emergence of *de novo* genes from scratch, which is expected to be rare. We are now characterizing the coding potential of intergenic ORFs at the transcriptional and translational levels to compare the rate of transcription/translation gain or loss versus ORF birth and death. Our results show that intergenic regions contain a large pool of ORFs readily available for natural selection to draw from during species formation.

Leducq JB. *et al.* (2016) Speciation driven by hybridization and chromosomal plasticity in a wild yeast. Nature Microbiol. 1: 15003. doi:10.1038/nmicrobiol.2015.3.

P2056B Few Nuclear-Encoded Mitochondrial Gene Duplicates Contribute to Male Germline-Specific Functions in Humans Compared to *Drosophila*. *M. Eslamieh*, A. Williford, E. Betrán. University of Texas at Arlington, Arlington, TX.

The analysis of nuclear-encoded mitochondrial genes (N-mt genes) in *Drosophila* has shown that most of the duplicated N-mt genes acquired testis-biased expression. These genes tend to be old and have energy-related functions. They are often relocated and many originate through retroposition, a duplication mechanism that appears to facilitate the acquisition of testis expression. These specific patterns reveal strong selection for retention of these duplicates in flies. Since selection could be different for mitochondrial functions in male germline because males do not pass the mitochondria to the offspring and are under strong male-male competition to fertilize the females, we predict that the same selective pressures could operate in other species lineages. To test this, we analyzed the entire set of genes annotated as N-mt genes in the human genome. We found 290 N-mt genes (i.e., 18%) that can be grouped into gene families which are the product of 164 gene duplication events. While around 32% of the new genes showed tissue-specific expression and testis had the highest number of tissue-specific duplicates, the percentage of this kind of duplicates was small (6%) compared to *Drosophila* (54%). Unlike *Drosophila* duplicates, human testis-specific duplicated genes are young and have diverse functions. These differences might reflect differences in the selective pressures for mitochondrial function during spermatogenesis and fertilization between *Drosophila* and humans, differences in the response to these pressures or differences in the efficiency of selection between these species.

P2057C Reconstruction of gene regulatory networks in the developing gonad of the common snapping turtle using ARACNe opens new perspectives for the study of temperature-dependent sex determination. *L. Guo*, T. Rhen. University of North Dakota, Grand Forks, ND.

For many reptiles, sex is determined by the incubation temperature of the egg during embryonic development, a process known as temperature-dependent sex determination (TSD). Previous studies in reptiles with TSD have examined homologs of genes that play a role in sex determination in mammals. However, the traditional candidate gene method lacks the ability to discover novel sex-determining genes and sometimes can be misleading. To complement the candidate gene method and accelerate progress in the field of TSD, we conducted a transcriptome-wide analysis of gene expression and gene function using next generation sequencing technology. We constructed and annotated a reference transcriptome for the common snapping turtle, *Chelydra serpentina*, one of the most widespread and abundant turtles in North America. With differential gene expression analysis comparing gonadal transciptomes between male-producing and female-producing

temperatures, we identified several hundred candidate genes for TSD. With these genes, we reconstructed a gene regulatory network underlying TSD using the algorithm for the reconstruction of accurate cellular networks (ARACNe), an algorithm that is able to identify direct gene-gene interaction with high precision and sensitivity. Our transcriptome analyses not only shed new light on the study of TSD but also offer important insights into sexual development in all vertebrates and sex associated disorders in humans.

P2058A Investigating the evolutionary pathways towards extremely AT rich genomes. *W. Hao*. Wayne State Univ, Detroit, MI.

Genomic nucleotide content (usually measured as GC content) varies widely among species, the most extreme (AT-rich) of which is in the mitochondria of yeasts. These extreme genomes provide a unique opportunity to study the evolution of genomic nucleotide landscape. In this study, we sequenced six complete mitogenomes of the *Saccharomycodes ludwigii* yeast, all of which have <10% GC content. Our comparative genomics analyses observed variable intron presence/absence patterns in the large ribosomal subunit (*rnl*) gene and cytochrome *c* oxidase subunit I (*cox1*) gene, and variable lengths of AT-rich tandem repeats. The whole genome alignments among these mitogenomes showed mosaic sequence patterns, suggesting perhaps frequent mitochondrial DNA recombination. We found no evidence of accelerated substitution rates in these *Saccharomycodes ludwigii* mitogenomes, when compared against other yeast mitogenomes. Thus, mutational pressure and reduced recombination, both of which can lead to increased AT content, are unlikely the main driving force leading to the extreme AT mitogenomes in *Saccharomycodes ludwigii.* We tend to believe that the proliferation of AT-rich tandem repeats via replication slippage and/or unequal crossing-over plays an important role in driving the extreme AT richness in these mitogenomes.

P2059B Evolution of gene regulation in nutrient starvation response between free-living and commensal yeast. *Bin He*, Xu Zhou, Erin O'Shea. Harvard University, Cambridge, MA.

The genomic processes behind local adaptation can be revealed by applying functional genomics in a comparative framework. Here I present work on the evolution of phosphate starvation response bewteen the model organism, *S. cerevisiae*, and a close relative, *Candida glabrata*. *C. glabrata* is a *post-genome-duplication* species, same as *S. cerevisiae.* Yet since their divergence, it has acquired abilities to colonize mammal gut. Preliminary studies of its phosphate starvation response revealed a puzzling difference in the regulation of the pathway. While the master transcription factor PHO4 in *S. cerevisiae* requires a co-factor (PHO2) to function, *this dependence is lost* in *C. glabrata*. Interestingly, the ortholog of the PHO2 in *C. glabrata* still functions and binds adjacent to most of the Pho4 sites, yet its Pho4 is able to bind and activate gene expression independent of Pho2. Using transcriptome profiling and chromatin IP, we mapped genome-wide binding sites of both PHO4 and their co-factor in each species' genome, and identified PHO4 dependent genes, respectively. We found that (1) loss of dependence on the co-factor results in expansion of PHO4's targets. (2) accompanying cis co-evolution modulates the extent of target expansion in *C. glabrata*. (3) functional annotation of PHO4 targets in *C. glabrata* suggests cross-activation of genes involved in other stress responses. Remarkably, a survey of PHO4 orthologs in divergent yeast species revealed two independent events for loss of PHO2-dependence, both in commensal species, suggesting the change is likely beneficial in that context.

P2060C Dynamics of mitochondrial genome evolution during speciation by hybridization. *Mathieu Henault*[1], Jean-Baptiste Leducq[1,2], Guillaume Charron[1], Yves Terrat[2], Jesse B. Shapiro[2], Christian R. Landry[1]. 1) Institut de Biologie Intégrative et des Systèmes, Département de Biologie, PROTEO, Université Laval, Québec, Canada; 2) Département des Sciences Biologiques, Université de Montréal, Montréal, Canada.

Hybridization between species or diverged populations may lead to individuals with mixed ancestry that are reproductively isolated from their parents, resulting in species formation. Although the genomic outcomes of speciation by hybridization have been extensively investigated, the dynamics of organelle genome evolution and its contribution to speciation in this context remain to be fully investigated. Here we study the dynamics of mitochondrial evolution during hybrid speciation in species where mitochondrial genomes are bi-parentally inherited. We used North American lineages of *Saccharomyces paradoxus* that are characterized by partially overlapping distributions and distinct responses to environmental conditions that suggest ecological divergence. The lineage SpC* was recently discovered and shown to be an incipient species that resulted from hybridization between the sister lineages SpC and SpB in their overlapping distribution. Analysis of three SpC* mitochondrial genomes revealed two mostly SpC-like haplotypes and one mostly SpB-like haplotype that all contain introgressed regions, showing extensive mitochondrial genetic admixture in the process of hybridization. These data suggest that mitochondrial genomes readily recombine in hybridizing lineages. We tested this hypothesis experimentally by analyzing the mitochondrial genomic haplotypes of experimental F1 SpB-SpC hybrid strains and showed that frequent recombination events occur within few generations. We are currently examining the adaptive potential of the new mitochondrial haplotypes produced by recombination. Our results show that mitochondrial genomes recombine in hybridizing species where mitochondria are bi-parentally transmitted.

P2061A Detailed structure and variation of complex satellite DNA loci in *Drosophila melanogaster*. *D. E. Khost*, D. Eickbush, A. M. Larracuente. University of Rochester, Rochester, NY.

Large blocks of tandemly repeated heterochromatic sequences called satellite DNAs (satDNAs) can make up a substantial fraction of most eukaryotic genomes. SatDNAs evolve rapidly and may contribute to genetic incompatibilities between closely related species. The variation in heterochromatic sequences, including satDNAs, within species is associated with variation in fitness, genome-wide gene expression and male fertility. Despite their prevalence, satDNAs remain poorly understood and understudied, largely due to the technical difficulty in sequencing and assembling such large, highly repetitive areas of the genome. Advances in single-molecule real-time (SMRT) Pacific Biosciences (PacBio) sequencing help overcome the limitations of traditional sequencing methods for some repeat-rich sequences. We use PacBio sequencing to

reveal the detailed structure and organization of two complex satDNA loci in *Drosophila melanogaster:* a 120 bp repeat called *Responder (Rsp)* and a 260 bp repeat in the *1.688* gm/cm^3 satellite family (*260-bp*). We report on the optimal assembly strategies for regions rich in complex tandem repeats and the complete assemblies of *Rsp* and 260-bp as supported by computational and molecular validation. Both *Rsp* and *260-bp* show high levels of repeat homogenization within their arrays, particularly over the center, indicating that they are undergoing concerted evolution. Sequence variants of the repeats are non-randomly distributed, tending to be located near the distal and proximal ends of their arrays. The *Rsp* locus possesses several additional interesting levels of organization: two islands of transposable elements occur approximately 100 kb apart near the proximal and distal boundaries of the *Rsp* locus, but in an inverted orientation. The *Rsp* sequences in their vicinity have identical partners on opposite sides of the array. This structure suggests several recent, complicated duplication/inversion events and/or recent gene conversion have helped shape the *Rsp* locus. We also use Illumina sequence reads to investigate polymorphism in the organization and abundance of these satDNAs in population samples of *D. melanogaster* from across the globe. We show that the size and composition of the *Rsp* and *260-bp* loci vary across populations and infer the mechanism and location of array expansion/contraction to be through unequal crossing over at the array center. We find an approximately tenfold and fourfold variation in locus size for *Rsp* and *260bp*, respectively. Overall, the unprecedented level of detail that we have in our assemblies allows us to begin to answer fundamental questions about the evolution and dynamics of repetitive regions.

P2062B The Rate and Spectrum of Spontaneous Mutations in Social Amoeba *Dictyostelium discoideum.* . *S. Kucukyildirim*[1,4], W. Sung[3], T. G. Doak[1,2], M. Lynch[1]. 1) Indiana University, Bloomington, IN; 2) University of North Carolina Charlotte, NC; 3) National Center for Genome Analysis Support, Indiana University, Bloomington, IN; 4) Hacettepe University Ankara, Turkey.

Background: In this study we determine the rate and spectrum of spontaneous mutations for the social amoeba, *Dictyostelium discoideum*, a key model organism in molecular and cell biology. Like many other microbial eukaryotes, *D. discoideum* has unusual life history and genome features, for example its AT-rich genome (77.5%) and high percentage of simple sequence repeats, including in coding sequence. This work enables us to explore evolutionary forces and molecular mechanisms that may have shaped the mutation rate and spectrum of *D. discoideum*.

Results and Discussion: Whole-genome sequencing of 19 mutation accumulation lines of *D. discoideum* after an average of 3,000 cell divisions yielded a base substitution mutation rate estimate of 3.55×10^{-11} per site per generation, substantially lower than that observed for most eukaryotic and prokaryotic organisms. Transversions biased transitions and base-substitution changes yielded a mutation rate in the AT direction. The deletion rate is higher than the insertion rate, which is inconsistent with the previous studies showing an insertion bias in unicellular eukaryotes. The base-substitution mutation rate in *D. discoideum* is on the same order of magnitude of that of the ciliates *Paramecium tetraurelia* and *Tetrahymena thermophila.* Like ciliates, *D. discoideum* maintains a large effective population size, reducing the power of random genetic drift, such that selection is relatively unhindered to reduce mutation rates. This observation is consistent with the drift barrier hypothesis that mutation rates are inversely proportional to effective population sizes, and furthers our understanding of the evolutionary forces and molecular mechanisms shaping the evolution of mutation rate.

P2063C Mitochiondrial genome comparisons across major sea urchin families, with special focus on the emerging model *Tripneustes gratilla*. *A. Laruson*, F. Reed. University of Hawai'i at Mānoa, Honolulu, HI.

The sea urchin genus *Tripneustes* has historically served as a model species for theories on population divergence due to its impressive pan-tropical distribution with few well documented barriers to dispersal. While showing very little localized variation, analysis of microsatellite markers and mitochondrial COI sequences suggests that greater isolation exists within the Pacific Ocean than previously believed. A draft transcriptome has allowed for the generation of a full mitochondrial genome sequence from the central Pacific *T. gratilla,* and is compared to mitochondrial genome assemblies across major sea urchin families. The genus *Tripneustes* is placed in a broader phylogenetic context and tree node calibrations are used to date the origin of the lineage. Rates of codon variation in the 13 coding sequences of the mitochondrial genomes across the families are assessed, and pairwise d_N/d_S (ω) ratios highlight variable contraints on coding regions among the sea urchins. Increased understanding of the genomics of such a broadly distributed animal, with an available reference genome in *Strongylocentrotus purpuratus*, and an effectice Wright-Fisher approximating population, could allow for *Tripneustes* to be an effective model organism for population genomics.

P2064A Comparative Methylome Analyses Identify Epigenetic Loci of Transcriptional Regulation in the Human Brain. *I. Mendizabal*[1,3], L. Shi[2,8], T. E. Keller[1], G. Konopka[4], T. M. Preuss[5], T. Hsieh[6], E. Hu[2,7], Z. Zhang[2], B. Su[2], S. Yi[1]. 1) School of Biology, Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA; 2) School of Biology, Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA; 3) Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country UPV/EHU, Barrio Sarriena s/n, 48940 Leioa, Spain; 4) Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX. 75390, USA; 5) Division of Neuropharmacology and Neurologic Diseases & Center for Translational Social Neuroscience, Yerkes National Primate Research Center, Emory University, and Department of Pathology and Laboratory Medicine, Emory University School of Medicine. Atla; 6) Department of Plant and Microbial Biology and Plants for Human Health Institute, North Carolina State University, North Carolina Research Campus, Kannapolis, NC 28081, USA; 7) Kunming College of Life Science, University of Chinese Academy of Sciences, Beijing 100101, China; 8) The Molecular & Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI 48109-2200, USA.

Elucidating the impact of epigenetic modifications in the evolution of phenotypes remains as a fundamental yet unresolved question in biology. In particular, the role of epigenetic divergence on the evolution of human brains, which underwent an unparalleled degree of recent evolutionary innovation, may have deep implications for human cognition and neuropsychiatric diseases. Systematic analyses employing

unbiased epigenome-wide methods, and including outgroup species and large panel of individuals are needed to fully understand the role of epigenetic divergence on human brain evolution. Here we report comprehensive identification and analyses of differentially methylated regions (DMRs) in human brains. We used comparative whole genome bisulfite sequencing (WGBS) of human, chimpanzee and rhesus macaque prefrontal cortices (n=8), unbiased non-parametric tests, as well as targeted deep genomic and bisulfite sequencing in an independent panel of 37 individuals across six primate species. We identify 278 DMRs using conservative cutoffs. These DMRs conspicuously co-localize with functional genomic indicators of transcriptional regulation, including chromatin features and transcription factor binding profiles. In particular, we identify many loci annotated as intergenic yet exhibiting conspicuous chromatin signatures of active transcription in brains, indicating that they represent currently unannotated loci of human brain-specific transcription. Remarkably, a large number of DMRs are found in a spatially clustered manner and tend to participate in active chromatin loops, indicating evolutionary remodeling at the higher-order chromatin structure. Analyses of previously generated gene expression and epigenetic data also support a role of DMRs in regulation of transcription. In summary, we identify hundreds of human brain specific DMRs and demonstrate that the unique epigenetic profiles of the human brain are supported in a large number of samples across multiple species. We unravel human brain specific epigenetic changes at regulatory regions that shape gene expression divergence. Substantial reprogramming of the epigenomic landscape appears to contribute to the evolutionary specializations in our brains.

P2065B Exploring the last chromosome: Y-linked sequence variation in the house mouse. *Andrew Morgan*, Fernando Pardo-Manuel de Villena. University of North Carolina, Chapel Hill, NC.

Over 180 million years since their divergence from autosomes, mammalian Y chromosomes have evolved a gene repertoire highly specialized for male reproduction. Y chromosomes are also a valuable tool for population genetics and phylogeny because they are inherited without recombination from a single parent. The Y chromosome of the house mouse (*Mus musculus*) is exceptional in that it is almost entirely euchromatic and has important roles in speciation. However, its complex repetitive structure has hindered analyses by high-throughput sequencing. We sought to fill this gap by performing a systematic survey of Y-linked variation in mouse. We have compiled whole-genome sequencing data for 132 male samples including wild-caught and laboratory representatives of each of the three subspecies of the house mouse (*Mus musculus domesticus*, *M. m. musculus* and *M. m. castaneus*) and transcriptome data from outgroup specices. First, we show that both the short and long arms of the Y chromosome of the three subspecies are differentiated by megabase-sized structural variants. Next we apply several complementary approaches to obtain a catalog of sequence variants in the non-ampliconic portion of the Y chromosome. Using laboratory strains with known pedigree relationships, we estimate the average mutation rate on Y to be $9.1 \pm 1.8 \times 10^{-9}$ per site per generation. Finally we use approximate Bayesian computation to compare patrilineal and matrilineal demographic patterns in wild mice. We find evidence for increased geographic differentiation and reduced effective population size on the Y compared to the mitochondria, and attribute this to the combined effects of background selection and variability in male reproductive success.

P2066C The evolution of sexual dimorphism of recombination rate in house mice. *April Peterson*, Bret Payseur. The University of Wisconsin-Madison, Madison, WI.

Although most studies of genetic diversity assume that the recombination rate is constant, this important parameter varies among individuals. Heterochiasmy, or - the sexual dimorphism in recombination rate - is one of the most striking forms of this variation. The theoretical foundation for recombination rate and heterochiasmy evolution is well established, yet these theoretical models remain largely untested due to a lack of empirical data. We aim to remedy this gap by expanding the range of empirical measurements and developing a framework for interpretation of observed patterns within an evolutionary context. Using an immunohistochemical approach that enables measurement in single oocytes and spermatocytes, we quantify variation across three wild-derived inbred strains of house mice (*Mus musculus*) in the genome-wide recombination rate, the synaptonemal complex length and the position of crossovers. We observe faster evolution in the genomic recombination rate of male house mice and divergence in the synaptonemal complex length across inbred strains. These data represent a subset of a larger panel of divergent inbred strains to be sampled across the murine phylogeny. When complete, these measures of heterochiasmy will enable us to test hypotheses for sex-specific evolution of genome-wide recombination rate.

P2067A Genomic disintegration in woolly mammoths on Wrangel island. *Rebekah L. Rogers*, Montgomery W. Slatkin. University of California, Berkeley, Berkeley, CA.

Woolly mammoths (*Mammuthus primigenius*) populated Siberia, Beringea, and North America during the Pleistocene and early Holocene. Recent breakthroughs in ancient DNA sequencing have allowed for complete genome sequencing for two specimens of woolly mammoths (data from Palkopoulou et al. 2015). One mammoth specimen comes from mainland populations ~40,000 years ago when mammoth populations were robust. The second, a 4300 yr old specimen, is derived from an isolated population on Wrangel island where mammoths formed an isolated population with an effective population size 50-fold smaller than previous populations. These extreme differences in effective population size offer a rare opportunity to test nearly neutral models of genome architecture evolution within a single species. Using these previously published mammoth sequences, we identify deletions, retrogenes, and non-functionalizing point mutations in the genomes of these two specimens. In the Wrangel island mammoth, we identify a larger number of deletions, a larger proportion of deletions affecting gene sequences, and an increased number of premature stop codons. We also identify more retrogenes, consistent with elevated retroelement activity. This accumulation of detrimental mutations is consistent with genomic meltdown in response to low effective population size in the dwindling mammoth populations on Wrangel island, consistent with nearly-neutral theory.

P2068B *Cis*-acting variation in gene expression dynamics within and between *Saccharomyces* species. *Ching-Hua Shih*[1,2], Justin Fay[1,2]. 1) Dept. of Genetics, Washington University in St. Louis, MO; 2) Center for Genome Science and Systems Biology, Washington University, St. Louis, MO.

Variation in *cis*-regulatory sequences has been shown to modulate gene expression levels or developmental patterns. However, temporal control of gene expression may be as important to fitness as steady-state levels. To determine whether *cis*-regulatory sequence variation contributes to gene expression dynamics as well as steady-state levels we measured allele-specific expression within multiple yeast hybrids during the transition from fermentative to respiratory growth.

We found 961-3045 genes with allele-specific differences in expression that changed over time. In comparison, we found 466-863 genes with allele-specific differences in expression either during growth on glucose or ethanol. Using logistic regression, we found that single nucleotide polymorphisms (SNPs) in promoters regions are associated with a 1.02-1.04 odds ratio (OR) of allele specific expression. In contrast, insertions and deletions (InDels) are associated with a 1.11-1.15 OR of allele-specific expression.

We conclude that there is nearly as much *cis*-acting variation that affects temporal changes in gene expression as affects steady-state levels alone. Our observation that InDels have a stronger association than SNPs suggests a difference in how expression levels and dynamics are modulated.

P2069C Degeneration and positive selection of a non-recombining chromosomal inversion underlying behavioral polymorphism in the white-throated sparrow. *D. Sun*[1], I. Huh[1], D. Maney[2], S. Yi[1]. 1) Georgia Institute of Technology, Atlanta, GA; 2) Emory University, Atlanta, GA.

The white-throated sparrow is a common North American songbird with two adult plumage morphs known as white- and tan-striped. The morphs differ in behavior, with white birds exhibiting more aggression and exhibiting less parental care than tan birds during the breeding season. The phenotypic differences are associated with multiple chromosomal inversions in the second chromosome. White birds are heterozygous for the inversions (ZAL2/ZAL2^m), whereas tan birds are homozygous (ZAL2/ZAL2). Tan and white morphs are maintained in relatively equal proportions in the population via a strong disassortative mating (nearly all mating pairs consist of one white and one tan bird). To elucidate the genetic and molecular basis of these fundamental phenotypic differences between the morphs, we compared the whole genome sequences of a rare superwhite bird (ZAL2^m/ZAL2^m) to the reference tan genome (ZAL2/ZAL2). We also incorporated RNA-seq data from a sample of 22 individuals that included tan, white and superwhite birds. Utilizing such rich genomic and transcriptomic resources, we can distinguish genomic scaffolds that reside within and outside the inversions. As expected, F_{ST} analyses indicate little recombination between the ZAL2 and ZAL2^m chromosomes within inversion. The inverted scaffolds are on average 1% divergent at the nucleotide level. Despite such small genetic differences, ZAL2^m scaffolds exhibit signs of degeneration via accumulation of slightly deleterious nonsynonymous substitutions, as expected due to the suppression of recombination. On the other hand, we also found signatures of adaptive evolution in both protein-coding and regulatory regions. Intriguingly, regions that exhibit signs of positive selection in ZAL2 and ZAL2^m chromosomes can be assigned to distinctive functional categories. Positively selected genes in ZAL2 are related to the immune system, whereas those in ZAL2^m are enriched for muscle structure development, locomotion and neuron differentiation. Our study provides new insights into how genetic differentiation can lead to phenotypic divergence in this unique vertebrate model system.

P2070A Enhancer activity of vertebrate ultraconserved elements in fruit flies. *T. Takano-Shimizu*, T. Ohsako, T. Matsuda, M. Tomaru. Kyoto Institute of Technology, Kyoto, Kyoto, JP.

Comparative genomics has identified a large number of non-coding segments that have been highly conserved over hundreds of millions of years of vertebrate evolution. To examine their functions *in vivo*, we generated over 300 Drosophila transgenic lines, each containing a unique human conserved noncoding segment inserted upstream of a core promoter fused to a GAL4 or a QF gene, and then assessed their enhancer activities. We observed reporter GFP expression in one or more tissues of embryos and larvae in all segments tested. Therefore, it is evident that human conserved segments can function as enhancer in Drosophila. Indeed, our assay system is more powerful and effective than the previously described mouse assay, which detected reporter gene expression only in a half of tested segments. What is more, induced GFP expression is biased toward the brain and central nervous system of Drosophila. This result may imply that human conserved segments share some unknown enhancer functions despite lacking sequence homology.

P2071B Divergent patterns of marsupial-eutherian genomic imprinting revealed from RNA-seq analysis in the opossum, *Monodelphis domestica* . *Xu Wang*[1], Kory Douglas[2], John VandeBerg[3], Paul Samollow[2], Andrew Clark[1]. 1) Cornell Univ, Ithaca, NY; 2) Texas A&M Univ, College Station, TX; 3) Southwest Primate Research Center, San Antonio, TX.

Among the ~150-200 imprinted genes identified in mouse and human, only 20 marsupial orthologs have been examined to date, and eight of these were found to be imprinted. Here we ask, what is the marsupial imprinting status for the remaining 130 eutherian imprinted genes, and are there any marsupial-specific imprinted genes? We profiled genome-wide allele-specific expression (RNA-seq), histone modifications (ChIP-seq) and DNA methylation (PyroMark) in fetal brain and extra-embryonic membranes from reciprocal crosses of two opossum lines, providing an unbiased survey of parent-of-origin effects. Among 68 genes known to be imprinted in eutherians (and having an opossum ortholog), 52 were covered with sufficient informative SNPs to score allelic expression. Only three (<6%) were found to be imprinted in opossum, and 48 display biallelic expression, reflecting a striking lack of conservation of imprinting status. We also discovered and validated eight marsupial-specific imprinted genes that are not known to be imprinted in any other species. Surprisingly, three of these are non-coding lincRNA genes with no homology to any eutherian sequences, but they are present and are highly conserved in other marsupial species and non-mammalian

vertebrates including chicken. Three of the rest five protein-coding imprinted genes were paralogous to eutherian genes, resulting from recent gene family expansions in opossum. Mechanistically, our epigenetic profiles confirmed that opossum-specific imprinted genes are regulated in the same way as eutherians by differential promoter methylation, despite the evolutionary fluidity of the imprinting profile. We estimate that opossums imprint only 30-40 genes, or about one-fifth the number imprinted by eutherian mammals. The smaller number and non-overlapping nature of imprinted genes could be due to the primitive placentation and shorter gestation time in marsupials compared to eutherians. Our study provides the first imprinting profile in a marsupial and sheds light on the regulation and evolution of genomic imprinting in mammals.

P2072C Comparative genomics of the *Daphnia pulex* species complex. *Z. Ye*[1], S. Xu[1,2], K. Spitze[1], J. Asselman[3], X. Jiang[1], M. Pfrender[3], M. Lynch[1]. 1) Indiana University, Bloomington, IN; 2) 2Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, Canada; 3) University of Notre Dame, Notre Dame, Indiana.

Comparison of closely related genotypes from populations with distinct population sizes can help us understand the impact of effective population size on genome evolution. For this purpose, we present a high-quality genome assembly of *Daphnia pulex* (PA42) and perform comparative analysis with the previously sequenced genome of *Daphnia pulex* (TCO) in the same species. PA42 is similar with TCO at the orthologous gene level, with average protein identity 98.8% and more than 60% of orthologous proteins identical. Nonetheless, there is a highly elevated number of genes in the genome annotation of TCO, with ~8,000 excess of the genes being due to inclusion of genes of bacterial origin or inaccurate annotation . This view is supported by the high GC content, lack of introns, and high sequence coverage of these suspicious genes. The reduced effective population size of the source population of TCO relative to that of PA42 makes the comparison of these two genomes an excellent platform to understand the short-term impact of effective population size on eukaryotic genome architecture. Consistent with the view that reduced effective population size can facilitate the fixation of transposable elements (TEs) and newly gained introns, we observe more proliferation of TEs and higher frequency of gained introns in the TCO genome.

P2073A Origin and spead of *de novo* genes in *Drosophila*. *Li Zhao*, David J. Begun. Department of Evolution and Ecology, UC Davis, Davis, CA.

The traditional view of evolutionary mechanism holds that novel functions result from the modification of ancestral functions by natural selection. *De novo* gene origination was considered to be almost impossible until recent studies revealed that genes may arise from ancestrally non-genic sequence. Though such genes have been observed in several species, the dynamics of their origin and subsequent spread within populations remain completely obscure. Here I present results on the abundance, properties, expression genetics, and population dynamics of segregating and fixed *de novo* gene in *Drosophila melanogaster* populations from multiple tissues and developmental stages. These genes appear to derive primarily from ancestral intergenic, unexpressed sequences. Natural selection appears to play a significant role in the spread of these genes. These results reveal a heretofore unappreciated dynamism of gene content.

P2074B Evolution trajectories of snake genes and genomes revealed by comparative analyses of five-pacer viper. *Q. Zhou*. Zhejiang University, Hangzhou, Zhejiang, CN.

Snake's numerous fascinating features distinctive from other tetrapods necessitate a rich history of genome evolution that is still obscure. To address this, we report here the first high-quality genome of a viper, *Deinagkistrodon acutus* and comparative analyses to other species of major snake lineages and lizard. We mapped the evolution trajectories of transposable elements (TEs), developmental genes and sex chromosomes onto the snake phylogeny. Besides the dynamic lineage-specific expansion, many TEs may have been rewired into the regulatory network of brain genes in viper, indicated by the associated expression of TEs and nearby genes restricted to the brain. We characterized signatures of adaptive evolution in olfactory, venom and thermal-sensing genes, and also functional degeneration in genes associated with vision and hearing. Phylogenetic distribution of the degenerating *Hox* and *Tbx* limb-patterning genes support a successive loss of forelimbs then hindlimbs during snake evolution. Finally, we showed snake Z and W sex chromosomes have undergone at least three times of recombination suppression at the ancestor of advanced snakes, with the W chromosomes forming a gradient of degeneration from basal snakes to advanced snakes. These results, together with all the genes identified as undergoing adaptive or degenerative evolution episodes at respective snake lineages forge a framework for our deep understandings into snakes' molecular evolution history.

P2075C The Genomics of Drug Consumption in *Drosophila melanogaster*. *B. Baker*, T. F. C. Mackay. Department of Biological Sciences, Program in Genetics and W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC 27695-7614.

Abuse and addiction to psychostimulants like cocaine and methamphetamine present a worldwide health issue. *Drosophila melanogaster* presents a model system to identify genetic and transcriptional networks that underlie variation in effects of drug exposure that can serve as a blueprint for subsequent studies on humans. We have derived an outbred advanced intercross population (AIP) from 37 of the sequenced inbred wild-derived lines of the *Drosophila melanogaster* Genetic Reference Panel (DGRP). The lines are maximally genetically divergent, have minimal residual heterozygosity, are not segregating for common inversions and are not infected with *Wolbachia pipientis*. We assessed voluntary consumption of 4% sucrose, 4% sucrose + 1 μg/μL cocaine and 4% sucrose + 0.3 μg/μL methamphetamine among 11 of the 37 DGRP lines that were parents of the AIP. We found significant variation among the lines, in both sexes, for consumption of both drugs, with estimates of broad sense heritability for cocaine consumption of H^2 = 0.57 (females) and 0.63 (males) and for methamphetamine consumption of H^2 = 0.45 (females) and 0.39 (males). Thus, there is genetic variation in the DGRP lines used to construct the AIP for voluntary drug consumption. Further, several DGRP lines consume over twice as much drug + sucrose as sucrose alone, and this phenomenon is both sex- and drug-specific.

We will present data from extreme quantitative trait locus genome wide association mapping of cocaine and methamphetamine consumption using the AIP.

P2079A Moving beyond the joint-scaling test for line cross analysis: An information-theoretic approach to estimating the composite genetic effects contributing to variation among generation means. *J. P. Demuth*[1], H. Blackmon[2]. 1) University of Texas at Arlington, Arlington, TX; 2) University of Minnesota, Saint Paul, MN.

The pace and direction of evolution in response to selection, drift, and mutation are governed by the genetic architecture that underlies trait variation. Consequently, much of evolutionary theory is predicated on assumptions about whether genes can be considered to act in isolation, or in the context of their genetic background. Evolutionary biologists have disagreed, sometimes heatedly, over which assumptions best describe evolution in nature. Methods for estimating genetic architectures that favor simpler (i.e., additive) models contribute to this debate. Here we address one important source of bias, model selection in line cross analysis (LCA). LCA estimates genetic parameters conditional on the best model chosen from a vast model space using relatively few line means. Current LCA approaches often favor simple models and ignore uncertainty in model choice. To address these issues we introduce Software for Analysis of Genetic Architecture (SAGA), which comprehensively assesses the potential model space, quantifies model selection uncertainty, and uses model weighted averaging to accurately estimate composite genetic effects. Using simulated data and previously published LCA studies, we demonstrate the utility of SAGA to more accurately define the components of complex genetic architectures, and show that traditional approaches have underestimated the importance of epistasis.

P2080B Genetic variation in male attractiveness: it's time to see the forest for the trees. *S. Drobniak*[1], Z. M. Prokop[2]. 1) Jagiellonian University, Krakow, PL; 2) Jagiellonian University, Krakow, PL.

The studies of sexual attractiveness are notoriously univariate. Quantitative genetics has repeatedly emphasized the need for considering multivariate phenotypes, especially if their undelrying traits are involved in genetic correlations of varying sign and magnitude. This bias arises mainly in situations when female preference (which defines the selection gradient acting on attractive male traits) is not aligned with the direction of the dominant axis of genetic variance in the complex of attractive traits.

Here we reiterate the important conclusion about considering full multivariate phenotypes instead of univariate isolated traits. We demosntrate how not accounting properly for a multivariate character of attractiveness traits may significantly bias conclusions that are drawn from studies of sexual selection. We discuss the influence of the number of traits, the imbalance of their attractiveness (i.e. difference between female selection gradients acting on specific traits) and their genetic correlations on the outcome of sexual selection for indirect genetic benefits. We provide also a short review of the existing literature that demonstrates how the published estimates of heritabilities in male attractiveness reflect this bias. Among the published accounts of genetic variance in male attractiveness those obtained using specific, isolated traits have substantially higher heritabilities than genuine attractiveness traits (measured as the female response to male phenotypes). We also review existing studies that publish G-matrices of multiple male attractiveness traits and demonstrate that the heritability of the true multivariate male attractiveness in those trait complexes can be an order of magnitude lower than the observed haritability of isolated characters.

Our results have important implications for the evolutionary genetics of sexual selection. They suggest that in many cases the indirect genetic benefits from mating may be greatly overestimated if one uses only isolated univariate male phenotypes. They also emphasize the need for using truly multivariate assays of male attractiveness, ideally by subjecting males to direct assessment by females, and scoring female responses as the male attractiveness index.

P2081C A comparison of PCR-based and GBS-based methodologies to fine-map anthracnose resistance loci in sorghum. *T. J. Felderhoff*[1], L. M. McIntyre[1], A. Saballos[2], J. W. Olmstead[1], W. Vermerris[1]. 1) University of Florida, Gainesville, FL; 2) Chromatin, Inc, Alachua, FL.

Colletotrichum sublineola is an aggressive fungal pathogen that causes the disease anthracnose in the bioenergy crop sorghum. The resulting leaf blight and stem rot can cause yield reductions of up to 70%. The sorghum acreage in the southeastern U.S. is increasing steadily due to the species' ability to produce high yields under limited inputs. Successful cultivation of sorghum in this region, where *Colletotrichum sublineola* is endemic, is contingent upon anthracnose resistance. We generated a biparental mapping population of 135 F_4 and F_5 sorghum lines to identify anthracnose resistance genes in the highly resistant line 'Bk7'. The population was phenotyped in three Florida environments and genotyped by sequencing (GBS) following a customized filtering procedure that retained informative markers. Two resistance loci were identified - on chromosomes 7 and 9 - using association analysis between the GBS-derived markers and phenotype using Fisher's exact test. Both loci contained multiple candidate resistance genes. Fine mapping of the locus on chromosome 9 was performed on a BC_1S_1 population generated from four sweet sorghum cultivars derived from the sorghum lines 'Bk7' (also used as parent in the abovementioned mapping population) and 'Mer 81-4'. The efficacy of fine-mapping with PCR-based SNP markers was compared to that using GBS. In both instances the size of the resistance locus could be reduced substantially, but the GBS procedure provided much greater resolution, at a substantially higher cost. This case formed the basis for a comparison of the merits of both genotyping methods under a number of different scenarios (population size, marker density, labor cost) to assist in deciding on the most effective genotyping procedure. Supported by the Southeastern Sun Grant Center/USDA-NIFA Award No. 2010-38502-21854, USDA-BRDI Award No. 2011-10006-30358, and US DOE award No. DE-SC0014439.

P2082A An additive genetic model is often not sufficient for predicting individual phenotypes. *S. Forsberg*[1], Ö. Carlborg[1,5], J. Bloom[2], M. Sadhu[2], L. Kruglyak[2,3,4]. 1) Division of Computational Genetics, SLU, Uppsala, SE; 2) Department of Human Genetics, University of California, Los Angeles, Los Angeles, California 90095, USA; 3) Howard Hughes Medical Institute, University of California, Los Angeles, Los Angeles, California 90095, USA; 4) Department of Biological Chemistry, University of California, Los Angeles, California 90095, USA; 5) Division of Computational Genetics, Uppsala University, Uppsala, SE.

Ever since Mendel, genotypetophenotype (GP) mapping has been the defining feature of genetics. The complete GPmap for a trait provides the expected phenotype (genotype value) for all possible combinations of alleles across all genes affecting it. Thus, instead of looking at the effect of every allele averaged across all genetic backgrounds (the marginal effect), the GP-map provides the phenotypic effect of each unique allele combination. If the joint effect of two or more loci departs from simply adding up the marginal effects at each locus, a simple additive model will fall short in predicting the phenotypic effects revealed in the GP-map. Geneticists have for many years debated whether such non-additive patterns are of importance, or if additive models are enough to describe the genetic architecture of a studied trait. The perhaps most important piece of information needed to resolve this debate, i.e. what the true multilocus GPmaps that are modeled actually look like, is however largely missing. Here, we use a large experimental yeast population to perform an extensive, empirical estimation of highorder GP-maps affecting a large number of quantitative traits. Using these as a basis, we illustrate how the estimates obtained from statistical quantitative genetic models will depend on various features of the underlying GPmaps. Specifically, we show that a large additive genetic variance does not necessarily imply that genetic interactions is of little importance, thereby illustrating how variance component analyses can be missleading when making inferences about the genetic architecture of complex traits. We also show how additiveonly genetic models can lead to poor predictions of individual phenotypes.

P2083B Estimation of genetic parameters for growth, yield and carcass quality traits in a fast-growing strain of Atlantic salmon. *J. A. Gallardo*[1], C. J. Soto[2]. 1) Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Valparaíso, CL; 2) Salmones Camanchaca S.A., Puerto Montt, CL.

In this study, the heritability and genetic correlations of the principal productive traits of interest related to growth, yield and carcass quality in a population of domesticated Atlantic salmon (Salmo salar) of Lochy strain were estimated. To do this, 199 families belonging to the company Salmones Camanchaca's breeding program were selected. Salmon were growed in a mariculture center as well as a freshwater fish farm. Regarding the fish farmed at sea, heritabilities of medium and high magnitude were established, for traits associated to growth (0.29 ± 0.04 to 0.43 ± 0.05) and for yield traits when were quantified based on carcass weight (0.39 ± 0.04 to 0.40 ± 0.04). Yield traits in terms of percentage and quality had low heritabilities (0.06 ± 0.01 to 0.02 ± 0.03), except for the color trait of TRIMD fish fillet, which had an average heritability (0.22 ± 0.05). Genetic and phenotypic correlations were high between the main growth and yield traits of ranges 0.79 to 1.0, but low and medium - some even negative -between them and those associated with quality, ranging from 0.13 to -0.69. In the particular case of fish fillet color, this trait should be regarded in the selection criteria, but others traits like gaping or descaling have not suficient genetic variation to be included in the selection criteria.

P2084C Tracing the signature of gene expression across time in *D. melanogaster* artificially selected for long and short sleep duration. *S. T. Harbison*, Y. Lin, Y. Serrano-Negron. National Heart, Lung, & Blood Inst, Bethesda, MD.

Sleep is highly conserved across taxa, and thought to be crucial for life. The suite of genetic factors that contribute to variation in sleep in natural populations, however, remains unknown. We conducted an artificial selection study coupled with RNA-Seq in order to identify gene networks associated with phenotypic changes over time. Using a previously created outbred population of flies constructed from ten *Drosophila* Genetic Reference Panel lines having extreme long and short night sleep duration, we constructed six selection populations. Two populations were designated as short-sleeper populations, two as long-sleeper populations, and two populations served as an unselected control. Each generation, we assayed sleep in each population. We chose the 25% most extreme sleepers as parents for the subsequent generation in the short- and long- sleeper populations; we chose 25% of the flies at random to be parents in the subsequent generation of controls. After 13 generations of selection, night sleep duration in the long-sleeper populations averaged 642.2 ± 3.83 and 667.8 ± 2.97 minutes; 104.3 ± 6.71 and 156.2 ± 8.76 minutes in the short-sleeper populations; and 563.4 ± 7.62 and 542.3 ± 7.91 minutes in the control populations. Night and day average bout length and sleep latency exhibited a correlated response to selection for night sleep across generations, while night and day bout number, day sleep, and waking activity did not. We harvested total RNA from homogenates of 10 flies of each sex per generation and sequenced poly-A selected libraries. We are currently analyzing the sequence data to detect patterns of expression across selection scheme, sex, and generation. Our goal is to detect gene expression networks that are crucial to these phenotypic changes.

P2085A Functional validation of loci contributing to nicotine resistance in *Drosophila*. *C. A. Highfill*, S. K. T. Nguyen, J. Tran, X. Wang, T. R. Moldenhauer, S. J. Macdonald. University of Kansas, Lawrence, KS. 66045.

All species posses detoxification pathways enabling them to respond to environmental toxins. Given the widespread use of pesticides, and the frequent development of resistance to these compounds by crop pests, it is critical to understand the genetic basis of xenobiotic resistance in insects. Previously, we used the *Drosophila* Synthetic Population Resource to map four QTL contributing to resistance to nicotine, a compound employed as a natural insecticide by some plants. RNA-seq showed that both cytochrome P450 genes under QTL1, *Cyp28d1* and *Cyp28d2*, and one of ten UDP-glucuronosyltransferase (Ugt) genes under QTL4, *Ugt86Dd*, were differentially-expressed between susceptible

and resistant strains. Here we carry out RNAi, quantitative complementation tests, overexpression experiments, and mutant analysis to functionally validate the effects of these loci on nicotine resistance, and attempt to identify causative sequence variants. Ubiquitous RNAi knockdown of *Cyp28d1* and *Ugt86Dd*, as well as targeted knockdown in the anterior midgut, reduced nicotine resistance, providing evidence these genes are involved in the phenotype. To determine whether these loci harbor segregating variation influencing resistance we crossed susceptible and resistant strains to a range of deficiencies and insertional mutants. These quantitative complementation tests revealed functional allelic variation at *Cyp28d1* and *Cyp28d2*, and suggested that multiple nicotine resistance factors are present within the QTL4 region, consistent with the idea that several of the Ugt genes are involved. Sequencing the *Ugt86Dd* open reading frame revealed a 22bp coding deletion segregating in our multi-parental mapping panel, with the four most susceptible founder haplotypes at the QTL all harboring the deletion allele. We constructed overexpression genotypes using both *Ugt86Dd* alleles, and found that nicotine resistance is significantly greater following anterior midgut overexpression of the insertion allele compared to the deletion allele, implying the variant has a functional role. To further test the effect of this polymorphism we generated two mixed populations derived from *Drosophila* Genetic Reference Panel; one founded with all seven DGRP strains homozygous for the deletion allele, and one founded with a random seven strains homozygous for the insertion. Flies from the population fixed for the deletion allele were more susceptible for nicotine, supporting the idea that this variant directly contributes to nicotine resistance. We have now successfully generated custom *Ugt86Dd* mutants using the CRISPR-Cas9 system, and future experiments will help elucidate the effect of the locus on nicotine resistance.

P2086B Analyzing the Effects of Naturally Occurring Genetic Variants in the Sphingosine-1-Phosphate Receptor Family. *J. T. Hornick*[1], S. Nguyen[2], P. Benegal[3], Y. I. Kawasawa[1], G. L. Moldovan[1], J. R. Broach[1]. 1) Pennsylvania State University College of Medicine, Hershey, PA 17033; 2) University of Pennsylvania, Philadelphia, PA 19104; 3) Princeton University, Princeton, NJ 08544.

The sphingosine-1-phosphate receptor (S1PR) family has been identified to be critical to a multitude of cellular functions including: cytoskeletal rearrangement, cellular motility and invasion, angiogenesis, immunological processes, and vascular maturation. In addition, the family is ubiquitously expressed throughout cell types. S1P receptors are also clinically relevant, being targeted in multiple sclerosis, cancers and recently COPD. Here we have used multiple model systems to explore how natural variants (SNPs) in human populations affect the sphingosine-1-phosphate (S1P) family of G protein-coupled receptor receptors. Variants rs61734752 (A11D) in the N-terminus of S1PR1 and rs3745268 (R60Q) and rs117064827 (V286A) in transmembrane domains 1 and 7, respectively, of S1PR2 significantly decreased the potency of the natural ligand, S1P. Variant rs35483143 (L318Q), in the C-terminus of S1PR5, disrupts the ability of the receptor to couple to Gα12, one of the two cognate Gα proteins through which the receptor elicits downstream responses. RNA sequencing analysis of transfected HEK293T cells showed decreases in transcripts pertaining to cytoskeletal rearrangement, cell motility, and RhoGEF effector signaling when comparing the variant and wild type receptors. Analysis of F-actin dynamics reveal a substantial decrease in overall F-actin in variant receptor transfected cells. Further analysis of variants is being undertaken to investigate the signaling defects to cellular and developmental homeostasis.

P2087C Integrated Genetic Analysis Platform (IGAP) for Web-based Interactive Association Analysis and Visualization of Large Scale Genotype/Phenotype Data. *G. Jun*. UTHealth School of Public Health, Houston, TX.

Modern genetic association studies require analysis of millions of variants often with hundreds or thousands of phenotypic variables. Association analysis typically involves many steps of human interventions, such as parsing variant annotation, unification of missing data identifier, sample ID matching, identification of population or family structures, stratification of input data according to population or other structural batches, and parsing phenotypic labels for traits of interest and covariates. Genotype and phenotype data are usually provided in human readable formats, but not with strictest format guidelines for automated pipelines. Due to these issues, a significant amount of analysts' time is spent on rectifying input files both for genotypes and phenotypes, and also on visualizing and parsing various quality metrics. These tasks typically require a fair amount of computer programming, and such (often in-house) programs are not being reused widely because every project has a slightly different data structure from each other. It is important to automate these steps, because computing time is cheap, and will get cheaper in the future, while an analyst's time is not. We propose a new web-based interactive pipeline for genetic association analysis, named Integrated Genetic Analysis Platform (IGAP) that provides automated, re-usable framework for large-scale genetic association analysis. IGAP is a web-based front-end environment that provides encapsulation of a collection of external tools and pipelines including PLINK, Merlin, and EPACTS. It accepts and provides easy data conversion tools between common genotype formats (VCF, PLINK and Merlin) from web browser interface, and also parses metadata from VCF file's INFO field to generate functional groups with minimal user interventions. A user can easily visualize population structures using PCA or MDS using back-end tools and data converters, and resulting plot is dynamically loaded onto the web browser. Phenotype data are stored in MySQL database together with meta-data from genotypes, and provides an interactive interface for common tasks such as matching sample IDs and defining subsets of samples for a given project. It invokes association analyses and visualizes results on the web browser. IGAP provides a flexible and easy-to-use interactive environment for various types of genetic analyses.

P2088A Automated tracking and analysis of sleep-like behavior in *Drosophila* larvae. *C. Kim*[1], S. Harbison[1], Q. Gaudry[2]. 1) National Heart Lung and Blood Institute, Bethesda, MD; 2) University of Maryland, College Park, MD.

Sleep is universally conserved among animals and invertebrates, but its purpose remains elusive. Although many studies have provided insights into the genetic architecture of sleep in adult flies, little is known about this behavior in the early developmental stages of the fly. Here we introduce a method based on machine vision to continuously measure kinematic parameters in third instar larvae during the first 4 hours of the dark cycle. We are measuring larvae from the *Drosophila* Genetic Reference Panel (DGRP) in order to associate genetic polymorphisms

with rest and activity correlates. Preliminary results indicate that significant genetic variation among the lines is present for activity and rest measurements. This powerful approach enables the construction of behavioral profiles of individual larvae using high-throughput behavioral screening.

P2089B The genetic basis of the Drosophila IIS pathway response to changing nutrition. *Elizabeth G. King*, Patrick D. Stanley, Anna M. Perinchery, Vincent S. Farinella. University of Missouri, Columbia, MO.

Given a finite amount of resources, a central requirement of all organisms is optimizing the allocation of those resources to competing anatomical structures and physiological functions, which necessitates the coordination of multiple organ systems within the nutritional environment. The insulin/insulin-like growth factor signaling (IIS) pathway is known to influence the coordination of nutrition with metabolism and allocation of nutrients, but little is known about the genetic basis of standing variation in the pathway. Most studies to date of the IIS pathway have followed a "one gene at a time" approach. In this regard, they have been very successful at determining the pathway's role in the regulation of metabolism and resource allocation and of the effects of altering each gene in isolation. In stark contrast to this success, we know little about the genetic basis of *natural* variation in the IIS pathway, metabolism, and resource allocation. We utilize a large multiparental mapping population, the *Drosophila* Synthetic Population Resource to map QTL for transcript levels of the genes encoding the core components of the IIS pathway in three different nutritional environments. We identify the loci responsible for the response of IIS expression in different nutritional conditions and discuss the extent to which different loci control IIS expression in different environmental conditions.

P2090C A decrease in soybean seed protein is associated with an increase in domestication traits. *E. E. Large*, E. Beche, T. La, X. Niu, G. Shannon, A. Scaboo. University of Missouri, Columbia, MO.

Domesticated soybeans (*Glycine max*) are an important source of protein for livestock in the Americas and an increasingly popular food source for humans around the world. Soybeans were originally domesticated 6,000-9,000 years ago in China and share a common ancestor with wild soybeans (*G. soja*). Both species share the same number of chromosomes (2n = 40) and crosses between the two species generate fertile hybrids. The two species exhibit distinct phenotypes with most modern domesticated *G. max* having large yellow seeds with upright plants and most *G. soja* having small black seeds with weedy vines. The past eighty years of soybean breeding in the United States has led to significant increases in yield but at the cost of producing less protein per seed. Furthermore, there is a growing demand to produce soybean seeds with increased cysteine, methionine, and lysine content in order to optimize monogastric animal growth. Eighty *G. soja* soybean lines, representing the broadest possible diversity of wild soybean germplasm, were screened for seed protein and amino acid composition. We identified a handful of elite *G. soja* lines with high seed protein and favorable amino acid profiles. The sixth generation of two populations derived from elite *G. soja* crosses with *G. max* were subsequently evaluated for domestication traits and seed protein. Preliminary results suggest protein content is linked or overlapping with loci controlling soybean domestication. These hybrid populations, therefore, provide clues to locate genetic determinants of protein and amino acid composition in domesticated soybeans.

P2091A The genetic basis of temperature sensitivity in a mutationally induced trait. *Jonathan Lee*[1], Matthew Taylor[1,2], Amy Shen[1], Ian Ehrenreich[1]. 1) University of Southern California, Los Angeles, CA; 2) University of Washington, Seattle, WA.

Determining how genetic variation alters the expression of heritable phenotypes across conditions is important for agriculture, evolution, and medicine. Central to this problem is the concept of genotype-by-environment interaction (or 'GxE'), which occurs when segregating genetic variation causes individuals to show different phenotypic responses to the environment. While many studies have sought to identify individual loci that contribute to GxE, obtaining a deeper understanding of this phenomenon may require defining how sets of loci collectively alter the relationship between genotype, environment, and phenotype. Here, we identify combinations of alleles at seven loci that control how a mutationally induced colony phenotype is expressed across a range of temperatures (21, 30, and 37°C) in a panel of yeast recombinants. We show that five predominant multi-locus genotypes involving the detected loci result in trait expression with varying degrees of temperature sensitivity. In analyzing the genetic basis of GxE in our system, we demonstrate that the involved alleles contribute to temperature sensitivity in different ways. While alleles of the transcription factor *MSS11* specify the potential temperatures at which the trait can occur, alleles at the other loci modify temperature sensitivity within the range established by *MSS11* in a genetic background- and/or temperature-dependent manner. We are now working to characterize how different combinations of these causal alleles modulate temperature sensitivity at the molecular and systems levels. Our goal is to establish a clear portrait of how genetic variation and the environment together alter global gene regulation, thereby resulting in GxE.

P2092B Genome-wide association mapping identifies SNPs influencing the plastic response of lifespan and age-specific fecundity to diet in *Drosophila melanogaster*. *J. Leips*[1], M. Durham[1], E. Stone[2], M. Magwire[2], P. Daya[1]. 1) University of Maryland Baltimore County, Baltimore, MD; 2) Department of Genetics, North Carolina State University, Raleigh, NC.

Many phenotypes exhibit a plastic response to variation in environmental conditions, and genetic variation in this plastic response provides the opportunity for selection to act on plasticity itself. Although genetic variation in plasticity is well documented in many taxa, the genes that regulate plasticity are largely unknown. In this study, we investigated the genetic basis of natural variation in the plastic response of age-specific fecundity and lifespan to changes in dietary protein content using the *Drosophila melanogaster* Genetic Reference Panel. Variation in the plastic response of fecundity to diet was associated with 491 polymorphisms and 292 genes, and variation in the plastic response of lifespan to diet was associated with 29 polymorphisms and 15 genes. We used our data to test predictions of two hypotheses on the genetic

mechanisms of plasticity, the allelic sensitivity hypothesis (the plastic response of traits is regulated by the genes that affect the trait means) and the gene regulation hypothesis (plasticity is regulated by genes that are independent of those contributing to trait variation). We found evidence to support both mechanisms; however, our data suggest that the gene regulation mechanism is the more common mechanism. This decoupling of trait genes from those regulating their plastic response reduces the potential constraints of pleiotropy, facilitating adaptive independent evolution of the trait means and their plastic response to environmental variation.

P2093C A Bayesian approach for the imputation of genotypes on observed markers in complex pedigrees. *D. Leroux*, S. Jasson. MIAT, Université de Toulouse, INRA, Castanet-Tolosan, FR.

We present a method to tackle the challenges posed by the modern genotype data sets in quantitative genetics and allow for quick imputation of parental origin probabilities (POP) in complex populations, for each marker, and given any number of allelic observations.

QTL analysis and genetic cartography software traditionally use parental origin (PO) data on a set of observed markers to perform their computations. This data used to be easily encoded in simple pedigrees such as backcrosses, bi-parental series of selfings, or the like. The most common encoding, introduced by Mapmaker, uses the letters ABHCD to denote PO in populations with two ancestral lines and is the result of a hand-made inference: restricting the data to markers that are homozygous on both ancestors yet distinct, one can immediately derive the PO for any individual in the population. Unfortunately this method doesn't generalize to the multi-parental population designs that are the modern trend. With complex population structures such as multi-parental generalized intercrosses (MAGIC), the allelic observations are often not informative enough to simply derive PO the old way, mainly due to limited polymorphism in regard to the number of ancestral lines.

We present a technique to infer the POP on all individuals in a given pedigree of any size and structure given observations on any subset of individuals. We represent the pedigree as a Bayesian network with discrete variables. Each variable corresponds to an individual in the pedigree and its domain is the Cartesian product of the possible PO and observed alleles (typically SNP data). This paradigm makes full use of all the available information for each individual, including the information from its relatives, to compute the POP. We also provide an algorithm to efficiently compute in amortized linear time the junction tree corresponding to the pedigree, which leads to exact inference on each marker of the PO/allele probabilities by means of simple belief propagation. This algorithm takes into account non-trivial reentrant individuals to compute the proper joint probabilities where required. We finally show how these results can be used in QTL analysis by extracting the POP for the individuals that have also been phenotyped and to genetic cartography by extracting the selection variable of each meiosis in the pedigree to create datasets that can be treated like back-crosses by the cartography software. An implementation of this method is in progress and will be made available soon to the research community.

P2094A Genetic basis of thermal tolerance in *Saccharomyces* species. *Xueying Li*, Ping Liu, Kim Lorenz, Justin Fay. Washington University in St. Louis, St. Louis, MO.

The genetic basis of phenotypic evolution is one of the central questions in evolutionary biology. It still remains unresolved how many mutations underlie species' divergence and what is the distribution of their effect sizes. The challenge of genetic analysis between species lies in their reproductive barriers and besides candidate gene studies, few large-scale, high-resolution studies have characterized the genetic basis of species' differences. Here, we performed two genome-wide screens to systematically dissect the genetic architecture of phenotypic divergence between two yeast species, *S. cerevisiae* and *S. uvarum*. These two species differ in a number of traits, the most prominent of which is thermal tolerance. In the first screen, we transformed a library of *S. cerevisiae* genes into *S. uvarum* and measured their phenotypic effects by competitive growth assays, aiming to identify single genes with independent effects underlying phenotypic divergence. We also carried out a non-complementation screen by crossing the *S. cerevisiae* deletion collection to *S. uvarum*, in order to identify genes with effects that depend on a complete complement of the *S. cerevisiae* genome. Our preliminary results suggest no single genes of large effect underlies the divergence, with the exception of mitochondrial DNA. The results of our interspecific genetic analysis are consistent with a model of multiple changes of small effect and contrast with those of intraspecific genetic analysis.

P2095B Comparison of normalization and differential expression analyses using RNA-Seq data from 726 individual *Drosophila melanogaster*. *Y. Lin*[1], K. Golovnina[2], Z.-X. Chen[2], H. Lee[2], Y. Serrano Negron[1], H. Sultana[2], B. Oliver[2], S. Harbison[1]. 1) National Heart Lung and Blood Institute, Bethesda, MD; 2) National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD.

In order to determine whether heritable differences in gene expression could be detected among individual flies, we performed a multi-factor experiment using RNA extracted from 768 flies. We harvested RNA from individual flies using 16 inbred lines from the *Drosophila* Genetic Reference Panel. These flies were reared in three separate biological replicates. The RNA was successfully sequenced for more than 98% of the flies, and the genotype and sex of each sample were verified by using a 'bar code' and Spearman correlation respectively. Application of this verification procedure resulted in 726 sequences remaining for further analysis. To identify the optimal analysis approach for the detection of differential gene expression among genotype, sex, environment, and their interactions, we investigated the effects of three different filtering strategies, eight normalization methods, and two statistical approaches. We assessed differential gene expression among factors and also performed a statistical power analysis using the eight biological replicates per genotype, environment, and sex in our data set. We found that two to five biological replicates were required in order to have adequate statistical power depending upon the factors analyzed. Some common normalization methods, such as Total Count, Quantile and RPKM normalization, did not align the data across samples. Analyses applying the Median, Quantile, and Trimmed Mean of M-values normalization methods were sensitive to the removal or retention of genes with low expression in the data set. The two statistical approaches, a generalized linear model with a negative binomial distribution and an ANOVA

model, yielded strikingly different results. Our favored analysis approach was to normalize the read counts using the *DESeq* method, to apply a generalized linear model assuming a negative binomial distribution using either *edgeR* or *DESeq* software, and to remove genes with very low read counts after the statistical analysis.

P2096C Genetic dissection of variation in sleep using the *Drosophila* Synthetic Population Resource. *S. J. Macdonald*, B. R. Smith. Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

In humans there is considerable genetic variation for the duration and quality of sleep, and studies have implicated a lack of sleep as a risk factor for a range of health problems. *Drosophila*, in addition to being widely employed as a model genetic system to understand fundamental aspects of the control of complex trait variation, is recognized as an important translational model for the study of human health and disease. Flies exhibit a sleep-like state, and similar to mammals show increased rest following a period of sleep deprivation, an age-related decline in the duration of sleep, and reduced rest following exposure to caffeine. Here, we use *Drosophila* to dissect natural variation in sleep, identify loci for future functional testing, and facilitate experimental exploration of the mechanistic basis of variation in sleep. As with all complex, polygenic traits identifying the molecular pathways and causative genes responsible for phenotypic variation is challenging. Thus, we employed a multi-tiered approach, encompassing high-resolution QTL mapping, expression QTL (eQTL) data, and functional validation with RNAi. We initially measured a battery of sleep traits in multiple individuals from each of 600 heterozygous genotypes derived from the *Drosophila* Synthetic Population Resource (DSPR). The DSPR is a large set of multiparental advanced generation intercross lines that facilitates powerful mapping of QTL to small genomic regions. We observed extensive genetic variation in sleep traits in the population, and successfully mapped a number of QTL that collectively explain significant fractions of variation in sleep. Under the assumption that some fraction of phenotypic variation is due to changes in gene regulation, true causative genes implicated by QTL may often additionally segregate for *cis*-eQTL. Merging sleep QTL data with a large *Drosophila* head transcriptome eQTL mapping dataset from the same population allowed us to refine the list of plausible candidate causative sleep loci. This set includes genes with previously characterized effects on sleep (e.g., *timeless*), in addition to novel candidates. Subsequently, we employed adult nervous system specific RNAi to functionally test the effects of several loci, identifying significant effects on sleep following knockdown of the *Dopa decarboxylase* and *dyschronic* genes. The genes we identify are highly likely to harbor causative, regulatory variation contributing to variation in sleep-like phenotypes.

P2097A The genetics of giant sperm in *Drosophila*. *M. Manier*[1], S. Pitnick[2], J. Belote[2], S. Dorus[2]. 1) George Washington University, Washington, DC; 2) Syracuse University, Syracuse, NY.

Spermatozoa are the most morphologically variable cell type among Metazoa, and the *Drosophila* lineage displays the greatest variation in sperm length, ranging from 0.3 mm to 5.8 cm. Available evidence suggests that sperm length in this group is evolving rapidly, driving by postcopulatory sexual selection via sperm competition and cryptic female choice. Longer sperm outcompete shorter sperm and especially in longer female sperm storage organs. To investigate the genetic basis of sperm length in *Drosophila*, we used a QTL sequencing approach that identified ~500 SNPs enriched within or near ~300 protein coding genes. We prioritized candidate genes based on known patterns of expression during spermatogenesis, GO functions, patterns of rapid evolution, and number of SNPs identified within the gene. The effect of candidate gene disruption on sperm length was assessed using GAL4/UAS RNAi or insertion mutation lines. We also characterized allelic variation and differential gene expression for a subset of candidate genes in populations previously selected for long or short sperm.

P2098B *IRF4* haplotype diversity and associations with hair, eye and skin pigmentation in a Brazilian admixed population. *C. T. Mendes-Junior*[1], A. L. E. Pereira[1], N. C. A. Fracasso[2], L. Marcorin[1], G. Debortoli[2], J. D. Massaro[3], A. L. Simões[2], E. A. Donadi[3], E. C. Castelli[4], M. L. G. Oliveira[2]. 1) Universidade de São Paulo (Departamento de Química, FFCLRP-USP), Ribeirão Preto, SP, Brazil; 2) Universidade de São Paulo (Departamento de Genética, FMRP-USP), Ribeirão Preto, SP, Brazil; 3) Universidade de São Paulo (Departamento de Clinica Médica, FMRP-USP), Ribeirão Preto, SP, Brazil; 4) Universidade Estadual Paulista (Departamento de Patologia, FMB-UNESP), Botucatu, SP, Brazil.

The *Interferon Regulatory Factor 4* gene, located at chromosomal region 6p25-p23, encodes a DNA-binding transcription factor, expressed exclusively in immune system cells and melanocytic lineages. The rs12203592 SNP (intron 4) has been associated with presence of freckles, hair, eye and skin color. Functional studies in human and mice melanin-containing cells revealed that this SNP is directly involved in the regulation of *IRF4* expression, suggesting a clear role in melanocyte pigmentation. In spite of these findings, the *IRF4* diversity in admixed populations has not been evaluated so far. In order to verify if other variation sites spread across the *IRF4* gene may be associated with human pigmentation, the regulatory and coding (9 exons and part of their flanking introns) regions were analyzed by next-generation sequencing in a Brazilian admixed population sample. The population sample was composed of 228 unrelated individuals from São Paulo State, which were stratified according to eye (blue, green, hazel, light-brown, and dark-brown), hair (red, blond, dark-blond, light-brown, dark-brown and black) and skin (light, intermediate and dark) pigmentation, as well as regarding the presence of freckles and intensity of hair greying. DNA libraries, including other pigmentation genes, were prepared using the Haloplex Target Enrichment System (Agilent) and sequenced at the MiSeq platform (Illumina). CutAdapt, BWA and GATK packages were used for trimming adaptor sequences, alignment and genotype calling, respectively. Missing alleles and haplotypes were inferred by using the PHASE method. A total of 105 variation sites were identified. Eighteen of these SNPs presented strong association (OR > 10) with at least one pigmentation feature. However, if the Bonferroni correction for multiple tests is taken into account, only two associations, both of them involving the rs12203592 SNP, remain significant: allele T associated with light skin and blue eyes. This result is in agreement with previous reports that the rs12203592*T allele leads to reduced IRF4 activation and reduced tyrosinase expression, leading to sun sensitivity and blue eyes. A total of 101 different haplotypes were inferred. When haplotypes were

subdivided in promoter, coding and 3'UTR haplotypes, 17, 29 and 37 different haplotypes were observed, respectively. Various associations were identified, particularly involving the most frequent promoter haplotype, the two most frequent coding (only one of them with allele rs12203592*T) and the most frequent 3'UTR, all of them with light skin, blue eyes, brown hair and hair greying. These results suggest that other variation sites besides rs12203592, when considered in a haplotype background, are associated with human pigmentation. Financial Support: CAPES, CNPq (309572/20142 and 448242/2014-1) and FAPESP (2013/15447-0).

P2099C Genetic constraints on the learning of a complex song phenotype. *D. G. Mets*, M. S. Brainard. HHMI/Univ California, San Francisco, San Francisco, CA.

Learning reflects the influence of experience on genetically determined circuitry. Both the ways in which experience shapes behavior during learning and the ways in which genetics shape non-learned phenotypes have been widely studied. However, little is known about how experience and genetics interact to determine complex learned phenotypes. Vocal learning in songbirds provides a rich system for investigating experiential and genetic contributions to learning; the output of learning (song) is quantifiable and we can manipulate both the experiential contributions (through computer tutoring) and genetic contributions (through breeding). Here we examine the relationship between experiential and genetic contributions to learning of an ethologically relevant phenotype, the tempo of song production (quantified in syllables produced per second). Distinct genetic lines of the Bengalese finch (*Lonchura striata domesitca)* were bred from parents with different song tempos. When juvenile birds of a given line were tutored with synthetic songs that varied only in tempo, they developed adult songs with tempos that varied with the tutor song. Hence, as expected, the structure of song was shaped by experience. However, when the tempo of the tutor song was held constant, juveniles from different genetic lines developed song tempos that strongly correlated with those of their fathers, even though these juveniles had never heard their fathers sing. Thus, under controlled tutoring conditions, we found an unexpectedly strong genetic contribution to the mean song tempo. We further investigated the interaction between genetics and experience by tutoring several genetic lines (that expressed slow, medium and fast songs) on a set of synthetic songs that differed only in tempo. We again found that, across all lines, tutoring experience influenced song tempo. However, each line learned different amounts of the stimulus tempo, revealing a significant gene by environment interaction (GXE; a non-linear interaction between genetic and experiential contributions to song tempo). Taken together, these findings demonstrate a strong genetic contribution both to the mean tempo of song and to the degree to which tempo is influenced by experience. Our results provide a striking demonstration of how genetics can both shape and constrain the influence of experience on a complex learned phenotype.

P2100A The genetic basis of the coordination of nutrition and energy allocation in a synthetic population of *Drosophila melanogaster*. *E. Ng'oma*, M. A. Reed, W. Fidelis, E. G. King. University of Missouri, Columbia, MO.

Allocation of energy is critical to biological structure and function. Availability of nutrients to support life varies tremendously across time and environments. Given a limited resource pool, organisms allocate differentially to functions such as somatic maintenance, reproduction and storage. This differential allocation has a direct bearing on, for instance, how and why organisms age, why species differ extensively in reproductive output, and is thought to constrain the evolution of fitness. We use a multiparental mapping population to explore the genetic basis of the coordination of allocation with resource availability. We phenotyped a suite of interrelated allocation phenotypes in multiple dietary regimes to uncover a complete picture of the genetic basis of the coordination between resource availability and resource allocation, using the DSPR resource lines. We identify several genomic locations that influence how resources are partitioned differently when resource availability varies. These loci will also serve as focal candidates we will track over time in a future selection experiment for divergent allocation strategies.

P2101B Proteome-wide association studies identify biochemical modules associated with a wing size phenotype in *Drosophila melanogaster*. *H. Okada*, A. Ebhardt, S. Vonesch, R. Aebersold, E. Hafen. Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland.

The manner by which genetic diversity within a population generates individual phenotypes is a fundamental question of biology. To advance the understanding of the genotype-phenotype relationships towards the level of biochemical processes we perform the first proteome-wide association study (PWAS) of a complex quantitative phenotype. We quantify the variation of wing imaginal disc proteomes in *Drosophila* genetic reference panel (DGRP) lines using SWATH mass spectrometry. In spite of the very large genetic variation (1/36 bp) between the lines, proteome variability is surprisingly small, indicating strong molecular resilience of protein expression patterns. Proteins associated with adult wing size form tight co-variation clusters that are enriched in fundamental biochemical processes. Wing size correlates with some basic metabolic functions, positively with glucose metabolism but negatively with mitochondrial respiration and not with ribosome biogenesis. Our study highlights the power of PWAS to filter functional variants from the large genetic variability in natural populations.

P2102C Genetics of skeletal evolution in unusually large mice from Gough Island. *Michelle Parmenter*[1], Melissa Gray[1], Caley Hogan[1], Irene Ford[1], Richard Cuthbert[2], Peter Ryan[3], Karl Broman[1], Christopher Vinyard[4]. 1) University of Wisconsin-Madison, Madison, WI, USA; 2) The Royal Society for the Protection of Birds, The Lodge, Bedfordshire, UK; 3) University of Cape Town, Cape Town, South Africa; 4) Northeast Ohio Medical University, Rootstown, OH, USA.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

Organisms on islands often undergo rapid morphological evolution, providing a platform for understanding mechanisms of phenotypic change. One example is the skeleton, which shows unusual patterns in island populations and forms an integral part of the vertebrate body plan. Although the genetic basis of skeletal variation has been studied extensively in laboratory strains, particularly in the house mouse *Mus musculus domesticus*, the genetic determinants of skeletal evolution in natural populations remain poorly understood. We used house mice living on the remote Gough Island, the heaviest wild house mice on record, to understand the genetics of rapid skeletal evolution in nature. Compared to a mainland reference strain from the same subspecies (WSB/EiJ), the skeleton of Gough Island mice has enlarged considerably. We used X-ray images to measure 16 skeletal dimensions (at 5, 10, and 16 weeks of age) that are designed to capture major changes in skeletal anatomy. Quantitative trait locus (QTL) mapping in a large F_2 intercross between Gough Island mice and WSB/EiJ reveals a total of 198 QTL that control skeletal dimensions. QTL exhibit modest, mostly additive effects and Gough Island alleles are associated with larger skeletal size at most QTL. A large proportion of QTL co-localize across skeletal traits and body weight, an observation that may be explained by a high degree of pleiotropy. Our results provide a rare portrait of the genetic basis of skeletal evolution in an island population, and position the Gough Island mice as a model system for understanding the mechanisms of rapid evolution in nature.

P2103A Validation of candidate anthracnose resistance genes in sorghum via Brome Mosaic Virus-mediated gene silencing. *S. P. Rao*, T. J. Felderhoff, L. Stutts, W. Vermerris. University of Florida, Gainesville, FL.

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most widely grown crop in the world and provides food, feed, fuel and fiber in over a hundred countries. Sorghum is an ideal bioenergy crop for the southeastern United States due to its high biomass potential on poor soils with limited inputs. Expansion of sorghum in this region, however, is contingent on resistance to the aggressive fungal pathogen *Colletotrichum sublineolum* (Henn.), the causal agent of anthracnose that can reduce grain, sugar and biomass yield by as much as 70%. We have identified a major anthracnose resistance locus on chromosome 9 using a biparental mapping population derived from the inbred lines Bk7 (resistant) x Early Hegari-Sart (susceptible). The population was genotyped by sequencing and phenotyped in three environments. The locus is 1.2 Mb in size and contains 12 candidate resistance genes. The genetic proof that one or more of these genes are responsible for conferring resistance requires showing that inactivation of the gene in a resistant line results in a susceptible phenotype, or introducing the allele of the resistant parent in a susceptible line and demonstrating acquisition of resistance. As few sorghum lines are amenable to transformation, and genome editing is not yet feasible, the best alternative is virus-induced gene silencing (VIGS) of candidate resistance genes with the help of the tripartite Brome Mosaic Virus (BMV). We are in the process of cloning short fragments of cDNA in one of the viral sequences and will generate infectious RNA transcripts through *in vitro* transcription. Three-week old resistant sorghum seedlings will be inoculated with a mixture of transcripts. Two weeks post viral inoculation, the plants will be challenged with *Colletotrichum* conidia to assess the effect of different constructs on host-pathogen interaction. Elucidating the identity of the resistance gene(s) will facilitate the breeding of disease-resistant sorghums, and provide information on the mechanism of disease resistance. Supported by USDA-BRDI Award No. 2011-10006-30358 and US DOE award No. DE-SC0014439.

P2104B Species diversity and sexual dimorphism of ethanol sensitivity in Drosophila. *M. H. Reich*, R. M. Graze. Auburn University, Auburn, AL.

Drosophila is a key model system in the genetics of ethanol sensitivity and tolerance. Ethanol sensitivity is defined as the overall sensitivity to the effects of ethanol exposure, ultimately resulting in sedation. Tolerance occurs when sensitivity is reduced after one or more initial ethanol exposure/s. Molecular studies have identified genes involved in sensitivity and tolerance in *Drosophila melanogaster*, with the aim of understanding their role in alcoholism and alcohol related diseases. However, ethanol is an important abiotic factor for many species of Drosophila and ethanol sensitivity and tolerance vary across the genus. In some species, including in *D. melanogaster*, the response to ethanol is also sexually dimorphic. Ethanol sensitivity and tolerance have not been assayed for all the currently sequenced Drosophila species in both males and females, and little is known about how sexual dimorphism in sensitivity or tolerance differs across species. Here we report the results of systematic sedation assays for sensitivity and tolerance in males and females across a diverse group of sequenced species of Drosophila. These experiments place diversity of ethanol sensitivity and tolerance, including sexual dimorphism, in a phylogenetic context. This will allow for a more complete understanding of how phenotypic diversity in the ethanol response is related to differences in the regulatory response to ethanol, in each sex.

P2105C Is genetic architecture predictable? Modeling the roles of mutation, recombination and selective forces in shaping allelic variation. *D. L. Remington*, M. Augustinovic. University of North Carolina at Greensboro, Greensboro, NC.

Research over the last few decades has led to contradictory insights on the genetic architecture and evolution of quantitative traits. Thus, key questions about the relevance of large-effect genes identified in some studies for understanding adaptation and evolution are yet to be resolved. To address these issues, models of quantitative trait variation need to incorporate the contribution of mutations at multiple sites within genes and the potential for recombination between these sites. In addition, predictable patterns of genetic architecture may be specific to particular combinations of evolutionary forces. We are using quantitative genetic simulations to test whether evolutionary genetic models incorporating different modes of selection can explain discordant results from different study systems. In contrast with other modeling efforts, we incorporate both the cumulative effects of multiple mutations in the same gene and realistic rates of intragenic recombination in our simulations. Preliminary results suggest that sharply contrasting genetic architectures may arise in a single population under stabilizing selection versus populations under divergent selection for contrasting phenotypic optima. Unlike the highly polygenic architecture generated

under stabilizing selection, divergent alleles at only a few loci soon come to explain most of the differentiation between populations under scenarios examined so far. However, large allelic effects tend to arise from the cumulative effect of many mutations in multiple potentially-recombining segments of the same locus. These results appear to support previous suggestions that micromutational models and differentiation at large-effect loci are not mutually exclusive. We discuss extension of our modeling to test whether "stepping stone" differentiation along environmental gradients produces similar or different patterns, and address insights into the roles of preexisting variation vs. novel mutations in adaptive divergence.

P2106A Virulence QTLs and Genome-wide Recombination Rates in *Cryptococcus*. *Cullen Roth*[1], Sheng Sun[2], R. Blake Billmyre[2], Joseph Heitman[2], Paul M. Magwene[1,3]. 1) University Program in Genetics and Genomics, Duke University, Durham, NC; 2) Department of Molecular Genetics and Microbiology, Duke University, Durham, NC; 3) Department of Biology, Duke University, Durham, NC.

Cryptococcal disease is estimated to affect 1 million people and kill approximately 600,000 annually. Environmental isolates of *Cryptococcus neoformans*, and its relative *Cryptococcus deneoformans*, vary in their pathogenicity, ranging from benign to hyper-virulent. Key traits that contribute to virulence, such as the ability to grow at human body temperature (37°C), have been identified. However, little is known about the genetic basis of this phenotypic variation. Here we investigate the genetic basis of virulence-related traits using quantitative trait locus (QTL) mapping in *C. deneoformans*. We crossed strains of two different genetic backgrounds (approximately 5 SNPs/kb), generated haploid segregants via bisexual and unisexual mating, and obtained genotypes for 100 segregants using short-read sequencing. We inferred recombination breakpoints using SNP genotype data and calculated a genome wide average recombination frequency of ~7 kb/cM. Five virulence related traits were phenotyped: high temperature growth (at 39°, 40°, 41°, and 42°C), growth at high pH, melanin production, amphotericin B resistance, and fluconazole resistance. We used a marker regression framework to identify QTL underlying variation in these virulence phenotypes. QTLs with major effects were identified for high temperature growth (chromosomes 2 and 12), amphotericin B resistance (chromosome 2), and fluconazole resistance (chromosome 2 and 7). Interestingly, we observed several candidate pleiotropic QTLs, one is located on chromosome 2 and appears to mediate both high temperature growth and amphotericin B resistance, and another is located on chromosome 12 and plays a role in both high temperature growth and melanin production. These results provide novel genome wide estimates of recombination and advance our understanding of the genetic basis underlying virulence in *Cryptococcus*.

P2107B Natural variation in behavior: finding the causal genes in *Drosophila*. *Thomas L. Turner*, Alison Pischedda, Veronica A. Cochrane, Jackson Runte, Wesley G. Cochrane. University of California, Santa Barbara, CA.

We are interested in understanding natural variation in behavior at the genetic and neurological levels. Male courtship behavior in *Drosophila* is a model system for connecting genes, brain, and behavior using molecular genetics, but little is known about how these behaviors vary or evolve. We used a multi-parent panel of recombinant inbred lines to map natural variation in a key parameter of the Drosophila courtship song (inter-pulse interval), and found several loci that explain modest fractions of song variation. The challenge for this and similar studies is then to map these QTL to the gene level for behaviors which don't have obvious candidate genes. We are using a two-step approach. First, we use visible markers to select for recombination in intervals of arbitrarily small size. Benign fluorescent markers can be inserted anywhere in the genome with CRISPR, making this a powerful approach. Second, once we have mapped a QTL to a potentially causal gene, we are using the reciprocal hemizygosity test to validate that gene and quantitatively estimate its effect on our trait. These fine-mapping and validation steps should have broad applicability in other systems and for validating candidate genes from all genome-wide methods.

P2108C Investigating mitochondrial and viral genome contributions to phenotype in *Saccharomyces cerevisiae*. *Sriram Vijayraghavan*[1], Pooja Strope[1], Daniel Skelly[2], Fred Dietrich[1], Paul Magwene[2], John McCusker[1]. 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC; 2) Department of Biology, Duke University, Durham, NC.

Determining how genotype translates to phenotype is a complex issue. While rapid progress has been made in linking nuclear genome variations to population traits, much less is known about whether and how extra-chromosomal elements shape phenotypic changes. Using the yeast *Saccharomyces cerevisiae* as our model, we analyzed the phenotypic contributions from two classes of non-chromosomal elements—mitochondrial DNA and endogenous double-stranded RNA viruses. *S. cerevisiae* shares highly conserved cellular processes with humans, is ideally suited for Mendelian as well as non-Mendelian genetics, and is markedly similar to clinically relevant pathogenic fungi, making it ideal for our studies.

Through a comprehensive sequence analysis of mitochondrial DNA from 100 geographically diverse strains of *S. cerevisiae*, we firstly identified a wide range of mitochondrial DNA-associated variations including copy number changes, single nucleotide polymorphisms, indels, and presence/absence of introns within select mitochondrial genes. Subsequently, we created a series of iso(genic)-nuclear diploid strains, each carrying a single, unique mitochondrial genotype, and tested these under diverse environmental conditions to systematically assess the contribution of individual mitochondrial genomes to phenotype. Our results indicate that mitochondrial variations significantly influence phenotypic outcome.

Additionally, we genotyped our 100-genome collection for the presence and absence of several naturally occurring viral dsRNA elements and found that up to a third of the population harbors the well-characterized dsRNA virus L-A. We observed pronounced L-A dependent phenotypic effects in our analysis of isogenic +/- L-A strain pairs. Interestingly, both mitochondrial and L-A genotypes were found to significantly influence several phenotypes, further underscoring the contributions of non-chromosomal genetic determinants to quantitative trait variations.

Our studies highlight the complexity of gene-environment interactions that ultimately determine fitness and survival. Future work would be aimed at identification of the precise genetic loci to understand the mechanistic basis of such phenotypic changes.

P2109A Quantitative genetics of skeletal traits in BXD recombinant inbred strain mice. *K. A. Warncke*[1], M. Guo[1], M. Henry[1], D. Alleyne[1,2], J. P. Kenney-Hunt[1]. 1) Westminster College, Fulton, MO; 2) University of Chicago, Chicago, IL.

The BXD recombinant inbred (RI) strain mice are well-characterized with a wide variety of published metabolic and skeletal phenotypes, as well as gene expression data. We perform a quantitative genetic analysis of femur, tibia, humerus, and ulna lengths in a set of 850 BXD mice of both sexes. Quantitative trait loci (QTL) and strain-by-sex interactions are identified for these traits. We also assess association between these identified QTL and published BXD QTL with effects on other skeletal traits. Additionally, we compare the genetic architecture of long bone traits in the BXD RI strains to the genetic architecture of these traits in other murine crosses to investigate intraspecies variation in genetic effects.

P2110B Hybrid male sterility in genetically diverse mice. *S. J. Widmayer*, C. D. McKenney, D. L. Aylor. Program in Genetics, North Carolina State University, Raleigh, NC.

Hybrid male sterility is frequently observed in the wild between two mouse subspecies – *Mus musculus musculus* and *M. musculus domesticus*. This complex trait drives reproductive isolation and ultimately speciation. We have replicated hybrid male sterility in the lab by crossing an inbred mouse strain representing *musculus*, PWK/PhJ, to four strains that are largely descended from *domesticus* (*Dom*). Hybrid males with PWK mothers exhibit a wide spectrum of abnormal reproductive phenotypic variation including reduced testes weights and azoospermia. Importantly, the reciprocal hybrids are reproductively normal and serve as experimental controls in this breeding scheme. We characterized reproductive phenotypes across a panel of 10 genetically distinct hybrid males at 8 weeks of age. PWKx*Dom* hybrid males exhibit significantly lower testis weight and sperm density than reciprocal hybrids. Additionally, we observe significant differences that are driven by the genetic background of each specific hybrid. For example, PWKxA/J F1 hybrid males and PWKxDBA/2J F1 hybrid males differ substantially in sperm count and relative combined testes weight despite sharing known sterility alleles. These findings suggest that unknown hybrid male sterility alleles segregate both between and within mouse subspecies.

P2111C Quantitative studies on gene-environment interaction in hitchhiking behavior of *C. elegans*. *H. Yang*[1], D. Lee[1], H. Kim[2], Y. Paik[2], E. Andersen[3], J. Lee[1]. 1) Seoul National University, Seoul, Korea; 2) Yonsei University, Seoul, Korea; 3) Northwestern University, Evanston, Illinois, United States of America.

Nictation is a dauer-specific hitchhiking behavior of *Caenorhabditis elegans*, which helps dauers to get on to other animals passing by. Nictation is only recently studied and little is known about the genetic and environmental factors related to this behavior. Here we show that several environmental factors including wind and temperature can affect nictation. We investigated how different isotypes respond to these environmental conditions. We are now screening QTL(s) that regulate variation in reaction norm of nictation behavior. We expect our study will help us to understand how gene-environment interactions regulate behavior.

P2112A Spatial and ecological determinants of genotype-by-environment interaction. *Rong-Cai Yang*[1,2], Chen Ding[1]. 1) University of Alberta, Edmonton, Alberta, CA; 2) Alberta Agriculture and Forestry, Edmonton, Alberta, CA.

Genotype-by-environment interaction (GxE) remains to be a major issue in plant and animal breeding as well as in evolutionary biology. Over the past decades, voluminous GxE literature has focused on the development of statistical methods to describe rather than to predict the GxE variability. In this presentation, we will describe a new geostatistical method to predict the GxE variability based on spatial and ecological variables that are easily available or derivable from the geography of environments and/or the origin of genotypes. The method will be illustrated using empirical data from a set of barley breeding trials and a set of pine provenance trials in western Canada.

P2113B Natural variation in sensitivity of rhabditid nematodes to microsporidia. *G. ZHANG*, M.-A. FELIX, C. DUBOIS. ECOLE NORMALE SUPERIEURE DE PARIS, PARIS, FR.

The nematode *Caenorhabditis elegans* is a powerful model for the study of host-pathogen interactions and their co-evolution. The microsporidian *Nematocida parisii* was the first characterized intracellular pathogen of *C. elegans* (Troemel et al. 2008). *N. parisii* proliferates in *C. elegans* intestinal cells and is transmitted horizontally through spores. By sampling rhabditid nematodes worldwide, we found ten new microsporidia species in 9 rhabditid nematode species. We tested the host range of these microsporidia species and measured intraspecific variation in sensitivity of the host. We further plan to detect genetic loci underlying this observed intraspecific variation in host sensitivity, using Quantitative Trait Locus (QTL) mapping.

Specificities of infection of seven nematode-infecting microsporidia were tested on four nematode species (*C. elegans*, *C. briggsae, Oscheius tipulae* and *O*. sp. 3). *N. parisii, N*. sp. 2 only infect *Caenorhabditis,* but not *O. tipulae* or *O*. sp. 3. To the infection of *N.* sp. 1, *Caenorhabditis* are more sensitive than *Oscheius*. *N*. sp. 3 infects both *Caenorhabditis* and *Oscheius* species. Microsporidia JUm408 and JUm1505 infect *Oscheius* spp., but not *Caenorhabditis*. Microsporidia JUm2551 infects both *Oscheius* species, JUm408 only infects *Oscheius* sp. 3, JUm1505 only infects *O. tipulae*.

Variation in sensitivity of ten wild *C. elegans* strains to *N.* sp. 1 infection was revealed by food consumption tests using GFP-labeled *E. coli* in the presence or absence of microsporidia, complemented by progeny production and longevity assays on a subset of these strains. For genetic

studies, we then chose JU2825 as the sensitive strain and JU1249 as the resistant strain. We started to map the genetic loci underlying their difference in sensitivity to *N*. sp. 1. We plan to generate bulk segregating populations after crossing them (Test group); in parallel, we have a Control group where we mixed JU2825 and JU1249 without crossing them (they reproduce by selfing). We will treat both groups with or without *N*. sp. 1. By following allelic proportions in the Control (uncrossed) group using pyro-sequencing of SNPs between the two strains, we can monitor the increase in frequency of JU1249 in the presence of microsporidia and determine when it approaches 100% in the population. Then the infected population in the Test (crossed) group should have recovered large numbers or progeny with beneficial alleles linked to relevant loci. We will pool-sequence each of the test populations and analyze the data for significant shifts in allele proportions along the genome of the infected versus uninfected populations, thus potentially revealing quantitative trait loci affecting sensitivity to *N.* sp. 1.

P2114C Genetic basis of octanoic acid resistance in *Drosophila sechellia*: functional analysis of a fine-mapped region. *J. D. Coolon*[1], J. M. Andrade-Lopez[2], S. M. Lanno[1], S. J. E. Shimshak[1], L. A. Sligar[2], P. J. Wittkopp[2]. 1) Wesleyan University, Middletown, CT; 2) University of Michigan, Ann Arbor, MI.

Drosophila sechellia is a species of fruit fly endemic to the Seychelles islands. Unlike its generalist sister species (*D. simulans*, *D. mauritiana,* and *D. melanogaster*), *D. sechellia* has evolved to specialize on a single host plant, *Morinda citrifolia*. Specialization on *M. citrifolia* is interesting because the fruit of the plant contains secondary defense compounds, primarily octanoic acid (OA), that are lethal to all other *Drosophila* species. Although ecological and behavioral adaptations to this toxic fruit are known, the genetic bases for evolutionary changes in OA resistance are not. Prior work showed that a genomic region on chromosome 3R containing 18 genes has the greatest contribution to differences in OA resistance between *D. sechellia* and *D. simulans*. To determine which gene(s) in this region might be involved in the evolutionary change in OA resistance, we knocked-down expression of each gene in this genomic region in *D. melanogaster* with RNA interference (RNAi) (i) ubiquitously throughout development, (ii) during only the adult stage, and (iii) within specific tissues. We identified three neighboring genes that decreased OA resistance when ubiquitously knocked-down. Tissue specific RNAi, however, showed that decreasing expression of two of these genes specifically in the fat body and salivary glands increases OA resistance demonstrating how specific changes in gene expression can differ from a global change in expression levels. Both genes have derived expression levels in *D. sechellia* and no changes in protein sequence suggesting differences in *cis*-regulation contribute to host specialization in *D. sechellia*.

P2115A A preliminary examination of genetic diversity in mantled howler monkeys (*Alouatta palliata)* in a fragmented forest in Costa Rica. *Marie-dominique Franco*[1], Amy Schreier[1], Matthew Barton[1], Nathaniel Pryor[1], Nancy Barrickman[2]. 1) Regis University, Denver, CO; 2) Salt Lake Community College, Salt Lake City, UT.

In most primates, one sex disperses from their natal group to reduce inbreeding. Habitat fragmentation may limit primates' dispersal opportunities, however, which has implications for species survival. We examined the influence of fragmentation on genetic diversity in wild mantled howler monkeys (*Alouatta palliata*) at La Suerte, Costa Rica. The La Suerte region has seen increased fragmentation over the past decade as forest has been cleared for fruit plantations. We hypothesized that genetic diversity would be lowest in the forest edges compared to the interior because fragment boundaries restrict dispersal opportunities for edge groups. In summer 2015 we collected 96 fecal samples from the howler population and selected 20 samples each from the interior and the edge of the forest, respectively, using GIS locations of sample collection sites. To examine DNA polymorphism, we isolated DNA from these samples and used two primer sets to perform PCR amplifications of selected microsatellite loci (Apm01 and Ab06). Analysis of the amplified fragments was carried out using BigDye Terminator v3.1 sequencing and run on ABI 3730xl instrumentation. We recorded observed (Ho) and expected (He) heterozygosity values under Hardy-Weinberg equilibrium and derived F-statistics indices (F_{IS}, F_{ST} and F_{IT}) to quantify genetic variation in the sub-populations (interior vs. edge). We subsequently assessed the genetic health of the howler monkeys using these indices. Our results show that genetic diversity is highest at the edges of the forest and lowest in the interior. The inbreeding coefficient for the edge population was F_{IS} = 0.45 and for the interior population F_{IS} = 0.58, suggesting inbreeding is occurring in the interior of the forest. In addition, the overall inbreeding coefficient was F_{IT} = 0.52, suggesting that the howler population as a whole is inbred. Overall, our results suggest that howler monkeys may be dispersing across open areas into neighboring fragments. These results may, however, be an artifact of our limited sample size. We therefore intend to analyze all samples collected in summer 2015 and those that will be collected in summer 2016. The results we present here are the initial stage of what we anticipate will become a long-term project examining the influence of habitat fragmentation on dispersal and genetic diversity in wild mantled howler monkeys.

P2116B Population genetics of the monarch butterfly, *Danaus plexippus*, in Mexico. *F. Pérez-Gálvez*[1], C. Chavez Mora[1], M. Ramirez Loustalot Laclette[1], E. Rendón-Salinas[2], E. Pfeiler[3], T. Markow[1,4]. 1) LANGEBIO-CINVESTAV, Irapuato, Mexico; 2) WWF-Mexico, Michoacan, Mexico; 3) CIAD Unidad Guaymas, Mexico; 4) University of California, San Diego, CA.

Population genetic variation and demographic history in migratory monarch butterflies, *Danaus p. plexippus* (L.), from four overwintering sites within the Monarch Butterfly Biosphere Reserve in central Mexico were assessed based on analyses of mitochondrial cytochrome *c* oxidase subunit I (COI) and subunit II (COII) gene segments in a total sample size of just under 100 individuals. Overall, haplotype (h) and nucleotide (π) diversities were relatively low, averaging 0.42 and 0.0006, respectively, in COI, and 0.63 and 0.0024 in COII. Analysis of molecular variance (AMOVA) of the concatenated COI and COII data set (n = 89; 1161 bp) indicated no significant population structure among the four sites. Estimates of demographic history based on the mismatch distribution and Bayesian skyline analyses of the concatenated

Mexican data set showed a population expansion dating to the late Pleistocene (~35,000 to 40,000 years before present), and then a stable and current effective female population size (N_{ef}) of just under 10 million.

P2117C A Second Coming of sechellia: Parallel Adaptation to a Toxic Fruit in *Drosophila yakuba*. *John Pool*[1], Amir Yassin[1], Vincent Debat[2], Héloïse Bastide[1], Nelly Gidaszewski[2], Jean David[2,3]. 1) University of Wisconsin - Madison, Madison, WI; 2) Muséum National d'Histoire Naturelle, Paris, France; 3) Centre National de la Recherche Scientifique (CNRS), Gif-sur-Yvette, France.

Among the *Drosophila* species of the melanogaster subgroup, the island endemic *D. sechellia* is unique for its specialization on noni fruit (*Morinda citrifolia*), which is toxic to related species, making it a renown system for the genetics of detoxification and ecological and diet shift. We recently discovered a second, younger case of *Morinda* specialization in the same clade, involving a unique population of *D. yakuba* from the island of Mayotte. *D. yakuba* was found exclusively amongst *Morinda* on this island, and it shows greatly increased preference and tolerance for the fruit compared with mainland populations. Partial prezygotic isolation is also present, in that island females strongly discriminate against mainland males. Based on a population genomic analysis, these traits have evolved within the past ~30,000 years. Therefore, genetic variation may still hold signals of natural selection at causative genes, providing a major advantage of this system. Using a new F_{ST}-based statistic called *Population Branch Excess* (*PBE*), we identified notable outliers for high genetic divergence in the island population, including several loci with known detoxification genes. We tested for an association between our *D. yakuba* outliers and *D. sechellia* trait mapping intervals, finding a significant enrichment for tolerance loci but not attraction to *Morinda*. Thus, some of the same genes may have contributed to *Morinda* specialization in these two species, reflecting a shared genetic tool kit for detoxification. The existence of a young specialist population of *D. yakuba* will provide noteworthy opportunities to study the genetics of ecological shift, reproductive isolation, and parallel evolution.

P2118A Genetic and environmental components of phenotypic and behavioral trait variation during lake sturgeon (*Acipenser fulvescens*) early ontogeny. *K. T. Scribner*, K. Dammerman, J. P. Steibel. Michigan State University, East Lansing, MI.

Quantifying the relative contributions of genetic and environmental effects and their interaction on phenotypic variation is vital to understand how populations respond to their environment. Adults can plastically respond to environmental conditions by selecting breeding and egg incubation locations that affect offspring traits during embryonic and larval development. Environmental conditions during incubation can also affect traits during later ontogenetic stages (i.e. ontogenetic contingency). Using a population of lake sturgeon (*Acipenser fulvescens*) from Black Lake, Michigan, we conducted field and common garden studies and evaluated whether larval phenotypes and behavior at different ontogenetic stages would vary among families whose eggs were incubated under different thermal and flow regimes in the laboratory, and associated with different micro-habitat conditions in river substrates in the field. A significant family-by-treatment interaction was detected for traits (body length, body area, head area) measured at hatch associated with different flow (high, medium, low) and temperature (10^{o}C, 18^{o}C, variable, ambient) treatments. The greatest range in phenotypic variance was observed among individuals reared in the most environmentally deviant conditions (warm temperature and high flow treatments). Traits measured at hatch from eggs in the stream varied due to the influences of stream micro-habitat variables, while levels of additive genetic variance covaried with age. Results demonstrate that phenotypic variation across sequential ontogenetic stages is dependent on physical stream conditions and additive genetic effects, although the relative contributions of effects differ across ontogenetic stages. Increasingly deviant environmental regimes may reveal cryptic genetic variation, potentially leading to differential survival between genotypes, thereby altering the genetic architecture of populations.

P2119B Quantification of behavioral and heritability correlates in prairie voles, a socially monogamous rodent. *Andrea Vogel*[1,2], Lisa McGraw[1]. 1) North Carolina State University, Raleigh, NC; 2) W.M. Keck Center for Behavioral Biology, Raleigh, NC.

Prairie voles (*Microtus ochrogaster*) are among the rare mammal species that have monogamous relationships and have become a model system for understanding the neurogenetic basis of social behaviors such as biparental care of young and pair bonding. Studying the strength of the pair-bond can inform researchers about complex mental health disorders with social components, such as autism, schizophrenia, depression, and anxiety. Although many prairie voles are monogamous, others will engage in extra-pair copulations, and many never form pair bonds at all. To begin to gain an understanding of the genetic basis for behavioral variation in this species, we examined socially-relevant behaviors including anxiety, alloparental care, and aggression against same-sex intruders. Furthermore, pair-bonding is hypothesized to create behavioral changes in the animals, so we examined these behaviors both before and after mating. We have determined that the related behaviors are not correlated with the amount of time spent with a partner, nor are any of the behaviors highly heritable. This experiment establishes the scope of individual variation of pair-bonding, which will be explored in more depth using neurobiology and genetic techniques.

P2120C Post-mating transcriptome profiles of *Drosophila novamexicana* females after con- and heterospecific copulation. *Y. Ahmed*, A. Clark. Cornell University, Ithaca, NY.

Post-copulatory sexual selection is a potent evolutionary force that can cause rapid divergence of reproductive proteins between closely related species. In many animal taxa, including *Drosophila*, several species are reproductively isolated exclusivley by strong gametic incompatibilities, which result in reduced fertilization in heterospecific copulations. One such species pair are the closely related *D. americana* and *D. novamexicana*, two members of the virilis group that show strong gametic incompatibilitiy when crossed in the lab, especially when *D. americana* males mate with *D. novamexicana* females (~1% fertilization). We are actively expoliting this system to better understand the genetic and molecular basis of post-copulatory sexual selection, in particular the role female proteins play in male-female gametic

interactions. Here we examine mating-induced transcriptome changes in *D. novamexicana* females after mating. In particular, we compare the regulatory response across three tissues (lower reproductive tranct, ovaires, and head) and between conspecific and heterospecific inseminations at several time-points post-insemination. The majority of transcripts that are affected by mating are shared between conspecific and heterospecific matings, but a subset are either uniquely responsive in either the conspecific or the heterospecific cross. We find that several Gene Ontology (GO) terms are enriched among mating-responsive genes, including proteolysis, immune response and proteasome degradation. We also find that several male-specific genes (both from the accessory glands and testes) show increases in abundance in the female reproductive tract after mating. These preliminary results suggest that mating and/or copulation trigger a coordinated regulatory response that can be disrupted when copulation occurs between species, and that the transfer of male RNAs during mating might have a functional consequence.

P2121A Population genetics models with selection for phylogenetic inference. *Jeremy Beaulieu*, Cedric Landerer, Russell Zaretzki, Michael Gilchrist, Brian O'Meara. University of Tennessee, Knoxville, TN.

Models used for phylogenetic inference typically ignore mutation, drift, and selection processes when analyzing protein-coding sequences, and thus try to fit the pattern with parameters that are not entirely based on population genetics. In other words, they do not allow different proteins to have different sensitivities to protein structure, incorporate gene expression levels, and/or strengths of selection. We develop a population genetics based model that uses biological parameters, such as energy cost of protein production, physical properties of amino acids, and levels of gene expression, to create more realistic models for DNA substitution for protein-coding genes. Our new model, which we refer to as SELAC (SELection on Amino acids and/or Codons), specifically infers the strength of selection on codon usage and amino acid sequence, sensitivity of protein function to different amino acid properties such as size and polarity, and mutation rates when inferring phylogenetic relationships among taxa. We use a rigorous simulation approach to show that our model can detect meaningful differences in the model parameters across genes under a variety of many different scenarios, including those that naturally violate the assumptions made by SELAC. We will also present preliminary phylogenetic analyses of several empirical genomic data sets for clades that represent different parts of the tree of life, which all show a dramatic improvement in model fit compared to traditional models. Our model also provides better estimates of the underlying branch lengths in the phylogeny, and can better predict empirical sequences, an indication of its overall adequacy.

P2122B A reverse ecology approach to understand the proximate and ultimate causes of phenotypic divergence during species formation. *C. Eberlein*, L. Nielly-Thibault, J.-B. Leducq, G. Charron, H. Maaroufi, C. R. Landry. Université Laval, Quebec City, Canada.

Research on species formation and adaptive divergence aims at identifying the proximate and ultimate causes of phenotypic differentiation, which involves complex interactions between genes and the environment. We used a reverse ecology approach to investigate the molecular bases of an on-going speciation event in the budding yeast *Saccharomyces paradoxus* in the North American deciduous forests. We applied whole-genome sequencing to identify candidate genes under positive selection and high-throughput phenotyping to determine the conditions into which these genes may play a role. We confirmed the fitness effect of protein divergence at candidate loci through direct allelic competition assays. This comprehensive approach allowed us to gain insights in the complex network of genes and environmental factors that may have shaped the fitness determinants of these two incipient species over the last 100,000 years.

P2123C Genomic analysis of ancestry in hybrid mice. *M. Frayer*[1], L. Turner[1,2], B. Harr[2], D. Tautz[2], B. Payseur[1]. 1) University of Wisconsin-Madison, Madison, WI; 2) Max Planck Institute for Evolutionary Biology, Plön, Germany.

Regions where related species' ranges overlap have the potential to become zones of hybridization and also have the potential to reveal the progression of speciation between those species. The evolutionary history and genetic barriers to reproduction can be revealed by the pattern of ancestry across the genomes of hybrid individuals. Ancestry junctions, historical recombination events between chromosomes of different ancestries, can be used as a measure of ancestry. However, empirical identification of these junctions is rarely performed. Combining whole genome sequencing with probabilistic inference of ancestry, we have identified ancestry junctions across the genome of a single hybrid individual from the well-studied house mouse hybrid zone between *Mus musculus musculus* and *Mus musculus domesticus* in Central Europe. We found that the average block length between junctions was small, but the variance in block length was high. These results show a complex history of hybridization, and may reveal that hybridization has been occurring in the zone longer than previously thought. This analysis demonstrates the power of genomic data in increasing our understanding of speciation.

P2124A Finding Hybrid Sterility Genes Between Two African Malaria Mosquitoes. *R. Green*[1], D. Turissini[1], S. Gamez[1], B. Cassone[2], B. White[1]. 1) University of California Riverside, Riverside, CA; 2) Ohio State University, Columbus, OH.

The Dobzhasnky-Muller model predicts that as species diverge over time they will accumulate genetic differences, which may be incompatible with each other when combined into the same genetic background. Despite the central importance of this process to diversification, identification of genes causing hybrid dysfunction has been primarily limited to model species. *Anopheles gambiae*, the principal mosquito vector of malaria in Africa, belongs to a complex of at least nine isomorphic species. In accordance with Haldane's rule, reciprocal crosses between *An. gambiae* and its sister species *Anopheles merus* produces completely sterile males, but fully fertile females. When we backcrossed F1 hybrid females to their maternal parental males, the resulting male progeny displayed a range of phenotypes from completely sterile to completely fertile. This phenotypic range provides us with the foundation for quantitative trait locus (QTL) mapping. We performed reciprocal backcrosses, phenotyped ~2500 males from those backcrosses, and genotyped each male using a total of ~12,000 markers across the

genome using a reduced representation sequencing approach. Our uniquely large quantitative genomics data set, when combined with testes-specific expression analysis, will allow us to identify a small number of hybrid-sterility candidate genes. Our current findings have shown us an intriguing result that points to a disruption sex chromosome inactivation during spermatogenesis that seems to be the cause sterility in only one of our reciprocal crosses. Not only will identification of hybrid-sterility genes provide insights into how and why postzygotic reproductive isolation barriers evolve, but they could also be important in the development of advanced sterile-insect control strategies aimed at reducing the role of the *Anopheles gambiae* complex in the African malaria transmission cycle.

P2125B Shared and species-specific transcriptional responses of barley (*Hordeum vulgare* L.) to generalist and specialist spider mite herbivores. *Robert Greenhalgh*[1], Huyen Bui[1], Alice Ruckert[2], Ricardo Ramirez[2], Richard Clark[1]. 1) University of Utah, Salt Lake City, UT; 2) Utah State University, Logan, UT.

With rising temperatures and increasing incidences of drought associated with climate change, spider mites, including the two-spotted spider mite (*Tetranychus urticae,* TSSM) and the Banks grass mite (*Oligonychus pratensis,* BGM), are becoming ever more important agricultural pests. An extreme generalist, the TSSM is documented to feed on more than 1,100 plant hosts, while the BGM, a grass specialist, feeds on cereal crops like maize, wheat, sorghum and barley. Historically, studies of plant-herbivore interactions have focused primarily on insects, while far less is known about how plants respond to mite herbivores, especially in grasses. To identify how plant defense pathways respond to both generalist and specialist mites, we compared time course RNA-seq data from barley (*Hordeum vulgare* L.) infested with TSSMs and BGMs. Strikingly, significant changes in the expression of hundreds of genes were detected at only two hours after mite infestation, and by 24 hours this number had increased to several thousand. As assessed by comparisons of TSSM- and BGM-induced responses at both time points, transcriptional reprogramming was strongly correlated for 3963 genes, including the down-regulation of genes involved in photosynthesis and the up-regulation of genes in the hormone signaling and secondary metabolic pathways. In particular, genes in the jasmonic acid biosynthesis and signaling pathway, as well as grass-specific secondary metabolic pathways, were among those with the strongest transcriptional changes. While many shared transcriptional changes were observed, the magnitude of barley responses to the specialist BGM was markedly stronger than that of the generalist TSSM, an observation that was reflected in enzymatic assays of infested leaves (e.g., peroxidase activity). In addition, 3261 genes exhibited BGM-specific changes in expression compared to only 535 genes unique to TSSM-infested plants. A potential explanation for this result is that the broad host range of generalist mite herbivores involves (at least in part) suppression of plant defenses, a finding that has been reported for some other herbivore species. In contrast, host adaptation for the specialist BGM may have involved the evolution of robust detoxification pathways for secondary compounds that typify members of the grass family upon which BGM is restricted.

P2126C Evidence for an epigenetic effect of kinship on fertility of flies (*Drosophila melanogaster)* induced by folic acid with reference to a possible similar mechanism in *Homo sapiens* at clinical dose levels. *M. L. Herbert*. Pinellas Country Dept Public Health , Largo, FL.

It has been shown that kinship determines fertility in humans as well as other mammals, birds, fish and insects. Evidence indicates that once kinship among a couple and their ancestors is taken into account there is no other important factor influencing fertility in a given species. At smaller populations the curve of fertility against kinship rises steeply as kinship rises until inbreeding is reached. At kinship less than an equilibrium point, fertility slowly falls with varying population time courses including: damped oscillation with rapid rise and slow fall seen in the wild, in the lab and by computer model; a two peaked pattern showing slow rise and rapid fall seen in humans, the wild and in computer model; and a single peak showing rapid rise and slow fall in humans, the wild, the lab and computer model, each pattern caused by a post zygotic mechanism or combined pre and post zygotic. Earlier we demonstrated damped oscillation in a population of captive fruit flies.

We hypothesized that the mechanism must be epigenetic and tried giving varying doses of folic acid to our flies looking for an effect.

We were able to demonstrate the two peaked pattern with slow rise and rapid fall characteristic of combined mechanisms.

We were surprised to discover that fertility depression can occur at roughly a dose of folic acid recommended for women. The number of possible confounding factors means that this may coincidental, but considering the importance of the issue this needs more study.

P2127A Robustness versus adaptation? *P. Jiang*[1], V. Sergey[2,3], M. Kreitman[1], J. Reinitz[1]. 1) University of Chicago, Chicago, IL; 2) Institute for Mechanical Engineering Problems, Sankt Petersburg, Russia; 3) ITMO University, Sankt Petersburg, Russia.

For multicellular organisms, a paradox exists between developmental robustness versus evolutionary novelty. On the one hand, in constant environment, robust developmental processes are preferred, in the face of weak or moderate genetic and environmental perturbations. On the other hand, in fluctuating or sudden change of environment, the least robust system will adapt the most quickly. Since environment cannot be in one mode forever, there must exist some balance between the degree of robustness and the ability to evolve. Here we propose a model, taking into consideration genes' combinatorial and pleotropic effects to determine phenotype for multicellular organisms and changing environment, to explore the relationship between robustness and adaptation. We mainly use forward population genetics simulations approach to address the question. We explored different parameter combinations that give rise to systems with different degree of robustness. We also find some connections of the current model to classic population genetics model.

P2128B Fisher's Geometric Model and the Cost of Reality. *K. Karkare*, R. B. R. Azevedo. University of Houston, Houston, TX.

Studies of adaptation strive to understand the relationship between genotype, phenotype, and fitness. Because mutations are the ultimate source of adaptive evolution, the distribution of fitness effects of mutations (DFE) provides critical information about adaptive processes. Fisher's Geometric Model of Adaptation (FGM) is a leading candidate for modeling adaptation because it captures the statistics of complex

gene interactions within a simple framework of mutation and selection. Despite its success fitting experimental data, the model's credibility is weakened because it does not explain all of the available data. FGM suffers an inherent 'cost of reality'. It cannot simultaneously satisfy a realistic DFE and maintain a realistic rate of adaptation. My work addresses if improved biologically plausibility of the underlying mutational and selective regimes can alleviate the cost of reality. I test multiple modifications to FGM and ultimately find that restricted mutational pleiotropy successfully relieves the cost of reality when a small proportion of traits are maladapted. This result harmonizes with previous work that shows restricted pleiotropy to be beneficial when few traits are maladapted because it allows those traits to adapt without disrupting the entire system. My work improves the statistical fit of a popular model of adaptation and finds evidence for an empirically supported mutational regime.

P2129C Identification and characterization of the *Taeniopygia guttata* (Zebra finch) sperm proteome. *T. Karr*[1], M. Rowe[2]. 1) Kyoto Institute of Technology, Kyoto, Kyoto Prefecture, JP; 2) Natural History Museum, University of Oslo, Oslo, Norway.

Spermatozoa exhibit remarkable variability in size, shape and performance, and are a key target of sexual selection due to both sperm competition and sperm-female interactions in sexually reproducing taxa. While knowledge of the evolutionary causes and adaptive significance of sperm morphological and behavioral variation is beginning to accumulate, our understanding of the genetic and molecular basis of sperm form and function remains limited. Here, we describe the first avian sperm proteome using LC-MS based discovery proteomics. The Zebra finch is an important model organism for several fields of study, including sperm competition. Thus, knowledge of the protein constituents of sperm may provide new insights into bird sperm biology and provide an important database for taxon-level comparative studies of sperm function. Sperm were isolated and purified from male seminal glomera (the site of sperm storage in passerine birds) and proteins separated by SDS-PAGE and subjected to trypsin digestion. The resulting peptides were analyzed by nano-electrospray mass spectrometry and resulting peptide spectra queried against the *T. guttata* proteome (uniprot.org) and protein assignments made using Protein Prophet. This approach identified 495 proteins of the zebra finch sperm proteome (ZfSP). Gene Ontology analysis of the major protein classes included numerous categories previously identified in both mammalian and insect sperm proteomes including metabolism/energetics (e.g., oxidoreductases, hydrolases, transferases) and cytoskeletal components of the sperm axoneme and acrosome (e.g., tubulins, dyneins and outer dense fiber proteins). In addition to these known categories, and compared to other defined sperm proteomes, the ZfSP is enriched in actin-related functions and associated processes at, or near, the membrane surface. We view these findings as an important first step in understanding the molecular basis of sperm form and function in a passerine bird, and believe our results will help shed light on the molecular mechanisms underpinning the outcomes of sperm competition and sperm-female interactions.

P2130A Genome-wide RAD genealogical analyses highlight the role of ancient genomic variation during rapid adaptation in threespine stickleback. *T. Nelson*, W. Cresko. Institute of Ecology and Evolution, University of Oregon, Eugene, OR.

Present genetic diversity, from the level of individual genes to genomic regions and entire chromosomes, is the product of the entire evolutionary history of a species. This deep history influences future adaptive potential. Recent advances in sequencing technologies have provided unprecedented views into patterns of genetic variation and spurred the field of population genomics, which aims to understand the distribution of genetic variation throughout genomes. While short sequence reads have greatly facilitated our understanding of genomic patterns of SNP variation, inference of deeper patterns of sequence evolution has been elusive. To facilitate genomic studies of haplotype evolution, we have use restriction site-associated DNA sequencing (RAD-seq) to generate phased sequence of length and quality comparable to Sanger reads — and thus amenable to genealogical analysis — at thousands of genomic loci. By comparing two populations of threespine stickleback (*Gasterosteus aculeatus*) to a sister species, the ninespine stickleback (*Pungitius pungitius*), we show that adaptive divergence in a freshwater pond population occurred primarily from standing genetic variation that originated long before that population was founded. Furthermore, we demonstrate that a chromosomal inversion under divergent selection is harboring a pair of anciently-evolved adaptive haplotypes. These data highlight the ability of RAD-seq to sample haplotypic variation at a genomic scale and help advance the field of populations genomics towards a deeper understanding of the genealogical structure of standing genetic variation and its importance for adaptation in the wild.

P2131B A karyological study of the artificial hybridization between *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus bidorsalis* (Geoffroy, 1809). *G.Chioma. Nzeh*, J. Olaoluwa Ademola. University of Ilorin, Ilorin, Kwara, NG.

This study was carried out to investigate the genetic factors responsible for the differential growth in the artificial hybrid of *C. gariepinus* and *H.bidorsalis*. Five hybrids obtained from the crossing of female *C. gariepinus* and male *H.bidorsalis* were used for the experiment. The live fish was injected intraperitoneally with 0.02% colchicine (1ml/100mg body weight) and left for four hours. Fish sacrificing was done by pitting. The anterior head kidney was used for karyotyping according to the air-drying method. The weights of the stunted growing fish are: 0.2Kg, 0.4Kg and 0.5 Kg, while the fast-growing weighed 0.8Khg and 1.5Kg. *C. gariepinus* has a standard karyotype of 2n = 56, while *H. bidorsalis* has 2n = 52. The hybrids showed differential growth rate. The hybrids were sterile; no gonads were observed within the first year. Karyological observation revealed variation in the hybrid's karyotype. Chromosome clumping was observed in hybrids that showed stunted growth. Further genetic studies should be carried out that will elucidate the genetic mechanism responsible for the differential growth of the hybrid.

P2132C Can Adaptive Evolution Undermine Canalization? The Case of Wing Size Evolution in High Altitude *Drosophila*. *John Pool*, Justin Lack, Matthew Monette, Evan Johanning, Quentin Sprengelmeyer, Amir Yassin. University of Wisconsin - Madison, Madison, WI.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

Many traits show canalization (robustness against genetic or environmental perturbation). But it's unclear how the buffering of a developmental process may be affected if adaptive evolution changes its phenotypic outcome. Highland Ethiopian *D. melanogaster* have evolved the largest thorax and wing length of any natural population. This size evolution is facilitated by an evolutionary tradeoff in which flies lay fewer but larger eggs, and it involves increases in both cell proliferation and somatic ploidy. Motivated by an unexpected frequency of wing vein abnormalities in Ethiopian inbred lines, we hypothesized that wing size evolution was accompanied by decanalized wing development in these flies. We repurposed a classic forward genetic approach (chemical mutagenesis) to test whether Ethiopian strains' wing development was more susceptible to genetic perturbation than a small-winged low altitude population (Zambia). Indeed, the offspring of mutagenized Ethiopian flies showed much higher rates of novel wing abnormalities, demonstrating reduced genetic robustness. Furthermore, wing size and decanalization cosegregated in the advanced generation offspring of a cross between Ethiopia and Zambia strains, implying that wing size variants undermined developmental buffering. Our results constitute the first natural example of a tissue's morphological evolution being associated with decanalized development, suggesting that decanalization might reflect an important cost of adaptation.

P2133A Effects of adaptive Neandertal introgression at the OAS locus on the modern human innate immune response. *A. J. Sams*[1], J. Nédélec[2,3], A. Dumain[2], V. Yotova[2], P. W. Messer[1], L. B. Barreiro[2,3]. 1) Cornell University, Ithaca, NY; 2) CHU Sainte-Justine Research Center, Montreal, QC, Canada; 3) University of Montreal, QC, Canada.

It is now clear the ancestry of all individuals living outside of sub-Saharan Africa is composed of roughly two percent Neandertal ancestry. Yet, it remains largely unclear to what extent this contribution from Neandertals impacts modern human biology, and further, to what extent it may have provided adaptive genetic variation to modern human populations. The immune system is one physiological system that harbors higher than typical amounts of genetic variation in order to provide a flexible set of responses to infection. Here we use coalescent simulation and population genetic approaches to demonstrate a signal of adaptive introgression in the 2'-5' oligoadenylate synthetase (OAS) gene cluster region of chromosome 12. The adaptive region encodes for three active OAS enzymes (OAS1-3) that are involved in the innate immune response to viral infection. In order to evaluate the functional consequences of the adaptive haplotype we infected primary macrophages and peripheral blood mononuclear cells from people with and without the Neandertal haplotype with a panel of bacteria, viruses, and viral-syntetic ligands. Our results show that human cells carrying the Neandertal-like haplotype show marked functional differences in the transcriptional regulation of *OAS3* in response to bacterial and viral agents which activate the type I interferon (IFN) production pathway. Additionally, we find that SNPs associated with the Neandertal haplotype are strongly associated with isoform usage in *OAS1* and *OAS2*. These results illuminate the phenotypic effects of Neandertal haplotypes into the regulation of innate immune responses and a potential functional explanation for adaptive introgression of the OAS locus into modern human populations.

P2134B GC-rich DNA is an inductor of adaptive response in MSCs. *Vasilina Sergeeva*[1], S. V. Kostyuk[1], N. N. Veiko[1], E. S. Ershova[1], L. V. Kameneva[1], E. M. Malinovskaya[1], N. N. Mordkovich[2], V. P. Veiko[2], N. A. Okorokova[2]. 1) Research Centre for Medical Genetics, Moscow, Russia; 2) Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia.

It has been established that cell-free DNA (cfDNA) circulates throughout the bloodstream, affecting cells. The characteristics of cfDNA depend on the physiological state of the organism. Diseases can cause GC-enrichment of the cfDNA pool (cerebral atherosclerosis, heart attack, rheumatic arthritis, cancer). As was shown previously, cfDNA pool is enriched with ribosomal GC repeat in cases of people working with gamma-radiation (N=230).

GC-rich cfDNA causes adaptive response in human adipose-derived mesenchymal stem cells (MSCs) via DNA damage response. It rapidly induces DNA breaks after 30 minutes of incubation (shown by means of single cell electrophoresis and flow cytometry), leading to increase in DNA reparation gene level *BRCA1* (6,5-fold) and anti-apoptotic genes *BCL2 (4,5 - fold)*, *BCL2A1 (5-fold)*, *BCL2L1 (3-fold)*, *BIRC3 (5-fold)*, *BIRC2(6-fold)* after 3 hours of incubation. Moreover, GC-rich DNA activates NF-kB signaling pathway: NF-kB translocates to the nucleus after 1 h of incubation (fluorescent microscopy) and genes of NF-kB signaling pathway: *MAP3K1, MAP4K4, NFKB1A, REL*, *IKBKB, RelA (p65), NFRKB, NFKB1* and *NFKB2* increase 2 – 5,5-fold (RT-PCR). Expression of NF-kB target proinflammatory cytokines genes *TNFα* (3,9-fold), *IL1B* (2,4-fold), *IL8* (2,8-fold), *IL6* (1,7-fold), *TNFRSF1A* (3,2-fold) increases. In addition, level of apoptosis decreases and proliferation rises, level of Ki-67 and PCNA proteins increases (flow cytometry). Treating MSCs with GC-rich cfDNA prior to exposure to damaging factors such as ionizing radiation positively affects cell survival - radiation induces less DNA breaks and less cell death in cell cultures treated with GC-rich cfDNA.

Conclusion. GC-rich fragments of the ribosomal repeat in the pool of cfDNA can be inductors of adaptive response development in cases of exposure to external damaging factors (ex. ionizing radiation).

P2135C The adaptive significance of natural genetic variation in the DNA damage response of *Drosophila melanogaster*. *Nicolas Svetec*, Julie M. Cridland, Li Zhao, David J. Begun. UCDavis, Davis, CA.

Despite decades of work, our understanding of the distribution of fitness effects of segregating genetic variants in natural populations remains largely incomplete. One form of selection that can maintain genetic variation is spatially varying selection, such as that leading to latitudinal clines. While the introduction of population genomic approaches to understanding spatially varying selection has generated much excitement, little successful effort has been devoted to moving beyond genome scans for selection to experimental analysis of the relevant biology and the development of experimentally motivated hypotheses regarding the agents of selection.

Solar UVB incidence is negatively correlated with latitude and an important agent of DNA damage in nature. Motivated by population genomic results, we investigated whether clinal variation in UVB incidence has led to genetic differences in the DNA damage response of *D.*

melanogaster populations. Using a combination of population genomics, transcriptomics, and organismal phenotypic analysis, we show that genetic variation in the DNA damage response in *D. melanogaster* is maintained by spatially varying selection due to latitudinal variation in solar UVB incidence.

P2136A Can the Y chromosome save males from the mother's curse? *J. Arvid Ågren*, Andrew G. Clark. Cornell University, Ithaca, NY.

Genomic conflicts often arise because not all genes are inherited in the same way. Opportunities for conflict between the biparentally inherited nuclear genome and the maternally inherited mitochondrial genome (mtDNA) seem especially favorable and conflicts between nuclear and mitochondrial genes over sex determination and sex ratio have been well documented. In general, because genes in the mitochondrial genome are strictly maternally inherited, mutations that are beneficial in females can spread in a population even if they are somewhat deleterious in males. This phenomenon has been dubbed the "mother's curse". The accumulation of male-biased mutations in the mitochondrial genome should lead to selection in males for compensatory nuclear modifier loci that alleviate the effect. The Y chromosome, being strictly paternally inherited, has been suggested as a good candidate for such modifiers. Recent work has countered classic predictions by finding that loci in the mitochondrion and the Y chromosome can affect the expression of nuclear autosomal loci. Moreover, several of the autosomal loci shown to be affected by mitochondrial variation overlap with autosomal loci affected by variation on the Y chromosome. The extent to which these loci are subject to mtDNA-Y chromosomal epistatic effects and determine male health and fitness, however, remains unknown. To experimentally examine the extent to which mitochondrial and Y-linked genetic variants interact to determine male fitness, we used *Drosophila melanogaster* strains from the five geographical locations (Ithaca, the Netherlands, Zimbabwe, Beijing, and Tasmania). Through crosses, we generated strains that differed only in the geographical origin of their mitochondrial genome and the Y chromosome. If Y-linked suppressors are important, co-evolved combinations of the mitochondrial genome and the Y chromosome from the same population should outperform novel combinations. We present evidence that the effect of mtDNA-Y interactions depend both the origin of the mtDNA and the Y chromosome, as well as on what aspect of male fitness measured. In light of these results, we outline a simple model for how mtDNA-sex chromosome interactions differ in an XY compared to a ZW sex chromosome system where the female is the heterogametic sex, and the W chromosome is always co-transmitted with the mitochondrion.

P2137B Positive selection and centrality in the yeast and fly protein–protein interaction networks. *D. Alvarez-Ponce*, S. Chakraborty. University of Nevada, Reno, Reno, NV.

Proteins within a molecular network are expected to be subject to different selective pressures depending on their relative hierarchical positions. However, it is not obvious what genes within a network should be more likely to evolve under positive selection. On the one hand, only mutations at genes with a relatively high degree of control over adaptive phenotypes (such as those encoding highly connected proteins) are expected to be "seen" by natural selection. On the other hand, a high degree of pleiotropy at these genes is expected to hinder adaptation. Previous analyses of the human protein–protein interaction network have shown that genes under long-term, recurrent positive selection (as inferred from interspecific comparisons) tend to act at the periphery of the network. It is unknown, however, whether these trends apply to other organisms. Here, we show that long-term positive selection has preferentially targeted the periphery of the yeast interactome. However, the opposite trend is found in flies: genes under positive selection encode significantly more connected and central proteins in the fly interactome. These observations are not due to covariation of genes' adaptability and centrality with protein length, protein abundance, expression level or, in the case of *Drosophila*, expression breadth. These results indicate that the distribution of proteins encoded by genes under recurrent positive selection across protein–protein interaction networks varies from one species to another.

P2138C GC content evolution in the light of nucleic acid molecular dynamics. *G. A. Babbitt*, E. E. Coppola. Rochester Institute of Technology, Rochester, NY.

Molecular evolutionary studies of genomic base composition and GC content have a long unresolved history of frequency-based methods of analysis applied only to DNA sequence data. However, this tradition ignores the physicochemical impacts of mutation that can affect the molecular dynamics of nucleic acid polymers. This is especially problematic during transcription, where mutation can influence the complex interactions at play in chromatin-mediated genomic regulation. Here, we investigate the two primary biophysical constraints required of eukaryotic transcription; a flexible/nuclear-compacted DNA and a stable/non-self-reactive RNA; both features which can be significantly affected by the molecular evolution of GC content in the genome. We utilize a 2x448 core Tesla supercomputer to conduct 2000 nanosecond scale GPU accelerated molecular dynamic simulations of mutation in randomized DNA and RNA sequence backgrounds, where we investigate mutational impacts on molecular dynamics while controlling for varying GC content. Additionally, in *Saccharomyces* yeast alignments, we employ simulations of neutral evolution to locally map natural selection acting on DNA flexibility and RNA stability. We find that different classes of mutation have very different GC-dependent impacts on dynamics, defined via shifts in atomic fluctuation and correlation, and indicating that GC-related molecular dynamics of nucleic acids are correlated and evolvable. In DNA, we report that low GC generally amplifies mutational impacts on molecular dynamics while increasing the impact of transversion over transition, providing a potent functional evolutionary constraint that may form a basis for transition bias observed in most genomes. In RNA, we observe that the molecular dynamic impacts of mutation depend largely upon their impact on uracil, the most interactive of the nucleobases. In *Saccharomyces* yeasts, we find evidence that many yeast genes have effectively decoupled the biophysical relationship between DNA flexibility and RNA stability by elevating GC. We also report a significant genome-wide signature whereby TFBS consensus sequences are partly defined by mutational impacts on DNA flexibility. We conclude that the historical assumption of genetic abstraction in molecular evolution is quite limiting, especially

regarding the role of nucleic acid polymer biophysics in interpreting broad patterns in molecular evolution related to genome packaging and control of transcription.

P2139A A genetic parallel between flightlessness evolution in the Galapagos cormorant (*Phalacrocorax harrisi*) and human skeletal ciliopathies. *A. BURGA*[1,2], W. WANG[3], P. C. WOLF[4], A. M. RAMNEY[5], C. VERDUGO[6], K. LYONS[3], P. C. PARKER[7,8], L. KRUGLYAK[1,2]. 1) Departments of Human Genetics and Biological Chemistry, UCLA, Los Angeles, USA; 2) Howard Hughes Medical Institute (HHMI); 3) Departments of Molecular, Cell and Developmental Biology and Orthopaedic Surgery, UCLA and Orthopaedic Institute for Children, Los Angeles, USA; 4) United States Department of Agriculture/Wildlife Services; 5) U.S. Geological Survey Alaska Science Center, Alaska, USA; 6) Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile; 7) Department of Biology and Whitney Harris World Ecology Center, University of Missouri-St. Louis, USA; 8) WildCare Institute, Saint Louis Zoo, Saint Louis, USA.

Changes in the morphology and size of limbs have played a key role in the adaptive evolution of species. Our own ancestors experienced such changes as they transitioned towards a bipedal, manually dexterous primate. However, despite the evolutionary importance of these modifications, we have a very limited idea of how these changes occur on a genetic and molecular level, especially in vertebrates. In order to fill this gap, we studied a recent case of extreme wing size reduction leading to flightlessness in the Galapagos Cormorant (*Phalacrocorax harrisi*). The Galapagos cormorant is unique in that is the only cormorant that lost the ability to fly among approximately 40 extant species. Their entire population is distributed along the coastline of the Isabela and Fernandina Islands in the Galapagos archipelago. *P. harrisi* has a pair of stubby wings, which are smaller than those of any other cormorant in spite of having the largest body mass, resulting in a significant deviation from the allometric relationship between wing length and body mass among flighted birds. We applied a joint predictive and comparative genomics approach to identify deleterious variants that affected the Galapagos Cormorant but were absent in three flighted close relatives. Among these variants, we found a significant enrichment for genes that cause skeletal ciliopathies when mutated in humans. Individuals affected by ciliopathies have small limbs and rib cages, mirroring the phenotype of *P. harrisi*. We show that *CUX1,* a highly conserved transcription factor, has a four amino acid deletion in *P. harrisi* that affects its regulatory domain. Moreover, we demonstrate that CUX1 controls the expression of cilia related genes and can promote the differentiation of chondrocytes. Our results suggest that the combined effect of deleterious variants in genes affecting chondrogenic differentiation led to the highly reduced wings of *P. harrisi.* .

P2140B Comparative Genomic Analysis of Zika Viruses between Southeast Asia and Microcephaly-Related South America Groups. *T. Chookajorn*[1], N. Kotanan[1], K. Kumpornsin[1], D. Loesbanluechai[2], M. Thammasatta[3], P. Auewarakul[1], P. Wilairat[1]. 1) Mahidol Univ., Bangkok, Thailand; 2) Chulalongkorn Univ., Bangkok,Thailand; 3) National Science and Technology Development Agency, Pathum Thani, Thailand.

Zika virus has become a global health crisis, causing devastating effects on lives of almost five thousand pregnant women and their newborns. One of the often overlooked facts is that Zika virus has been in circulation in Southeast Asia for several decades without distinct manifestations of neurological pathologies. An understanding of the cause for discrepancy between potentially mild Southeast Asia and more aggressive Zika viruses in South America could reveal the mechanisms underlying outbreaks and neurological damages of Zika infections in Brazil. We performed a comparative genomic analysis was performed to determine putative causations stemming from the virus. Phylogenetic analyses integrating geographical and time factors showed that Asian Zika virus might not be the direct source of South American outbreaks as previously speculated. The differences in amino acid residues between Southeast Asian and Brazilian Zika viruses are specifically clustered on the outer surfaces of Envelope and NS1 proteins. Comparative genomic analyses also revealed that only a selected few primer/probe sets currently in clinical use are capable of detecting Zika virus strains worldwide. Interestingly, the Envelope proteins of Dengue and Zika viruses show a remarkable degree of similarity especially at the surface residues. This pattern explains the cross-reactivity of Dengue virus antibodies to Zika viruses. It also suggests that therapeutic antibodies and vaccine candidates against Dengue viruses can target the Zika virus, thereby providing a strategy for using already available means to treat and prevent Zika virus infection.

P2141C Three blind mammals: Regressive evolution in the mammalian eye and the identification of new eye-specific *cis*-regulatory elements. *Nathan Clark*[1], Raghav Partha[1], Zelia Ferreira[1], Joseph Robinson[2], Bharesh Chauhan[1], Ken Nischal[1], Maria Chikina[1]. 1) University of Pittsburgh, Pittsburgh, PA; 2) University of California, Berkeley, CA.

After adopting primarily underground lifestyles, 3 independent lineages of subterranean mammals – the naked mole rat, star-nosed mole, and cape golden mole – evolved greatly reduced eyesight in a process known as regressive evolution. The multiple independent cases of regressive evolution allow an investigation of genomic patterns underlying this phenotypic convergence. Using new phylogenomic methods we found that hundreds of genes show increased evolutionary rates specifically in subterranean species, primarily due to a loss of functional constraint. These genes were predominantly involved in eye physiology, such as those encoding lens crystallins, photoreceptors and transduction pathways. Furthermore, bright-light color photoreceptors were clearly more degraded than dim-light receptors indicating they were lost earlier during regression. This finding suggests these species underwent a gradual transition to the subterranean environment, retaining vision in dim light for longer. Regressive evolution proceeded very differently between eye tissues; while lens and retinal genes are highly degraded, corneal genes remain under constraint, perhaps because they continue to provide a protective outer barrier for the vestigial eye. Moreover, genes important for the embryological development of all eye tissues remain highly conserved, potentially because they are expressed during the development of tissues outside the eye. For example, the coding portion of PAX6, a key transcription factor in the development of eye and the central nervous system, showed no signs of regression in subterranean mammals. We also explore an alternative explanation that development of early embryonic eye tissues is important for the canalized development of neighboring tissues such as the

brain. Observed developmental patterns in these species are consistent with this hypothesis. In contrast to its coding sequence, we found that PAX6's eye-specific enhancers were evolving at a much faster rate in subterranean species, likely due to relaxed constraint, relative to aboveground species. PAX6's enhancers active in other tissues did not demonstrate this pattern. This observation led us to perform a genome-wide screen to identify 40 new candidate eye-specific *cis*-regulatory sequences, which clustered near confirmed eye development genes. Thus, the results of convergent, regressive evolution provide a powerful means to assign functions to uncharacterized elements in the genome. We conclude that eye-related genes and regulatory elements show convergent patterns of loss and retention during repeated instances of regressive evolution, and that regression occurs differentially across tissues, physiology and development.

P2142A Plasmodium vivax mdr1 genotypes in isolates from successfully cured patients living in endemic and non-endemic Brazilian areas. *Maria de Fatima Ferreira-Da-Cruz*[1*], L. Gomes[1], Lavigne AR[1], Pina-Costa A.[1], Brasil P.[1], Almeida-De-Oliveira NK[1], Daniel-Ribeiro CT[1], Menard Didier[2]. 1) Fundacao Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil; 2) Institut Pasteur , Cambodia.

Plasmodium vivax (Pv) is the most widely distributed species causing the highest number of malaria cases in the world. In Brazil, Pv is responsible for approximately 84 % of reported cases. In the absence of a vaccine, control strategies are based on the management of cases through rapid diagnosis and adequate treatment, in addition to vector control measures. The approaches used to investigate Pv resistance to chloroquine (CQ) were in vivo studies because of the difficulty in keeping parasites in continuous in vitro culture. In view of the limitations related to follow-up of patients and to assessing the plasma dosage of CQ and its metabolites, an alternative approach to monitor chemo-resistance (QR) is to use molecular markers. Single nucleotide polymorphisms (SNPs) in the multidrug resistance gene pvmdr1 are putative determinants of CQ resistance (CQR), but such SNPs in Pv isolates from patients with good response to treatment should be further explored. The aim of this study is to investigate the mutations in the gene, supposedly associated to QR, in Pv isolates from successfully cured patients, living in Brazilian endemic and non-endemic areas. Blood samples were collected from 49 vivax malaria patients from endemic (Amazon Basin: 45) and non-endemic (Atlantic Forest: four) Brazilian regions and analysed for SNPs in the CQR-related Pv gene (pvmdr1). Among the 49 isolates genetically characterized for the gene pvmdr1, 34 (70 %) presented at least one mutation. T958M mutant alleles were the most frequent (73 %) followed Y976F (15 %) and F1076L (12 %). Single mutation was detected in 24 (70.5 %) isolates and double mutations in ten (29.5 %). The most common single mutant genotype was the 958M/Y976/F1076 (79 %), followed by 976F/F1076 (21 %) whereas 958M/Y976/1076L (60 %) and 976F/1076L (40 %) double mutant genotypes were detected. Single mutant profile was observed only in isolates from Amazon Basin, although double mutants were found both in the Amazon and Atlantic Forest regions. Interestingly, the genotype 958M/Y976/1076L was present in all isolates from the Atlantic Forest in the Rio de Janeiro State. Considering that primaquine (PQ) efficacy is highly dependent on concurrent administration of a blood schizontocidal agent and that PQ could not circumvent CQR, together with the fact that no pvmdr1 mutation should be expected in successfully cured patients, these findings seem to indicate that the pvmdr1 gene is not a reliable marker of CQR. Further investigations are needed to define a reliable molecular marker for monitoring Pv CQR.

P2143B Phylogenetic reconstruction using Wright-Fisher models of sequence evolution vastly out perform standard approaches. *M. A. Gilchrist*[1,3], B. C. O'Meara[1,3], R. Zaretzki[2,3], C. L. Landerer[1], J. M. Beaulieu[1,3]. 1) Dept. of Ecology & Evolutionary Biology, University of Tennessee, Knoxville, TN; 2) Dept. of Business Analytics & Statistics, Univerity of Tennessee, Knoxville, TN; 3) National Institute for Mathematical and Biological Synthesis, Knoxville TN.

Most phylogenetic reconstructions using sequence data eitehr ignore natural selection or incorporate it in a heuristic manner such as Yang and Nielsen's ω term for describing either stabilizing or diversifying selection. Here we present an alternative approach in which fixation probabilities are explicitly based on the Wright-Fisher model of population genetics undergoing gene specific, stabilizing selection the amino acid sequence of a given protein. Further, the mechanistic nature of our approach means our model is easier to justify biologically and the parameters estimated describe explicit biological processes such as a gene's average protein production rate or the predicted effect of an amino acid substitution on protein function. Despite being more parameter rich than traditional, heuristic models used in phylogenetics, our model consistently provides astronomically better AIC values when applied to a wide range of test datasets. Finally, because it is mechanistically derived, it is relatively straight forward to expand our model to incorporate additional or alternative biological processes.

P2144C First report of *Rhizoctonia solani* AG-4 on tomato in Pothwar region of Pakistan. *A. S. Gondal*, A. Rauf, G. Irshad, F. Naz. PMAS Arid Agriculture University, Rawalpindi, Punjab, PK.

Rhizoctonia solani Kühn (teleomorph = *Thanatephorus cucumeris* (Frank) Donk) is the most important soil-borne fungal pathogen cause causes significant establishment with typical symptoms of collar rot, seedling death, stunted growth and root rot in tomato. Fungus isolated from diseased plants was identified as *Rhizoctonia solani* by comparing its morphological characters. Isolates were further confirmed as AG-4 by hyphal fusion test with tester isolates and DNA analysis. Present study is the first report of R. solani AG-4 effecting tomato in Pothwar region, Pakistan.

P2145A Comparative genome-wide analysis and evolutionary history of haemoglobin-processing and haem detoxification enzymes in malarial parasites. *T. Kochakarn*[1*], P. Ponsuwanna[1], D. Bunditvorapoom[1], K. Kümpornsin[1], T.D. Otto[2], C. Ridenour[1], K. Chotivanich[1], P. Wilairat[1], N.J. White[1,3], O. Miotto[1,3,4], T. Chookajorn[1]. 1) Mahidol University, Bangkok, Thailand; 2) Wellcome Trust Sanger Institute, Cambridge, UK; 3) University of Oxford, Oxford, UK; 4) Wellcome Trust Sanger Institute, Hinxton, UK.

Intra-erythrocytic parasitism requires malarial parasites to digest haemoglobin and detoxify iron-bound haem. These tasks are executed by

haemoglobin-specific proteases and haem biocrystallization factors that are components of a large multi-subunit complex. Since haemoglobin processing machineries are functionally and genetically linked to the modes of action and resistance mechanisms of several anti-malarial drugs, an understanding of their evolutionary history is important for drug development and drug resistance prevention.

Maximum likelihood trees of genetic repertoires encoding haemoglobin processing machineries within *Plasmodium* species, and with the representatives of Apicomplexan species with various host tropisms, were created. Genetic variants were mapped onto existing three-dimensional structures. Genome-wide single nucleotide polymorphism data were used to analyse the selective pressure and the effect of these mutations at the structural level.

Recent expansions in the falcipain and plasmepsin repertoires are unique to human malaria parasites especially in the *Plasmodium falciparum* and *P. reichenowi* lineage. Expansion of haemoglobin-specific plasmepsins occurred after the separation event of *Plasmodium* species, but the other members of the plasmepsin family were evolutionarily conserved with one copy for each sub-group in every Apicomplexan species. Haemoglobin-specific falcipains are separated from invasion-related falcipain, and their expansions within one specific locus arose independently in both *P. falciparum* and *P. vivax* lineages. Gene conversion between *P. falciparum* falcipain 2A and 2B was observed in artemisinin-resistant strains. Comparison between the numbers of non-synonymous and synonymous mutations suggests a strong selective pressure at falcipain and plasmepsin genes. The locations of amino acid changes from non-synonymous mutations mapped onto protein structures revealed clusters of amino acid residues in close proximity or near the active sites of proteases.

A high degree of polymorphism at the haemoglobin processing genes implicates an imposition of selective pressure. The identification in recent years of functional redundancy of haemoglobin-specific proteases makes them less appealing as potential drug targets, but their expansions, especially in the human malaria parasite lineages, unequivocally point toward their functional significance during the independent and repetitive adaptation events in malaria parasite evolutionary history.

P2146B Is the evolution of innate immunity the next EvoDevo? Changes in molecular function and cellular signaling in an antiviral immune system across the animal phylogeny. *B. Kolaczkowski*, C. Pugh, K. Aadland. University of Florida, Gainesville, FL.

Although it is widely appreciated that a functioning innate immune system is required to support multicellular life, we know surprisingly little about how the immune system of early multicellular animals was put together, how it changed over animal evolutionary history or the consequences of those changes for the organism. Here we combine ancestral sequence reconstruction with *in vitro* molecular-functional analyses and comparative *in vivo* examinations in mammalian and early-animal model systems to characterize the evolution of the RIG-like receptor (RLR) signaling network, an important component of animal innate antiviral immunity. We find that RLRs originated in the earliest animal lineages, probably through fusion of an RNA-binding domain from Dicer with CARD signaling domains, coupled with the origination of a novel structural fold used for viral RNA recognition. RLRs diversified through gene duplication events followed by changes in domain architecture and adaptively-driven protein-coding changes that repeatedly altered RNA preference. We present evidence that RLRs recruited novel signaling partners at least twice in animal evolution, leading to changes in the RLR signaling network and resulting in alteration of cellular immune function. Our work highlights the dynamic nature of immune-system evolution, the roles that protein structural variants, amino-acid variants and genomic clusters can play in evolving novel protein-protein signaling networks and the potential importance of changes in immune-signaling networks for cellular and organismal function. In our view, further understanding how the innate immune system evolved will help us understand how differences in innate immunity may have contributed to animal biodiversity.

P2147C The lower limit of transcription error rate in the bacterium *Escherichia coli*. *Weiyi Li*, Jean-François Gout, Michael Lynch. Indiana University Bloomington, Bloomington, IN, USA.

Errors can occur at any level during replication and expression of genetic information. Genetic mutations are derived mainly from replication errors and have been extensively studied in evolutionary research. However, many details of the transcription error remain largely unknown. Transcription errors can indirectly give rise to misfolded proteins and impose a load on cellular integrity. Same as the rate of genetic mutation, the rate of transcription error is shaped by the directional process of selection and the random process of drift. However, transcription errors are fundamentally different from genetic mutations in many aspects. Firstly, unlike mutations that reside in genomes, transcription errors in RNA transcripts have short life spans and will finally be degraded. Secondly, there are multiple RNA copies of one gene and only a minor portion of them may carry transcription errors. These transient natures would dilute the fitness effect of transcription errors and selection is less sufficient to push down the rate of transcription errors. To investigate the lower limit of transcription error rate in the bacterium *Escherichia coli*, we took a novel rolling-circle sequencing approach and have accurately identified transcriptome-wide transcription errors. Our results indicate that the lower limit of transcription error rate in the bacterium *Escherichia coli* is 10^5 x higher than the corresponding genetic mutation rate.

P2148A A maximum pseudo-likelihood approach for estimating species trees. *L. liu*[1], L. Yu[2], S. Edwards[3]. 1) University of Georgia, Athens, GE; 2) Georgia Southern University, Statesboro, GA; 3) Harvard University, Cambridge, MA.

Several phylogenetic approaches have been developed to estimate species trees from collections of gene trees. We propose to use a pseudo-likelihood function of the species tree to obtain Maximum Pseudo-likelihood Estimates of Species Trees (MP-EST), with branch lengths of the species tree in coalescent units. The MP-EST method estimates species trees from a set of gene trees by maximizing a pseudo-likelihood function. The program can run independent searches (chains) in parallel using the parallel version of the program. Each chain starts with a different seed. The program will find the estimate of the species tree with the largest pseudo-likelihood score across chains.

P2149B Reduction of intergenic non-coding RNAs from the *HBS1L-MYB* locus linked to Thalassemia disease severity. *D. Loesbanluechai*[1], K. Leecharoenkiat[1], S. Fucharoen[2], O. Sripichai[2]. 1) Chulalongkorn University, Bangkok, Bangkok, TH; 2) Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, NakornPathom, TH.

Thalassemia is a hematological disease caused by imbalanced globin production. The degree of diseases severity depends on several factors including the expression of fetal globin which can compensate for loss in adult globin. Genome wide association studies were performed to identify loci that are linked to reduction in disease severity. The majority of polymorphic sites are clustered within the globin genes. A subset of key loci is located at or near the genes encoding transcription factors functionally associated with globin gene expression. The polymorphism at the intergenic region between *HBS1L* and *MYB* was shown to one of the top severity modifying factors. A set of short transcripts corresponding to the intergenic *HBS1L-MYB* region was found in RNA-seq analyses of erythroid cell lines and hematologic stem cells, indicating that this intergenic region is actively transcribed. Recently, the enhancer RNA was shown to play roles in transcription control. These small non-coding RNAs might be responsible for change in disease severity. Quantitative RT-PCR was carried out to determine and quantify the non-coding *HBS1L-MYB* transcripts in primary human erythroblasts from normal individuals and Thalassemia patients. Three clusters of transcripts were identified. Interestingly, erythroblasts from beta-thalassemia/HbE patients have significantly lower levels of the non-coding transcripts than those from individuals with normal globin genes, warranting further studies to determine the function of these non-coding *HBS1L-MYB* transcripts.

P2150C Folding and misfolding of evolutionarily young proteins. *Joanna Masel*[1], Scott Foy[1], Ben Wilson[1], Rafik Neme[2], Matt Cordes[1]. 1) University of Arizona, Tucson, AZ, USA; 2) Max Planck Institute for Evolutionary Biology, Ploen, Germany.

Random polypeptides are expected to form amyloids and other toxic aggregates, making de novo gene birth from non-coding sequences a difficult transition. We find that evolutionarily young mouse proteins contain more hydrophilic amino acids than old proteins do, which in turn are more hydrophilic than intergenic sequences would be if they were translated. Low hydrophobicity is presumably a precondition for avoiding harmful aggregation; computationally predicted aggregation propensity tracks amino acid composition. But surprisingly, when amino acid composition is held constant, young proteins (but not old proteins) actually have a higher predicted aggregation propensity than scrambled controls. Preliminary results suggest that this might be explained by the degree of dispersion of hydrophobic amino acids along the primary sequence. Previous work concluded that amino acids with different properties, e.g. polar vs. non-polar or hydrophobic vs. hydrophilic, are overdispersed relative to a random ordering. We find that this conclusion holds only for the very oldest proteins, which are overrepresented in protein structural databases. Young proteins instead show significant underdispersion / clustering. We hypothesize that this clustering to form locally structured regions may be a precondition for a de novo evolved protein to fold, with a greater tendency to misfold/aggregate arising as an inevitable byproduct. Over long evolutionary timescales, more subtle folding strategies allow proteins to become more hydrophobic while still avoiding misfolding, and to have less clustering of their hydrophobicity while still being able to fold.

P2151A Assessing the compatibility of eukaryotic transcript evolution with *de novo* gene birth. *L. Nielly-Thibault*, C. Landry. Laval Univ., Quebec, Canada.

The emergence of new genes is often caused by the duplication or the fusion of ancestral genes. On their own, these mechanisms cannot explain the observation of species-specific genes and seemingly unrelated gene families. Recent studies have revealed cases of gene birth from non-genic ancestral DNA in various Eukaryotes. Such *de novo* gene birth may complement other mechanisms in the explanation of gene content variation between species. However, this phenomenon requires that regions of non-genic DNA acquire transcription and, in the case of protein-coding genes, translation. The roles played by cellular and populational processes in *de novo* gene birth are not yet well understood. We hypothesize that it is mainly driven by selectively neutral mutations generating and disrupting non-functional occurrences of sequence elements involved in transcription and translation. To test this hypothesis, we are using published data from the yeast *Saccharomyces cerevisiae* to assess the impact of mutations on the boundaries of transcribed and translated regions. As a first model, we are examining the evolution of transcriptional initiation and termination following the yeast whole genome duplication. Our results will clarify the impact of neutral evolutionary forces on eukaryotic transcripts, an important component of their potential as a raw material for *de novo* gene birth.

P2152B Improved accuracy of phylogenetic analyses by partitioning schemes that incorporate structural information. *Akanksha Pandey*, Edward Braun. University of Florida, Gainesville, FL.

Phylogenetic analyses of ancient evolutionary relationships, like the earliest divergences among metazoans, have often used protein sequences. Maximum likelihood (ML) analyses of aligned protein sequences typically use “empirical models” of evolution, where the parameters describing the instantaneous rate of change among amino acids is estimated from large-scale “training sets” of proteins. This creates the problem that the analyses essentially assume that all positions in the alignment exhibit similar patterns of evolution (with the exception of the overall rate, which is typically modeled by assuming rates at different sites are drawn from a gamma distribution). Effectively, one assumes that all sites evolve like the average site in the average protein in these analyses. However, it is clear that different sites in proteins exhibit substantial heterogeneity in their patterns of evolution. This variation probably reflects various structural and functional constraints. A number of approaches have been proposed to incorporate this heterogeneity into phylogenetic analyses, but it seems reasonable to postulate that incorporating structural information might provide a means to move toward a more realistic model of protein evolution. Here, we examine two straightforward and computationally efficient partitioning approaches that divide proteins into subset of sites using structural information (i.e. secondary structure and relative solvent accessibility). We then compared the performance of our model on

an alignment of 242 orthologous proteins for 19 metazoan taxa (104,840 sites with 16.9 % missing data). This dataset has been somewhat equivocal regarding the topology it supports but all of our analyses using the new structural partitioning schemes, with parameter estimates for each structural class, place ctenophores sister to all other metazoans. This is the topology found in a number of other analyses using more extensive taxon sampling so it is encouraging for the phylogenetic accuracy of this approach. Our approach is straightforward to implement in existing software and, based on information theoretic criteria, outperform available empirical models. The best-fitting partitioning scheme included both secondary structure and relative solvent accessibility and it used partition boundaries generated by programs in the SCRATCH package on a weighted consensus sequence for each protein. Estimates of parameters for different structural classes show many differences, suggesting that this approach provides a better overall fit to the evolutionary process.

P2153C Here and there, but not everywhere: the repeated loss of uncoupling proteins in reptiles and mammals. *T. S. Schwartz*[1], S. M. McGaugh[2]. 1) Auburn University, Auburn, AL; 2) University of Minnesota, Saint Paul, MN.

Uncoupling proteins in the inner mitochondrial membrane provide a proton "leak", thereby uncoupling the movement of protons down the gradient and the production of ATP. There are six uncoupling proteins (UCP1-6), the largest family of nuclear-encoded mitochondrial transporters. From an evolutionary perspective they provide dramatic steps in evolution including the evolution of endothermy, as well as regulation of ROS that are fundamental to rates of aging and oxidative stress. While the function of UCP1 in nonshivering thermogensis in mammals is well established known, the functions of the other uncoupling protein homologues is less clear. We conducted this study to fill a gap in literature related to the evolution of mitochondrial uncoupling proteins. The purpose of this study was two fold. First, we surveyed the available reptile genomes and transcriptomes for the presence of the different homologues to predicted gene losses in each group. Second, we determined whether there was evidence that positive selection acted on each UCP in the amniotes—mammals and reptiles. Across 66 species, alignments made from transcriptome and genome-derived coding sequence suggest that specific UCPs are missing in several clades, and we confirmed this by examining syntenic regions among different clades and BLAST searches across newly available genomes. The cumulative analysis suggests that all six of the UCP homologues are present in mammals. The *ucp1* gene was lost very early in the reptile lineage and the lost of UCP1 seems to be a repeated occurrence in multiple mammalian lineages despite its role in non-shivering thermogenesis. Further, among reptiles UCP2 was absent in birds, and UCP3 and UCP4 were absent in snakes. Branch-site tests suggest that positive selection acted on UCP2 in the branch leading to mammals and UCP4 on the branch leading to crocodiles. These results in the context of physiological comparisons can bring new insight into the functional roles of these proteins and how they vary across taxa.

P2154A Functional divergence of two young duplicate genes in *Drosophila*. *I. M. Ventura*[1,2], M. Long[1]. 1) Ecology and Evolution, University of Chicago, Chicago, IL; 2) CAPES Foundation, Ministry of Education, Brazil.

New duplicate genes can rapidly diverge from their parental copies in sequence and expression, and acquire novel or specialized functions. Understanding their divergence can shed light on the molecular mechanisms leading to evolutionary innovations. Here, we explore the functional evolution of *Zeus* and *Poseidon* (CG2053), two young genes that duplicated from the same parental gene, *Caf40*, in *Drosophila*. *Caf40* encodes a broadly expressed and highly conserved nucleic acid binding protein in eukaryotes, which is part of a regulatory complex that plays a prominent role in many aspects of transcriptional and translational regulation of many genes. Previous works have shown that *Zeus* affects the expression of a different set of genes from those regulated by *Caf40*, and acquired an important role in male fertility. Curiously, we recently found an older *Caf40* duplicate, which we call *Poseidon*, whose evolutionary trajectory resembles *Zeus*': both originated through retrotransposition of *Caf40*, are only expressed in testis and larval imaginal discs, and rapidly diverged in amino acid sequence, exhibiting signals of positive selection. In addition, both duplicates underwent a burst of amino acid replacements shortly after duplication, whereas more recent branches have rates consistent with purifying selection.

Strikingly, we found that many crucial residues that are invariable among Caf40 proteins from distantly related eukaryotes were substituted in Zeus and Poseidon (for instance, respectively 22% and 51% of the totally conserved sites in the Caf40 alignment were replaced in the duplicates), which suggests that fundamental properties of the original protein may have been altered in the young copies. Using RNAi knockdown in *D. melanogaster*, we also found that silencing *Zeus* results in a 30% decrease in egg to adult survival compared to controls. The impact on both viability and fertility suggests that the young gene was coopted in different tissues and developmental contexts. We will further test the hypothesis that the duplicate paralogs enrolled in new cellular processes by recruiting new protein partners. In addition, the independent evolution of duplicates of the same parental gene suggests that some molecular properties, such as the ability of engaging in protein interactions, may increase the probability of young genes being retained and evolving new functions.

P2155B Function of Ssl2 in RNA Polymerase II Transcription Start Site Scanning. *T. Zhao*, C. Kaplan. Texas A&M University, College Station, TX.

Protein-coding genes are transcribed by RNA polymerase II (Pol II) in three sequential stages: initiation, elongation, and termination. During initiation, Pol II cooperates with general transcription factors (GTFs), including TFIIB, TFIID, TFIIE, TFIIF and TFIIH, for DNA binding, promoter melting, transcription start site (TSS) selection, initial RNA synthesis, and the escape of Pol II from the promoter. TSS selection is the step that determines how many and how efficiently TSSs are used, but the selection mechanism is unclear. In *Saccharomyces cerevisiae*, Pol II finds TSSs through a "scanning" process by which TFIIH's translocase activity melts the promoter, and the Pol II machinery scans downstream for usable TSSs. Our studies support promoter scanning at all yeast promoters by showing that Pol II mutants alter TSS distributions universally in the stereotypical, polar fashion predicted by scanning models. The role of TFIIH in promoter scanning steps downstream of promoter melting is less

well understood. We have proposed a "shooting gallery" model for promoter scanning, where Pol II and TFIIH activities work together to determine the distribution of TSSs and promoter output in yeast. We have identified novel alleles of *SSL2* in genetic screens for *ssl2* mutants with putative initiation defects. Our screens have identified a number of *ssl2* alleles separable into distinct classes based on in vivo growth and transcription phenotypes. We propose that some of these alleles alter promoter scanning in ways that are distinct from how changes to the Pol II active site alter promoter scanning. Our goal is to determine how Pol II and Ssl2/TFIIH work concurrently to promote transcription initiation and influence promoter output.

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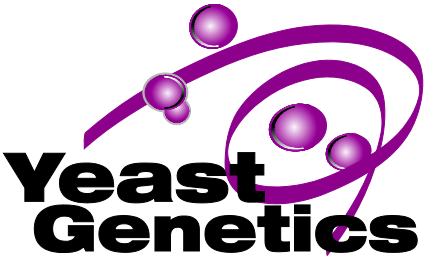

Poster Session Abstracts

Y3001A Elimi-NAD-ing fat in old mother yeast cells. *A. O. Beas*, J. C. Berude, J. J. Hsu, K. L. Zhou. Fred Hutchinson Cancer Research Center, Seattle, WA.

In an effort to identify and understand fundamental mechanisms of aging, we screened for changes that occur in the organelles of replicatively aging "mother" cells of the budding yeast, S. cerevisiae. In this screen we identified key changes, including an age-dependent accumulation of lipid droplets (LDs) as shown by staining of LDs and fluorescent tagging of known LD proteins. Classically, LDs are storage sites for energy in the form of neutral lipids (NLs) and therefore play important roles in energy metabolism and lipid-based signaling. To begin to understand the genetic network that underlies LD accumulation during aging, we carried out semi-targeted gene mutation analysis and screened for delays in the age of onset of LD accumulation. Surprisingly, deletion and/or overexpression (OE) of genes known to affect LDs had minor effects on LD accumulation during aging. In fact, deletion of only one of the four acyltransferases that synthesize triacylglycerol, the major NL of LDs, suppressed LD accumulation during aging. These data suggest that a limited number of biosynthetic pathways underlie LD accumulation during aging. Age-associated LD accumulation was also delayed by deletion of Longevity Assurance Gene 1, which encodes the founding eukaryotic ceramide synthase; interestingly, LAG1 was the first gene ever described that when deleted extends the lifespan of an organism (D'mello et. al. JBC 1994). These data suggest a role for sphingolipids in LD accumulation and correlates increased LDs with reduced lifespan. Furthermore, LD accumulation during aging was delayed by OE of the conserved and rate-limiting enzyme of the pathway for nicotinamide adenine dinucleotide (NAD+) biosynthesis. Because NAD+ levels have been shown to decline during aging in eukaryotes, our data suggests that the age-dependent accumulation of lipid droplets is influenced by the age-dependent decline in the levels of NAD+. The levels of NAD+ are regulated in yeast by processes such as biosynthesis, NAD+ dependent histone deacetylases (i.e., sirtuins), the salvage pathway, and the repertoire of NAD+ dependent metabolic reactions occurring at any given time. Lastly, we investigated whether LD accumulation during aging was sensitive to genetic manipulation of the processes that regulate NAD+ levels and to treatment with vitamin precursors of NAD+. We found that perturbations expected to increase NAD+ levels delayed/reversed age-associated LD accumulation, which suggests that the metabolic circuitry of old cells can be manipulated in ways that alleviate LD accumulation. We are now determining which NAD+ dependent metabolic processes influence NL metabolism with age and how those changes affect energy metabolism and lifespan. We speculate that our studies are relevant to the widespread obesity problem and its impact on the aging process and disease.

Y3002B Fin1-PP1 clears the spindle assembly checkpoint protein Bub1 from the kinetochore in anaphase. *M. Bokros*, C. Gravenmier, F. Jin, D. Richmond, Y. Wang. Florida State University, Tallahassee, FL.

The spindle assembly checkpoint (SAC) ensures faithful chromosome segregation during mitosis by preventing the onset of anaphase when the kinetochore is not attached. The mechanism for activation of the SAC has been well studied yet it remains ambiguous how the SAC is silenced after chromosome bipolar attachment. Recently we identified Bmh1, one of the two 14-3-3 proteins in S. cerevisiae, as a regulator of the SAC silencing. Data from our lab and others indicates that Bmh1 sequesters Fin1 and PP1, a key SAC silencing phosphatase, until early anaphase. We find this prevents premature SAC silencing. In addition, immediately following anaphase entry, the Cdc14 early anaphase release (FEAR) pathway promotes the release of Fin1-PP1 from Bmh1 allowing Fin1-PP1 to localize on the kinetochore. Importantly we find that kinetochore recruitment of Fin1-PP1 promotes the removal of the SAC protein Bub1 from the kinetochore during anaphase, thereby revealing a mechanism for SAC disassembly in anaphase. We are continuing to investigate the molecular mechanism for Fin1-PP1 mediated SAC disassembly and its physiological function during the cell cycle.

Y3003C How TORC controls growth through metabolism. *J. Chen*. UT Southwestern Medical Center at Dallas, Dallas, TX.

Nitrogen permease regulator 2 (Npr2) is a conserved negative regulator of target of rapamycin complex 1 (TORC1), which promotes cell growth and proliferation in nutrient-sufficient conditions. Npr2 forms a complex with Npr3 and Iml1, and can function as a GTPase activating protein (GAP) complex for the small GTPases important for TORC1 function. Npr2 is frequently deleted or mutated in cancers, suggesting it plays a critical role in cell growth control. Interestingly, yeast cells lacking Npr2 (*npr2Δ*) can grow faster or slower than wild type in a manner dependent on the growth medium. We dissected the metabolic changes that occur in *npr2Δ* cells under these different nutrient environments, which has revealed the consequences of deregulated TORC1 signaling in different contexts. These findings have started to help us understand what TORC1 is really doing to cellular metabolism to reciprocally regulate cell growth and autophagy.

Y3004A Cell-Cycle Control of a Pulse-Generating Network Restricts Frequency of Periodic Transcription. *C. Cho*, S. Haase. Duke University, Durham, NC.

Periodic transcription plays an essential role in eukaryotic cell cycle. We have proposed that a transcription factor (TF) network could function autonomously to drive genome-wide periodic transcription during the cell cycle in budding yeast *Saccharomyces cerevisiae*. In support of this model, multiple pulses of cell-cycle transcription persist even when budding yeast cells are arrested by perturbing the oscillation of S-phase and mitotic cyclin-dependent kinase (CDK) activity. However, how the dynamics of the TF network are coupled with the normal cell cycle remains unclear. Here we show that G1 cyclin-CDKs and Cdc14 phosphatase oppositely regulate the pulse-generating and pulse-transmitting capability of the TF network during cell cycle commitment and mitotic exit, respectively. Using an integrated genetic-genomic approach, we established that G1 cyclin-CDKs relieve the inhibition of the TF network by inhibiting transcriptional corepressors Whi5/Stb1 and E3 ubiquitin ligase complex APC-Cdh1. Importantly, genetic perturbation of Whi5/Stb1 and APC-Cdh1 in cells lacking G1 cyclin-CDK activity is necessary and sufficient for the TF network to drive a robust pulse of cell-cycle transcription. On the other hand, timely activation of Cdc14 in late mitosis is necessary for preventing ectopic pulses of cell-cycle transcription, potentially through reactivating Whi5/Stb1 and APC-Cdh1 to restore the

inhibition of the TF network. In conclusion, we propose that frequency control of periodic transcription in budding yeast is an emergent property of the global network with coupled CDKs and the pulse-generating network.

Y3005B The Yeast Genome Project: Exploring APD1. *C. A. Douds*, J. Keeney. Juniata College, Huntingdon, PA.

Yeast is considered to be "man's oldest industrial microorganism" and has been used by humans for over 5,000 years. Today, its use has expanded and *Saccharomyces cerevisiae* has become an ideal eukaryotic model due to its quick growth and easily manipulated genome. After its genome was sequenced in 1996, scientists came together to create a full encyclopedia of systemized gene functions characterized according to the Gene Ontology Consortium (GO). However, nearly twenty years later, the functions of more than 10% of these genes still remain unknown. The ORFan gene project aims to aid the characterization of the *S. cerevisiae* genome by exploring the function of specific open reading frames (ORFs). In order to classify these unknown ORFs, a putative pipeline has been constructed beginning with the identification of an ORFan of interest through the Saccharomyces Genome Database (SGD). This project focuses on YBR151W or *APD1*. The selected ORF is fluorescently tagged at the C and N terminals by PCR and visualized with fluorescent microscopy to verify cellular localization. Then a deletion strain is constructed and SGD reported phenotypes are verified. Verified phenotypes for *Δapd1* cells include increased H_2O_2 sensitivity and abnormal actin patch localization. Next, a comparison between the wild type and target gene knockout strains is analyzed through RNAseq. However, in this study RNAseq data has yet to be obtained. Instead, microarray analysis using SGD's SPELL was conducted to explore hypotheses. Further exploration into the putative function of *APD1* has led to studies involving actin patch localization, as well as the protein's potential role in oxidative stress mechanisms. We have observed the effects of actin patch localization under various forms of oxidative stress such as HU and H_2O_2 between wildtype and *Δapd1* strains.

Y3006C Mmf1p protects Hem1p from damage caused by the ubiquitous metabolic stressor, 2-aminoacrylate. *Dustin C. Ernst*, Diana M. Downs. University of Georgia, Athens, GA.

Members of the RidA/YER057c/UK114 protein family are observed throughout each kingdom of life. Despite being widespread, a biochemical role for these proteins *in vivo* remained elusive until work in *Salmonella enterica* showed RidA prevents free 2-aminoacrylate (2AA) accumulation. Strains lacking RidA experienced 2AA stress, revealed as damage to a variety of metabolic enzymes and decreased fitness. The generation of 2AA in *S. enterica* was dependent on the activity of pyridoxal 5'-phosphate (PLP)-dependent serine/threonine dehydratases (IlvA/TdcB; EC 4.3.1.19). Previous studies of *Saccharomyces cerevisiae* RidA homologs, Mmf1p (mitochondrial) and Hmf1p (cytosolic), showed the lack of Mmf1p resulted in a *petite* growth phenotype. Given the paradigm described for *S. enterica*, we hypothesized that 2AA accumulation, and the resulting damage to a PLP-dependent enzyme, was responsible for the growth phenotypes observed for *S. cerevisiae* Δmmf1 strains. The nuclear encoded serine/threonine dehydratases, Ilv1p (anabolic) and Cha1p (catabolic), are both localized to the mitochondrial matrix. When Ilv1p and Cha1p are simultaneously disrupted, respiratory activity is maintained in a Δmmf1 background. These results showed that diminished respiratory activity in the absence of Mmf1p is a consequence of 2AA formation. The paradigm in *S.* enterica suggested that the target for 2AA stress would be a PLP enzyme. Our data show that the first step in heme production, catalyzed by PLP-dependent aminolevulinic acid (ALA) synthase (Hem1p; EC 2.3.1.37), is inhibited in the absence of Mmf1p under conditions where 2AA is predicted to accumulate. Consistent with previous reports, diminished ALA production in *S. cerevisiae* adversely impacted respiration, perhaps highlighting a role for Mmf1p in maintaining Hem1 activity.

Y3007A Global Analysis of Molecular Fluctuations Associated with Cell Cycle Progression in *Saccharomyces cerevisiae*. *Ben Grys*[1], Helena Friesen[2], Oren Kraus[3], Adrian Verster[2], Brendan J. Frey[2,3], Charles Boone[1,2,4], Brenda J. Andrews[1,2,4]. 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) The Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada; 3) Electrical and Computer Engineering, University of Toronto, Toronto, Ontario, Canada; 4) Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada.

The regulation of protein expression, turnover, and localization has been recognized as imperative for eukaryotic cell cycle progression. However, there has been no systematic study of proteomic fluctuations throughout the cell cycle in eukaryotes. By combining Synthetic Genetic Array (SGA) technology with high-throughput fluorescence microscopy of the ORF-GFP fusion collection, we have generated image-based data for ~75% of the yeast proteome. Our strategy involves scoring diagnostic fluorescent markers that indicate cell cycle position, which permits the computational classification of yeast cells into one of six predetermined cell cycle stages, and subsequently quantifying protein abundance and localization for each member of the GFP collection, We automated cell cycle classification using a supervised neural network-based approach that functions with ~97% accuracy. With mean GFP-pixel intensity as a metric for protein abundance, we determined how the entire visible budding yeast proteome fluctuates over the course of the cell cycle. We have also adapted our neural network classification method for the automated assignment of GFP-fusion proteins to 21 different subcellular compartments. After applying statistical analyses, we have resolved those GFP-fusion proteins that change in abundance and/or localization in a cell cycle-dependent manner. When combined with cell cycle transcriptional information, and ribosome profiling, this unique platform will provide a resource that can be mined to better characterize existing pathways of cell cycle control, while also identifying novel players in the regulation of cell growth and division. On a broader scale, our dataset will allow us to study pre- and post-translational gene regulation in an ordered and highly conserved biological process, providing a unique opportunity that is not possible with existing eukaryotic data.

Y3008B Interplay between Rfa2 N-terminal phosphorylation and Rad53 dephosphorylation in regulating exit from a checkpoint in the presence of persistent DNA damage. *Stuart Haring*[1], Timothy Wilson[1], Jessica Kesson[1], Nolan Miles[1], Trevor Baumgartner[1], Sarah Klein[2],

Sakina Khaku[2], Andre Walther[2]. 1) North Dakota State University, Fargo, ND; 2) Cedar Crest College, Allentown, PA.

Replication Protein A (RPA) is a heterotrimeric, single-stranded DNA binding complex with critical roles in DNA metabolism and cell cycle progression. While human RPA is observed to be hyper-phosphorylated in response to genotoxic stress, primarily on the N-terminus (NT) of the 32 kDa subunit (Rpa2), the Rfa2 NT of yeast Replication Factor A (RFA) had not been identified as a major site of phosphorylation upon DNA damage induction. Despite this, examination of Rfa2 NT phospho-mutants strongly suggested that the phospho-state of the Rfa2 NT plays a key role in cell cycle progression, especially when DNA damage persists. This phenomenon is termed checkpoint adaptation, and although it provides the cell the chance to survive and grow, it comes with the negative consequence of increased genomic instability that can ultimately lead to cellular disease.

Using *Saccharomyces cerevisiae*, it was demonstrated that in the presence of persistent DNA damage, Rfa2 NT hyper-phosphorylation is readily detected, and that the Rfa2 NT is required for checkpoint adaptation to occur. Phosphorylation of the Rfa2 NT is also observed upon prolonged exposure to an array of genotoxic agents. Using checkpoint kinase deletion strains, some kinases responsible for phosphorylation of the Rfa2 NT during genotoxic stress have been identified.

Additionally, a chimeric yeast Rfa2 containing the human Rpa2 NT was examined. This chimeric protein was previously demonstrated to be hyper-phosphorylated in response to an array of DNA damaging agents. The yeast Rfa2 containing the human Rpa2 NT displayed earlier and more robust phosphorylation after stress than cells containing wild-type (WT) Rfa2. Furthermore, this robust phosphorylation of the chimeric Rfa2 promoted dephosphorylation of Rad53 and even earlier and more efficient checkpoint adaptation than in WT Rfa2-containing yeast cells.

Taken together, the data suggest a model where Rad53 deactivation (necessary for checkpoint bypass) and Rfa2 NT phosphorylation are linked to coordinate checkpoint adaptation in yeast. Specifically, Rfa2 NT phosphorylation drives Rad53 dephosphorylation to promote bypass of the checkpoint despite the continued presence of DNA lesions. Understanding how the Rfa2 phospho-state influences cell cycle progression in the presence of genotoxic stress will assist in deciphering the molecular signaling mechanisms cells employ to maintain the integrity of their genomes.

Y3009C The function of Sgo1-centromere recruitment pathway and spindle assembly checkpoint silencing. *F. Jin*, Y. Wang. Florida State University, Tallahassee, FL.

Accurate chromosome segregation during cell division is critical to maintain genomic stability. Kinetochore-microtubule bipolar attachment, in which sister chromatids attach to microtubules emanating from opposite spindle poles, ensures chromosome segregation fidelity. Improper kinetochore microtubule attachments activate the spindle assembly checkpoint (SAC) to prevent anaphase onset. Following kinetochore bi-orientation, the SAC is silenced allowing for anaphase onset. The aurora kinase Ipl1, and a centromere-associated protein Sgo1, sense bi-orientation defects and delay anaphase entry. Previous works from our lab and others have found that Ipl1 and Sgo1 in *Saccharomyces cerevisiae* prevent SAC silencing in the presence of tension defects. How Sgo1 prevents SAC silencing is still unknown. Bub1 kinase facilitates Sgo1-centromere recruitment through phosphorylates H2A and Sgo1 further recruits Rts1. In this study, we find *bub1* kinase dead (*bub1-KD*), phosphodeficient H2A (*H2A-121A*), and *RTS1* deletion mutants all exhibit premature SAC silencing. In the presence of syntelic chromosome attachments, these mutants enter anaphase prematurely and result in high levels of chromosome missegregation. Moreover, these mutants suppress the anaphase entry delay of *dam1* phospho-mimicking mutant. Therefore, the centromere recruitment of Sgo1 plays a major role in preventing SAC silencing.

Y3010A Regulation of lifespan by vitamin B_6 metabolism-related genes in yeast. *Y. Kamei*, Y. Mukai. Nagahama Institute of Bio-Science and Technology, Nagahama, Shiga, JP.

Lifespan is thought to be largely determined by the combined effects of genetics and environmental factors including stress and nutrients. The effects on individual lifespan by a large amount of nutrients, such as carbohydrates and amino acids, and a limited amount of nutrients, such as vitamins, are well-studied. However, molecular mechanism of cellular lifespan determination by vitamins is not elucidated. Here, we show that pyridoxal 5'-phosphate (PLP), a biologically active form of vitamin B_6 (VB6) consisting of pyridoxine, pyridoxal, and pyridoxamine, is a key factor to regulate cellular lifespan of budding yeast. Through microarray analysis of yeast aging cells, we found that the expression of *SNZ1* gene encoding PLP synthase was increased in senescent cells. Yeast cells synthesize PLP via the salvage pathway as well as *de novo*, and import extracellular VB6 by a plasma membrane VB6 transporter Tpn1p. Deletion of *SNZ1* and *TPN1* shortened yeast replicative lifespan, which is defined by the number of daughter cells that a mother cell can generate before dying. Simultaneous deletion of *SNZ1* and *TPN1* severely shortened the lifespan. Excess supplementation of pyridoxine to medium restored the lifespan of the Δ*snz1* and Δ*tpn1* mutants to wild-type level. *SNZ1* overexpression in the Δ*tpn1* cells and *TPN1* overexpression in the Δ*snz1* cells prolonged the lifespan to normal. These data strongly suggested that VB6 regulates yeast replicative lifespan. To confirm this, we measured the intracellular content of VB6 by a microbiological assay and that of PLP by an enzymatic assay in the wild-type and mutant strains. The contents of VB6 and PLP in Δ*tpn1* mutant were decreased. Pyridoxine supplementation to Δ*tpn1* mutant culture recovered the VB6 and PLP contents to wild-type level. Overexpression of *SNZ1* in the Δ*tpn1* cells did not change the VB6 content but elevated the PLP content. These results reveal that the short lifespan of Δ*tpn1* cells is caused by reducing the PLP content. Unexpectedly, the VB6 and PLP contents of Δ*snz1* mutant were comparable to that of wild-type strain. We found that the VB6 and PLP contents were increased in senescent cells of both wild-type and Δ*snz1* mutant strains, suggesting that the elevated PLP level in old cells is due to increasing VB6 uptake by Tpn1p but not due to increasing de novo PLP synthesis by Snz1p. We propose that PLP is required for maintenance of cellular lifespan against aging.

Y3011B The role of protein acetylation in stress defense. *Jeffrey Lewis*[1], Rebecca Sides[1], Aaron Storey[2], Alan Tackett[2]. 1) University of Arkansas, Fayetteville, AR; 2) University of Arkansas for Medical Sciences, Little Rock, AR.

Cells face a staggering array of environmental challenges during their lifetimes. Not surprisingly, cells have evolved sophisticated rapid-response mechanisms to deal with acute environmental changes. Our focus has been on post-translational lysine acetylation, which was originally discovered as a histone modification over fifty years ago. Recent proteomic studies have identified thousands of acetylated proteins in diverse organisms, rivaling phosphorylation in terms of number of targets. While the sheer size of the "acetylome" points to its importance, we have little idea how acetylation affects protein function for the vast majority of targets. The striking enrichment of stress defense proteins as acetylation targets suggested an underappreciated role for protein acetylation in stress defense. We now provide evidence that protein acetylation plays a key role in stress defense. First, we have identified a post-translational mechanism for thermotolerance that requires properly coordinated protein acetylation. Second, through quantitative proteomics we observe extensive acetylome remodeling during acute heat stress. Intriguingly, we find increased acetylation of choline kinase (Cki1p) during heat shock, which in turn regulates Cki1p activity. Because Cki1p plays a known role in cell membrane turnover, this provides an exciting new link between protein acetylation and cell membrane metabolism during heat stress.

Y3012C A genome-wide screening identifies novel filament-forming metabolic enzymes in *Saccharomyces cerevisiae*. *J. L. Liu*[1], Q. J. Shen[1], H. Kassim[1], Y. Huang[1,2], H. Li[1,3]. 1) Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3PT, United Kingdom; 2) Southwest University, Chongqing 400715, China; 3) Institute of Physics, Chinese Academy of Sciences, Beijing 100190, China.

Compartmentation via filamentation has recently emerged as a novel mechanism for the regulation of metabolism. In order to identify filament-forming metabolic enzymes systematically, we performed a genome-wide screening of all strains available from an open reading frame-GFP collection in *Saccharomyces cerevisiae*. We discovered 9 novel filament-forming proteins and also confirmed those identified previously. Out of the 4,159 strains, we found 23 proteins, mostly metabolic enzymes, that are capable of forming filaments in vivo. In silico protein-protein interaction analysis suggests that these filament-forming proteins can be clustered into several groups, including translational initiation machinery, glucose and nitrogen metabolic pathways. Using glutamine-utilising enzymes as examples, we found that the culture conditions affect the occurrence and length of the metabolic filaments. Furthermore, we found that two CTP synthases (Ura7p and Ura8p) and two asparagine synthetases (Asn1p and Asn2p) form filaments both in the cytoplasm and in the nucleus. Live imaging analyses suggest that metabolic filaments undergo sub-diffusion. Taken together, our results identify novel filament-forming proteins in *Saccharomyces cerevisiae* and suggest that filamentation of metabolic enzymes is more general than currently appreciated.

Y3013A TOR pathway mediates cytoophidium assembly in *Schizsaccharomyces pombe*. *J. L. Liu*, L. Hulme, K. Wesley. Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3PT, United Kingdom.

The essential metabolic enzyme CTP synthase catalyses CTP formation and has been demonstrated to form large, evolutionarily conserved intracellular filaments, termed cytoophidia (meaning cellular serpents in Greek). The exact functional role of cytoophidia, or how formation and maintenance is regulated remains unanswered. By monitoring cytoophidium formation in a GFP tagged CTP synthase background in *Schizsaccharomyces pombe,* we have discovered that the TOR (target of rapamycin) kinase pathway regulates cytoophidium formation. The TOR pathway is highly conserved and is essential for regulation of cell growth and response to nutrient deprivation. We show that the TOR signalling cascade is involved in cytoophidium assembly. Moreover, this relationship is specific to the TORC2 complex. This study connects cytoophidium assembly and the TORC2 complex which is potentially relevant considering the increasing evidence implicating these two major pathways in cancer development.

Y3014B Candida albicans Pho84 is required for anabolic TOR signaling, stress responses and virulence determinants. *Ning-Ning Liu*[1*], Priya Uppuluri[2], Kicki Ryman[3], Ylva Engström[3], Per Olof Ljungdahl[3], Julia R. Köhler[1]. 1) Boston Children's Hospital/Harvard Medical School, Boston, MA; 2) UCLA David Geffen School of Medicine, Torrance, CA; 3) The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden.

Systems of phosphate homeostasis are required for virulence of bacterial pathogens. In a transposon mutant screen for new elements of *Candida albicans* Target of Rapamycin (TOR) signaling, we found a homolog of the major phosphate plasma membrane transporter in *S. cerevisiae*, Pho84, to be required for normal TOR activity. The link between Pho84 and TOR signaling in *C. albicans* does not seem to consist in pH homeostatic mechanisms, but appears to go through the EGO complex component Gtr1. Mutants in *PHO84* are hypersemsitive to cell wall stress and oxidative stress, and fail to eliminate reactive oxygen species. Their hyphal morphogenesis is misregulated, and they are defective in biofilm formation. They are unable to damage host cells in ex vivo models, and are killed more efficiently by neutrophils. In a wild type Drosophila model, they are nearly avirulent. The macronutrient phosphate is required for DNA replication, ribosome biogenesis, expansion of membranes and key metabolic processes like glycolysis and oxidative phosphorylation. We found its acquisition to be connected to critical functions in *C. albicans* pathogenesis.

Y3015C Response of quiescent cells to exogenous DNA damage. *L. J. Long*[1], J. S. Welty[1,2], M. A. Osley[1]. 1) University of New Mexico School of Medicine, Albuquerque, NM; 2) University of Pittsburgh, Pittsburgh, PA.

Under certain conditions, such as nutrient deprivation, cells exit the cell cycle and enter G0, a state of quiescence. Quiescent cells are viable but non-growing and can re-enter the cell cycle when appropriate stimuli are provided. Quiescence is an important feature of adult stem cells, where it provides a pool of cells for self-renewal or differentiation into appropriate cell lineages. Quiescent cells must respond to exogenous stresses such as DNA damage so that deleterious mutations are not transmitted to progeny upon differentiation. Our data show that quiescent

cells are more sensitive to UV irradiation when compared to growing cells. We find that the majority of UV lesions are removed from quiescent cells before these cells re-enter the cell cycle. Moreover, we observe that the frequency of UV-induced mutations is significantly higher in quiescent cells than in G1 arrested cells. We are currently using genetic, molecular, and genomic approaches to investigate the hypothesis that UV induced lesions are directly repaired in G0 cells through the gap-filling activity of mutagenic translesion DNA polymerases.

Y3016A Regulation of lifespan by phosphate starvation response factors in budding yeast. *Y. Mukai*, T. Maruhashi, S. Jiang, Y. Kamei. Nagahama Institute of Bio-Science and Technology, Nagahama, Shiga, JP.

Lifespan of organisms is determined by genetic factors and environmental factors, such as stress and nutrients. Calorie restriction is well studied to extend organismal and cellular lifespan in a variety of eukaryotes from yeast to mammals. Phosphate, an essential nutrient for all organisms, has been reported to be involved in lifespan regulation: the mutant mice lacking *klotho* exhibits hyperphosphatemia to shorten lifespan. However, molecular mechanism of lifespan regulation by phosphate is not known. Here, we show that phosphate starvation response factors regulate replicative lifespan in budding yeast. Yeast replicative lifespan, the number of daughter cells produced by a mother cell, is usually measured on YPD medium, which contains high amounts of phosphate. To know the effect of extracellular phosphate on yeast lifespan, we measured replicative lifespan on synthetic high- and low-phosphate medium. Phosphate restriction did not extend the lifespan of wild-type strain. Deletion of *PHO80* and *PHO85*, which encode cyclin and cyclin-dependent kinase in phosphate starvation response pathway, respectively, shortened the lifespan under both high and low phosphate conditions. Pho80p-Pho85p phosphorylates and exports transcription factor Pho4p from the nucleus under high phosphate condition, resulting in no expression of phosphate starvation response genes. Simultaneous deletion of *PHO80* and *PHO4* restored the lifespan to wild-type level, indicating highly expression of the Pho4p target genes by deletion of *PHO80* causes short lifespan. We screened for Pho4p target gene mutations that recovered the lifespan of the Δ*pho80* cells, and found such mutations in *VTC1*, *VTC2*, and *VTC4* genes. The *VTC* genes are transcriptionally regulated by Pho4p and are required for vacuolar polyphosphate accumulation. Deletion of *PPN1*, which encodes vacuolar polyphosphate phosphatase, also shortened the lifespan as did that of *PHO80*. *In vivo* ^{31}P-nuclear magnetic resonance analysis showed that polyphosphate was accumulated in Δ*pho80* and Δ*ppn1* mutants compared with the wild type, but not in Δ*pho80* Δ*pho4* mutant, indicating that accumulation of polyphosphate shortens yeast replicative lifespan.

Y3017B Natural variation in the cell adhesin, FLO11, and its effects on biofilm formation. *H. A. Murphy*, B. A. Lenhart, B. K. Meeks. William and Mary, Williamsburg, VA.

A biofilm is a type of cooperative microbial community that is found wherever microorganisms are found— in aquatic and terrestrial systems, in living and dead tissues, on medical devices and dental surfaces. These communities are anchored to a surface and enmeshed in a protective extracellular matrix, properties which lead to increased resistance to antimicrobials and environmental stresses. Recent work has demonstrated that some natural isolates of *Saccharomyces cerevisiae* are capable of forming complex, structured biofilm-like colonies, a phenotype known as complex colony morphology (CCM). The flocculin, Flo11p, is required for CCM and is involved in cell-cell and cell-surface adhesion. In order to investigate the role of FLO11 in natural cellular interactions, the gene and its 2kb regulatory region were sequenced in 55 isolates from various geographic and ecological niches using PacBio technology. These data exhibited a large amount of genetic variation, suggesting that numerous alleles are segregating in the global population and that they may affect inter-cellular interactions. Allele swaps were performed among a subset of the strains to determine the effect of the alleles on biofilm formation and showed a detectable effect of allele status. Finally, mixed-strain colonies were assayed to determine whether the different FLO11 alleles changed the interactions between the individual cells in the biofilm.

Y3018C *GPH1* over-expression rescues glycogen and calcium accumulation defects in a *pgm2Δ* mutant strain of *Saccharomyces cerevisiae*. *K. Ngo*, A. Charales, R. McDonald, V. Bahram, A. Selvamani, D. Aiello. Austin College, Sherman, TX.

Phosphoglucomutase (PGM) plays an important role in yeast carbohydrate metabolism. It is responsible for interconverting glucose-1-phosphate (G1P) and glucose-6-phosphate (G6P). Pgm2 is the major isoform of PGM in *Saccharomyces cerevisiae.* Yeast that lack *PGM2* exhibit slow growth, sensitivity to cyclosporine A, high glycogen accumulation, and calcium homeostasis defects when metabolizing galactose. The purpose of this investigation is to determine whether or not hyperaccumulation of glycogen contributes to other phenotypes observed in the *pgm2Δ* mutants. The high glycogen accumulation can be rescued using two different approaches: decreasing glycogen synthesis or increasing glycogen breakdown. To decrease glycogen synthesis, knockouts of the two known glycogen synthase enzymes, *gsy1Δ* and *gsy2Δ*, were constructed in both wt and *pgm2Δ* strains, alone and in combination. *GPH1*, encoding glycogen phosphorylase, was over-expressed to enhance glycogen breakdown in the context of the wt and *pgm2Δ* strains alone, and in combination with the *gsy1Δ* and *gsy2Δ* mutants. Gph1 is responsible for catalyzing the breakdown of glycogen to G1P. The growth, glycogen accumulation, and calcium accumulation phenotypes of each strain were analyzed. Glycogen accumulation was monitored by both iodine vapor staining and by quantitative enzymatic assay. Loss of either isoform of glycogen synthase (*gsy1Δ* or *gsy2Δ*) failed to rescue *pgm2Δ* defects. However, we report here that *GPH1* over-expression partially rescues the *pgm2Δ* cyclosporine A growth defect when metabolizing galactose as a carbon source. Additionally, *GPH1* over-expression rescues the high calcium and glycogen accumulation defects observed in *pgm2Δ* mutants.

Y3019A Strategies for metabolic engineering and optimization of *S. cerevisiae* into microbiofactories for the production of terpenes. *P. Prochasson*, T. Johnson, B. Wenndt, A. Kirkland, J. Whitaker, K. Thomas, D. Saran, S. Park, B. Julien. Evolva, Lexington, KY.

S. cerevisiae has been used for millenniums to make wine, beers and bread. The rise and development of Synthetic Biology has turned many micro-organisms, especially *S. cerevisiae*, into microbiofactories allowing the production of a wide range of molecules from other organisms in

a sustainable way using fermentation.

Evolva produces many important high value molecules from the terpene family (valencene, nootkatone, and rebD and rebM [components of the artificial sweetener Stevia]) which all use the same metabolic pathway to produce the isoprene precursor farnesyl pyrophosphate (FPP) used by terpene synthases.

We present an approach of combinatorial genome integrations of the mevalonate pathway genes into *S. cerevisiae* to maximize carbon flux towards terpene production. The levels of key metabolites are monitored during fermentation as an indicator of desired changes in the metabolic flux towards FPP. Flux imbalance can negatively affect terpene production, particularly when scaled-up. Hence, we applied a stepwise engineering method and took advantage of the push and pull strategy to minimize the accumulation of inhibitory concentrations of metabolites and maximize carbon flux to product formation.

We will also discuss strategies for biological formation of nootkatone from valencene. Nootkatone is not only important as a flavor and fragrance molecule but it also has potential applications in insect control. The CDC has shown that nootkatone both repels and kills ticks and mosquitos, making it a promising agent to control the spread of Lyme disease carried by the black-legged tick, *Ixodes scapularis*, and of the Zika virus carried mainly by the yellow fever mosquito, *Aedes aegypti*.

Y3020B NASA's BioSentinel mission: using the power of yeast genetics in deep space. *S. R. Santa Maria*, D. Marina, S. Bhattacharya. NASA Ames Research Center, Moffett Field, CA.

Ionizing radiation presents a major challenge to human exploration and long-term residence in space. The deep-space radiation spectrum includes energetic particles that can generate a series of DNA lesions, including DNA double strand breaks (DSBs). DSBs represent a major disruption in the integrity of the genome, which can lead to cell death or the onset of carcinogenesis. DSBs can be repaired without errors via homologous recombination (HR), a conserved pathway in all eukaryotes from yeast to humans. While progress identifying and characterizing biological radiation effects using Earth-based facilities has been significant, no source duplicates the unique space radiation environment.

We are currently developing a biosensor-based nanosatellite to fly aboard NASA's Exploration Mission 1 (EM-1) in 2018. Our biosensor uses *S. cerevisiae* to measure DSBs in response to ambient space radiation. The BioSentinel payload will contain a series of yeast strains, including: (1) a wild type strain as a control for yeast health and unrepairable DNA damage, (2) DNA repair defective strains that cannot repair DSBs and serve as radiation sensitive controls, and (3) a DSB BioSensor strain that can only grow in the presence of DSBs via heteroallelic HR repair. The BioSensor strain contains genetic defects that prevent growth until and unless a radiation-induced DSB near a reporter gene activates the yeast's HR repair mechanisms. Thus, cell growth indicates a successful DSB-and-repair event. In BioSentinel, desiccated cells will be carried within microfluidic cards, and each card will be activated by medium addition at different time points over 18 months. Cell growth and metabolic activity will be tracked continuously via optical density. One reserve set will be activated only if a solar particle event (SPE) occurs during the mission. Biological measurements will be compared to data provided by onboard dosimeters and to Earth-based experiments.

BioSentinel will conduct the first study of biological response to space radiation outside Low Earth Orbit in over 40 years. BioSentinel will thus address strategic knowledge gaps related to the biological effects of space radiation and will provide an adaptable platform to perform human-relevant measurements in multiple space environments, including the International Space Station (ISS), on and around other planetary bodies, and other exploration platforms.

Y3021C Dissecting the role of the transcription factor Hap1 in *Saccharomyces cerevisiae* respiration and fitness. *K. C. Serdynski*, D. Boulton, C. Ramirez, S. Renna, M. J. Hickman. Rowan University, Glassboro, NJ.

Oxygen is crucial for the survival of many organisms, as it functions as the final electron acceptor in aerobic respiration and as a substrate in essential biosynthetic reactions. Several cell types experience hypoxia, or reduced oxygen levels, including human cells within tumors and tissues during cardiovascular disease. Cells of the yeast *Saccharomyces cerevisiae* are similar to human cells in that they adapt to hypoxia by mounting a large gene expression response. One of the mediators of this response is the heme-dependent transcription factor Hap1, functionally analogous to human Rev-Erb. Hap1 activity is regulated directly by binding the biomolecule heme, which converts Hap1 from a repressor to an activator of genes involved in aerobic respiration and other cellular processes. Heme biosynthesis requires oxygen, so the level of cellular heme is dependent upon oxygen level. Despite Hap1 playing a critical role in regulating cellular respiration, *hap1Δ* cells do not have an obvious phenotype during aerobic or hypoxic conditions. Thus, we took several approaches to test how Hap1 contributes to cellular function and fitness. First, we found that deleting the *HAP1* gene had no effect on replicative lifespan, showing that dysregulation of respiration does not affect aging. Second, we found through competitive growth assays that a strain without *HAP1* exhibited a lower fitness per generation compared to a wild type strain. This lower fitness was observed on both fermentable and non-fermentable carbon sources, demonstrating that Hap1 is required for optimal cellular fitness by promoting aerobic respiration. Third, we wanted to test whether Hap1 is important in fermentation as well, so we constructed a strain unable to undergo aerobic respiration. In this strain, deleting *HAP1* does not affect growth on glucose, indicating that Hap1 does not play a role in fermentative respiration. Together, our results suggest that while Hap1 is not absolutely required for respiration, Hap1 provides a fitness advantage by fine-tuning aerobic respiration.

Y3022A Nutrient starvation induces upregulation of α-mannosidase Ams1 in *Saccharomyces cerevisiae*. *M. Umekawa*[1], M. Ujihara[1], K. Makishima[1], S. Yamamoto[1], H. Takematsu[2], M. Wakayama[1]. 1) Ritsumeikan University, Kusatsu, JP; 2) Kyoto University, Kyoto, JP.

Cells have evolved the mechanisms to survive nutritional shortages in the environment. In *Saccharomyces cerevisiae*, α-mannosidase Ams1 is known to play a role in catabolism of *N*-linked free oligosaccharides in the cytosol. Although, this enzyme is also known to be transported selectively from the cytosol to the vacuoles by autophagy, the physiological significance of this transport has not been clarified.

To elucidate the regulatory mechanism of the activity of Ams1, we assessed the enzymatic activity of the cell free extract of the wild-type and various gene disruptants under different nutritional conditions. In addition, the regulation of Ams1 at both transcription and post-translation was examined. The activity of Ams1 was significantly increased upon the depletion of glucose in the medium. Interestingly, the activity of the enzyme was also stimulated by nitrogen starvation. Our data showed that the activity of Ams1 is regulated by the stress responsive transcriptional factors Msn2/4 through the protein kinase A and the target of rapamycin complex 1 pathways. In addition, Ams1 is post-translationally activated by Pep4-dependent processing in the vacuoles.

Y3023B Differential Acetylation of Protein N-Termini in Response to Nutrient Starvation. *S. Varland*[1], P. Van Damme[2,3], J. Hollebeke[2,3], F. Kryuchkov[1], H. Aksnes[1], K. Gevaert[2,3], T. Arnesen[1,4]. 1) Department of Molecular Biology, University of Bergen, Bergen, Norway; 2) Department of Medical Protein Research, Vlaams Instituut voor Biotechnologie, Ghent, Belgium; 3) Department of Biochemistry, Ghent University, Ghent, Belgium; 4) Department of Surgery, Haukeland University Hospital, Bergen, Norway.

N-terminal acetylation (Nt-acetylation) is a ubiquitous protein modification involving the transfer of an acetyl moiety from acetyl-CoA to the α-amino group of a nascent polypeptide. The modification is presumed irreversible and is catalyzed by a group of enzymes called N-terminal acetyltransferases (NATs). The NATs have defined substrate specificities, which are largely determined by the N-terminal sequence identity. The acetylation event can be partial or complete and occurs primarily cotranslational. The biological role of Nt-acetylation remains an enigma. Increasing evidence suggest that Nt-acetylation has diverse molecular functions depending upon the protein being targeted, such as protein degradation, protein folding, protein-protein interaction, and membrane targeting.

Another major type of protein acetylation is the more commonly known lysine acetylation. Unlike Nt-acetylation, lysine acetylation is a reversible process, due to the action of lysine deacetylases. An additional level of regulation is provided by the sensitivity towards the availability of acetyl-CoA. This is particularly the case for histone lysine acetylation where energy availability, and thus acetyl-CoA levels, is linked to altered expression of genes involved in metabolism.

Although the intricate linkage between lysine acetylation and metabolism has been highlighted by several studies, the effect of cellular energy status on Nt-acetylation remains unclear. To address this, we have used COFRADIC proteomics to perform a global analysis of Nt-acetylation in response to nutrient starvation. Our study shows that Nt-acetylation is largely unaffected by alterations in cellular metabolism. However, a minor group of cytosolic proteins are differentially acetylated at their N-termini. The majority of affected neo-N-termini presented the same acetylation trend as histones, becoming less acetylated at stationary phase. To our surprise, a group of affected mature N-termini were more acetylated in stationary phase as compared to active phase. Our results indicate that specific Nt-acetylation events are subjected to metabolic-dependent regulation. This is the first study concerning regulation of Nt-acetylation from a nutrient perspective, and thus provides an important insight to metabolic regulation of protein acetylation as an entirety.

Y3024C Identification of Unforeseen Functions of Ataxin-2, a Conserved Protein Linked to Neurodegenerative Disease. *Y. S. Yang*, X. Wu, B. P. Tu. UT Southwestern Medical Center, Dallas, TX.

Poly-glutamine expansions in ataxin-2 have been linked to neurodegenerative diseases, but the disease-driving mechanism remains elusive. Unexpectedly, we identified the yeast ortholog of ataxin-2, Pbp1p, in a screen for novel regulators of autophagy. Autophagy is a process that degrades cytoplasmic contents and damaged organelles to maintain cell viability during nutrient starvation, and is thought to be an effective mechanism for degrading aggregation-prone proteins linked to neurodegenerative diseases. Our work aims to understand how Pbp1p functions to induce autophagy in response to the metabolic status of cells. In efforts to understand the regulation mechanism, we have focused on its localization properties under different nutrient conditions and its effects on cellular metabolism. The potential function of ataxin-2/Pbp1p in autophagy hints that alterations in this function could play a key role in neurological pathogenesis.

Y3025A Phospholipid methylation regulates sulfur homeostasis in coordination with maintenance of the epigenome. *C. Ye*, B. M. Sutter, Z. Kuang, B. P. Tu. The University of Texas Southwestern Medical Center, Dallas, TX.

Phospholipids are the major component of cell membranes that compartmentalize metabolism and a variety of other processes. Not surprisingly, the regulation of the synthesis of phospholipids necessitates coordination with metabolic status. However, how cells might utilize membrane lipids in cellular and metabolic homeostasis is poorly understood. Among thousands of metabolic reactions, the transmethylation reaction is one of the most important and universal. The reaction transfers a methyl group from the donor S-adenosylmethionine (SAM) to a variety of cellular substrates, including proteins, lipids, nucleic acids, and cellular metabolites. These SAM-dependent methyltransferases engage in nearly every aspect of biology, thereby underscoring the importance of SAM homeostasis. I recently found in the yeast *Saccharomyces cerevisiae* that the methylation of particular phospholipid species is the major cellular consumer of SAM, and dysregulation of phospholipid methylation has remarkable consequences on other methylation events ongoing in the nucleus. Using a combination of approaches in genetics, metabolomics, and biochemistry, I addressed an underappreciated link between phospholipid metabolism and the maintenance of the epigenome. I propose a cellular strategy through which cells integrate epigenetic regulation with metabolic information from membrane lipids.

Y3026B Production of volatile aroma compounds by yeast during fermentation of Chinese Baijiu. *C. Zhang*[1,3], W. Li[2,3], J. Wang[2,3], Y. Chen[1,3], D. Xiao[1,3]. 1) Key Laboratory of Industrial Fermentation Microbiology, Ministry of Education, Tianjin University of Science and Technology, Tianjin, CN; 2) Tianjin Industrial Microbiology Key Laboratory, Tianjin University of Science and Technology, Tianjin, CN; 3) College of Biotechnology, Tianjin University of Science and Technology, Tianjin, CN.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

Appropriate levels of volatile aroma compounds are the most important factors influencing the quality of Chinese Baijiu, an alcoholic beverage from China, and the perception of Chinese Baijiu flavor and aroma is the result of a large amount of interactions between a great deal of volatile aroma compounds and sensory receptors. During fermentation of Chinese Baijiu, most of the volatile aroma compounds are owing to the metabolic activity of microbial community. Yeast, one of the most important microorganisms, plays an important role in formation of higher alcohols, esters and many other aroma compounds. Meanwhile, many environmental factors greatly affect the production of volatile aroma compounds, of which yeast-derived aroma compounds are affected by factors acting on the expression of relevant yeast genes. The known function and regulation of several individual genes are involved in volatile aroma metabolism of *Saccharomyces cerevisiae*. *ATF1* and *ATF2* genes code for alcohol O-acetyltransferases, key enzymes for the synthesis of acetate esters from higher alcohols. *EHT1*- and *EEB1*-encoded esterases are required for synthesis of the medium-chain volatile flavor esters. Isoamyl acetate is degraded into a higher alcohol by the catalysis of *IAH1*-encoded esterase. *BAT2* gene codes for a cytosolic branched-chain amino acid aminotransferase which plays an important role in the synthesis of branched-chain alcohols. *LEU1*-, *LEU2*-, and *ILV1*-encoded enzymes affect the production of higher alcohols in Harris pathway. Different environmental factors effect on the expression of these gene to have a large impact of volatile aroma production. Furthermore, in fermentation process of Chinese Baijiu, material, composition of distillers' grains, temperature and oxygen have great influences on the quality of base liquor.

Y3027C Newly made prion particles must overcome actin-based spatial quality control mechanisms. *A. L. Manogaran*, D. Lyke, J. Dorweiler, E. Legan, B. Wisniewski. Marquette University, Milwaukee, WI.

Prions are self-perpetuating misfolded proteins that can be passed during cell division. *De novo* formation of yeast prions naturally occurs at an extremely low frequency. Yet, increasing the level of the prion protein can induce higher prion frequencies, presumably because the abundance of protein molecules makes it more likely that a small percentage of proteins misfold and form prions. Using the prion [*PSI*$^{+}$], which is the misfolded version of the Sup35p translation termination protein, prion formation can be monitored by transiently over expressing the prion domain of Sup35p fused to GFP. A notable early hallmark of prion induction is the formation of cytoplasmic fluorescent rings. These ring-like structures are retained in the mother cell, and prion induction is dependent upon inheritance of prion particles by the daughter cells. Our previous work identified several genes that reduced prion induction. These genes fell into two groups: the first affects ring formation, and the second appears to be involved in later events after the ring has formed. Since most of the genes identified encoded proteins associated with the actin cytoskeleton, we further investigated the role of actin in prion formation. We found that low levels of the actin cable-disrupting drug, Latrunculin A, had no effect on ring formation, but increased prion induction frequency. Similar effects were identified in an actin mutant with documented cable defects, *act1-101*, suggesting that actin cables are important for later stages of prion formation. It was previously shown that altered actin networks in strains lacking *SIR2* reduce the asymmetrical retention of damaged proteins to the mother cell. To explore whether the actin effects on prion induction are due to spatial quality control, we induced prion formation in *sir2D* strains. We found that ring formation was again unaffected in *sir2D* strains, but prion induction frequency was significantly increased. Taken together, our data suggests that later prion events are dependent upon the ability to overcome spatial quality control mechanisms.

Y3028A Duplication of the budding yeast spindle pole body. *D. Rüthnick*, A. Neuner, E. Schiebel. Zentrum für Molekulare Biologie Heidelberg (ZMBH), DKFZ-ZMBH Allianz, Heidelberg, DE.

The functional equivalent of the centrosome in budding yeast is the spindle pole body (SPB). It is the principle microtubule-organizing center. For centrosomes and SPBs it is crucial that they, just like DNA, duplicate exactly once per cell cycle. During this process the parental structure serves as a nucleation platform for the assembly of the new complex. A major difference in duplication of SPBs and centrosomes arises from the fact that budding yeast undergoes a closed mitosis without nuclear envelope (NE) breakdown. Therefore the new SPB has to be embedded into the NE to fulfill its microtubule organizing functions. The process of SPB duplication starts in anaphase with the elongation of the half bridge into a full bridge. In a next step, the satellite, a miniature version of the SPB, develops at the distal end of this bridge structure on the cytoplasmic side of the NE. After the Start point of the cell cycle the satellite grows into the duplication plaque, which simultaneously becomes inserted into the NE. In the a final step, after the assembly of the nuclear side of the SPB components, the bridge is severed in its center and the two SPBs become the poles of the mitotic spindle.

Our lab is particularly interested in the two major characteristics of the SPB cycle: restricting SPB duplication to one event per cell cycle and insertion of the duplication plaque into the NE. In order to get new insights into these topics we uncoupled duplication plaque formation from NE insertion. We then ask whether the overly large duplication plaque subsequently can be inserted into the NE. For this, we applied standard fluorescence microscopy, super-resolution microscopy (PALM, dSTORM, SIM) and electron microscopy next to a variety of molecular techniques. Our analysis identified novel properties of SPB components and showed that structures as big as 1 µm can be inserted into the NE post-assembly.

Y3029B A new experimental system to study meiotic non-allelic homologous recombination in yeast. *H. N. Conover*, J. L. Argueso. Colorado State University, Fort Collins, CO.

Nearly half of the genetic differences between individuals are rooted not in their nucleotide DNA sequences, but instead come from changes in chromosome structure known as copy number variations (CNVs). Such structural variation causes genes within certain genomic regions to deviate from the “normal” two copies found in a standard diploid cell. *De novo* gene copy number variations (CNV) are now recognized as a significant source for a wide range of human diseases, including autism spectrum disorder. However, the cellular mechanisms and

environmental factors that contribute to copy number mutagenesis still are poorly understood. In the human germline, meiotic recurrent CNVs form through non-allelic homologous recombination (NAHR) between low copy repeat elements (LCRs). Such large repeated sequences are found at CNV hotspot regions of our genome, but are absent in *Saccharomyces cerevisiae*. To study LCR-mediated CNV pathways in this tractable model system, we modified a yeast chromosome through the introduction of engineered LCRs: 10 to 40 Kb segments of yeast DNA that we duplicated to simulate the NAHR substrates that exist in humans. The engineered LCRs flank allelic insertions of genes encoding resistance to geneticin and hygromycin (KanMX and HphMX, respectively) and the *SFA1-CUP1* cassette that confers dose dependent resistance to copper and formaldehyde (CuFA). The segregation of these markers in the haploid cell progeny within tetrads is used to identity and classify CNV events. Normal allelic recombination produces cells that express one of each drug resistance marker (never both together), and intermediate resistance to CuFA. In contrast, interhomolog NAHR between the LCRs produces tetrads with a pair of haploid cells expressing (i) double drug resistance and hyper-resistance to CuFA, and (ii) double drug sensitivity and hypersensitivity to CuFA. These spores carry, respectively, recombinant chromosomes with reciprocal segmental duplication and deletion. Tetrads that contain an intersister NAHR event have a pair of spores with (iii) single drug resistance and hyper-resistance to CuFA, and another (iv) sensitive to both drugs and hypersensitive to CuFA. Finally, intrachromatid NAHR between the LCRs produces tetrads in which only one spore has a deletion of the markers, without an associated duplication. This system allows us to not only measure the overall frequency of *de novo* meiotic CNV, but also to determine the relative occurrence of each of the NAHR classes. We are using this approach to investigate meiotic CNV mechanisms and to interrogate the CNV stimulation activity of a diverse panel of candidate meiotic genes and environmental copy number mutagens. The experimental system will be described in the poster, with our initial analyses of the role of LCR size and distance from each other on the formation of CNVs.

Y3030C Program number not assigned

Y3031A Coordination of meiotic cytokinesis by the GCKIII kinase, Sps1. *L. S. Huang*[1], S. M. Paulissen[1], C. J. Slubwoski[1,2], J. M. Roesner[1,3], B. C. Seitz[1]. 1) Univ Massachusetts, Boston, Boston, MA; 2) Beth Israel Deconess Medical Center, Boston, MA; 3) Merck, Boston, MA.

During sporulation, an atypical cytokinesis event occurs in which prospore membranes grow from the spindle pole bodies to engulf the meiotic nuclei. As the prospore membranes grow, they assume varying morphologies, first appearing as small cups, then forming elongated structures around the nucleus, before rounding up and closing. The leading edge of the growing prospore membrane is marked by a complex of proteins, which include Ssp1; these proteins are removed from the leading edge when the prospore membrane closes. We find that the GCKIII kinase encoded by *SPS1* is required for this process, with *SPS1* acting together with the sporulation-induced *SPO77* to promote prospore membrane closure. Cells lacking either *SPS1* or *SPO77* make hyper-elongated prospore membranes that sometimes do not close. Previous studies show that cells lacking *AMA1*, which encodes an activator of the meiotic anaphase promoting complex, also have partially penetrant prospore membrane closure defects. Our genetic analyses demonstrate that *SPS1* and *SPO77* act in parallel to *AMA1*, as double mutants of either *sps1* or *spo77* and *ama1* exhibit completely penetrant prospore membrane closure defects. We also find that *SPS1* acts through *SSP1*. We see that Ssp1 is stabilized and not properly removed from the leading edge at the time of prospore membrane closure in *sps1* mutants. Sps1 and Ssp1 are found in a complex, suggesting that the action of Sps1 on Ssp1 may be direct. Taken together, our results suggest that Sps1 regulates meiotic cytokinesis by promoting the proper degradation and/or removal of Ssp1. To determine how this cytokinetic event is coordinated with meiosis, we have examined how meiotic regulators interact with *SPS1*.

Y3032B The synaptonemal complex is dispensable for MutSγ-mediated crossover recombination during meiosis in budding yeast. *A. J. MacQueen*, K. Voelkel-Meiman, S. Morehouse, M. Parziale, Michelle Cheng. Wesleyan University, Middletown, CT, USA.

Meiosis is a specialized cell division that enables sexually reproducing organisms to generate reproductive cells with reduced chromosome ploidy. Crossover recombination is normally essential for successful ploidy reduction during meiosis, as crossovers create attachments that orient homologous chromosomes and facilitate their subsequent segregation apart from one another on the spindle. Thus, the meiotic cell must ensure a sufficient number of recombination events repair as interhomolog crossovers (instead of non-crossovers). Many of the events that process meiotic DNA repair intermediates into interhomolog crossovers occur within the context of a meiosis-specific tripartite structure called the synaptonemal complex (SC), which assembles along lengthwise-aligned homologous chromosomes. Mutants missing building block components or regulators of SC assembly typically exhibit reduced or absent crossover recombination. In several organisms including budding yeast, crossovers specifically associated with SC are mediated by MutSg, a set of proteins related to the prokaryotic MutS family of mismatch repair enzymes. Despite the correlation between SC and crossover formation, the functional relationship between the SC structure, SC components, and MutSg crossover recombination has remained obscure. Our analysis of several budding yeast mutants demonstrates unequivocally that the SC structure is dispensable for MutSg crossover formation. First, through an interspecies complementation experiment we found that the crossover promoting activity of budding yeast SC component, Zip1, can be replaced by *Kluyveromyces lactis* Zip1, yet *K. l.* Zip1 does not assemble SC in *S. cerevisiae* cells. Second, we found that mutants missing either of the SC central element proteins - Ecm11 or Gmc2 - exhibit no deficit in, but rather an increase in, MutSg crossovers. We furthermore identified a non-null *zip1* mutant that phenocopis *ecm11* and *gmc2* mutants: *zip1-N1* mutant meiotic cells fail to assemble SC and exhibit an elevation in MutSg crossovers. Our studies reveal that the SC structure is built of two classes of components: Zip1 represents an SC component with genetically separable activities that promote crossover recombination and SC assembly, while the central element proteins Ecm11 and Gmc2 solely function in SC assembly. Interestingly, we discovered that *ecm11* and *gmc2* mutants exhibit a mismatch repair defect during meiosis. The mismatch repair activity associated with SC central element proteins does not require Ecm11 SUMOylation, suggesting that it is independent of the SC structure *per se*. Thus both SC

transverse filaments and central element components collaborate in building SC structure that attenuates crossover recombination, while these proteins act independently of the SC structure to positively influence recombination in distinct ways.

Y3033C Coupling activation of the Smk1 MAPK to the completion of meiosis. *G. Omerza*, CW. Tio, T. Phillips, E. Winter. Thomas Jefferson University, Philadelphia, PA.

Smk1 is a meiosis-specific MAPK that controls spore morphogenesis in *S. cerevisiae*. While Smk1 is activated by phosphorylation of its T-X-Y activation loop similar to other MAPKs, this pathway does not require a MAPK kinase family member. Instead, the CDK activating kinase, Cak1, phosphorylates Smk1 on the T, producing a low activity form of the enzyme that is uniformly distributed throughout the cell early in meiosis. Smk1 autophosphorylates the Y in-cis at prospore membranes (PSMs) at anaphase II. This spatiotemporally-regulated autophosphorylation reaction is triggered by Ssp2, a PSM-localized protein whose translation is induced specifically at anaphase II. To interrogate the mechanism of this developmentally-regulated autophosphorylation reaction, Smk1 activation has been reconstituted in bacterial cells and with purified proteins. Our data indicate that Ssp2 binding to Smk1 is sufficient to trigger Smk1 autophosphorylation. We previously reported that the meiosis-specific bridging protein for the anaphase promoting complex (Cdc20 homolog), Ama1, regulates Smk1 activation. We find that Ama1 controls Smk1 autophosphorylation by influencing the interaction of Smk1 and Ssp2 at the PSM. Thus, despite the timely production and localization of Smk1 and Ssp2 to PSMs in the *ama1Δ* background, they fail to form a complex. We will present a model in which Ama1 triggers the productive interaction of Ssp2 and Smk1, thereby coupling the completion of the meiotic chromosome cycle to spore wall differentiation.

Y3034A Kel1p mediates yeast cell fusion through a Fus2p and Cdc42p-dependent mechanism. *Jean Smith*, Mark Rose. Princeton University, Princeton, NJ.

Cell fusion is ubiquitous among eukaryotes. However, little is known about the molecular mechanism. In yeast, as cells polarize growth toward the mating partner, the tip of the shmoo comes into contact with its partner, forming a zone of cell fusion. As such, the shmoo tip comprises a hub for proteins necessary for cell fusion. Fus2p, a key regulator of cell wall removal during fusion, forms a heterodimer with Rvs161p, an amphiphysin, and the complex localizes to the shmoo tip. We have shown that Rvs161p facilitates the membrane interaction required for cortical localization. However, domain mapping of Fus2p revealed that the last eight amino acids form a novel signal also required for localization, independent of Rvs161p. Point mutations and truncations in this region cause severe mating defects. Analysis of successive C-terminal Fus2p truncations suggested a model in which an auto-inhibitory mechanism interferes with localization via an upstream region. Previous evidence showed that Fus2p is retained at the shmoo tip by both Fus1p and actin-dependent pathways. Fus1p is a pheromone-induced transmembrane protein broadly localized to the shmoo tip. We find that the actin-dependent pathway requires the C-terminus, whereas the Fus1p pathway depends on more internal sequences. Thus, the C-terminal mutations affect both pathways through auto-inhibition. To identify additional proteins interacting with Fus2p, we performed a high copy suppressor screen of the C-terminal mutations, and identified Kel1p. *KEL1*, encoding a kelch-domain protein, was previously implicated in cell fusion, but its function in fusion is unknown. Localization suppression by Kel1p is dependent upon Fus1p, showing that it does not bypass the normal pathway. Kel1p and its homologue, Kel2p, are required for normal levels of Fus2p localization, and act through the actin-dependent pathway. Kel1p also plays a role in mating independent of Fus2p localization; a *kel1* deletion reduces cell fusion despite proper Fus2p localization. Moreover, overexpression of Kel1p can weakly suppress the fusion defect of *fus2*Δ, arguing that Kel1p has a Fus2p-independent function. Fus2p interacts with Cdc42p, a Rho-like GTPase that plays numerous roles in growth and morphogenesis, and the interaction is required for fusion. Overexpression of Kel1p suppresses the mating defect of a Cdc42p mutant that cannot bind to Fus2p, but suppression is dependent upon Fus2p. We hypothesize that Fus2p, Cdc42p and Kel1p form a complex during fusion. In support, Kel1p interacts with two different domains of Fus2p, dependent on Cdc42p. We propose that Fus2p localization is dependent on actin, Kel1p and Fus1p and that Kel1p enhances the activity of Fus2p/Cdc42p in cell fusion.

Y3035B *NDT80* dependent internal transcriptional initiation sites during budding yeast sporulation. *S. Zhou*, R. Sternglanz, A. Neiman. Stony Brook University, Stony Brook, NY.

Budding yeast sporulation is a developmental process that generates stress-resistant progeny under nutritient limitation. Sporulation is regulated by a cascade of transcriptional signals. The transcription factor *NDT80* is induced during sporulation, and drives the meiotic divisions and spore formation by inducing the transcription of hundreds of genes. The *NDT80* regulon consists of both sporulation-specific genes and constitutively expressed genes that are upregulated during sporulation. For these constitutively expressed genes, tiling array and RNA-seq studies indicate that alternative transcription start sites (TSS) are sometimes seen in sporulating cells as compared to vegetative growth.

Here we investigate a subset of the sporulation specific TSSs that initiate within ORFs and generate a shorter transcript and protein that could serve specific functions during sporulation. One such TSS initiates within an intron of the *MRK1* gene, encoding one of the four homologs of GSK-3 kinases in budding yeast. We find that an *NDT80* binding site within the intron is required for the induction of this shorter transcript and that the shorter transcript results in the expression of an N-terminally truncated form of Mrk1 that nonetheless contains the entire kinase domain. A survey of published datasets identified additional genes with internally initiated transcripts induced in mid-sporulation that have nearby *NDT80* consensus sites. We show using qPCR that four other genes have *NDT80*-dependent internal transcription initiation. We are currently investigating the regulation of these genes as well as the possible function of the truncated proteins during sporulation.

Y3036C *S. cerevisiae RTT105* mediates Ty1 Gag localization under stress. *J. Keeney*, J. Scales. Juniata College, Huntingdon, PA.

The mRNA of LTR-retrotransposons encodes the replication proteins (*gag* and *pol*) and serves as the genomic material for virus-like particles (VLPs), a cytosolic structure in which the element's genome is reverse transcribed. In the genome of Baker's yeast (*Saccharomyces cerevisiae*),

there are several families of such retrotransposons, including the abundant Ty1 element. In *S. cerevisiae*, genome-wide screens have identified numerous candidate genes that mediate Ty1 transposition activity. One of these candidates, *RTT105*, has previously been identified as a negative regulator of native Ty1 mobility in a genetic screen. Here, we show *RTT105* as a positive regulator of galactose-induced Ty1 mobility. *RTT105* is uncharacterized and contains no conserved domains of known function, thus the nature of the role of *RTT105* in Ty1 transposition activity remains unknown. We identified that the *rtt105* mutation does not interrupt Ty1 Gag protein processing nor cDNA synthesis within mature VLPs. In permissive conditions, Rtt105p localizes at discrete cytoplasmic Ty1 Gag foci, indicating a role in VLP assembly and maturation. Ty1 RNA has been shown to localize to the ER membrane in association with the signal recognition particle (SRP), facilitating virus-like particle assembly. In RNA-Seq analysis with isogenic *rtt105* deletion and wild type strains, we found a slight down-regulation of several subunits of the signal recognition particle (SRP) complex and a significant downregulation of the RNA subunit, scR1. Further, growth in stressful environmental conditions, such as the presence of galactose or glucose deprivation, triggers Ty1 Gag protein localization to the vacuole. Interestingly, an *rtt105* mutation inhibits movement of cytoplasmic Ty1 Gag protein to the vacuole in the presence of these stressors. Gag localization to the cytoplasm is reduced in an *rtt105* mutant as assayed by growth of strains containing a Gag::URA3 fusion construct on 5-FOA. These results illuminate an important step in the processing and regulation of retrotransposons and suggest an additional route by which the host cell actively maintains Ty1 mobility in variable environmental conditions.

Y3037A Early stage prion formation and the insoluble protein deposit (IPOD). *D. Lyke*, AL Manogaran. Marquette University, Milwaukee, WI.

The yeast prion [*PSI*$^{+}$] is the misfolded version of the translation termination protein Sup35p. The study of [*PSI*$^{+}$] has greatly enhanced our understanding of how prions propagate; yet the cellular mechanisms underlying how prions form are not well understood. It has been proposed that the initial misfolding and assembly of Sup35p aggregates accumulate at a site called IPOD, Insoluble Protein Deposit, which is tethered to the vacuole. To uncover other cellular pathways that may be involved in prion formation, we previously performed a small scale screen of deletion mutants. We identified several gene deletions that impaired early stages of prion formation and others that that affected later stages. Since most of the deletions possessed fragmented vacuoles, we originally hypothesized that vacuole integrity and the maintenance of its tethered component IPOD is essential for prion formation. Our current data indicate that vacuole fragmentation and prion formation are independent of each other. In fact, introduction of a centromeric plasmid alone was sufficient to cause fragmentation but not impact prion formation. However, it appears that gene deletions that impair early stages of prion formation did have altered localization of IPOD. This alteration in IPOD is not seen in wild type strains or gene deletions that affect later stages. Together our data suggest that although vacuole fragmentation does not affect prion formation, its tethered component IPOD likely plays an important role in the early stages of prion formation. Efforts to further characterize IPOD as it relates to prion formation will be presented. .

Y3038B Mechanisms of suppression of Cox1p degradation by Oma1p. *Gavin McStay*. New York Institute of Technology, Old Westbury, NY.

Cytochrome *c* oxidase is the terminal electron acceptor in the electron transport chain in many aerobic organisms. This multi-protein complex can be composed of a varying number of subunits depending on species. In the budding yeast, *Saccharomyces cerevisiae*, cytochrome *c* oxidase is made up of 11 subunits, 8 are derived from the nuclear genome and 3 are derived from the mitochondrial genome. To ensure these subunits associate at the correct time and place a family of assembly factors is required. In the case of the mitochondrial DNA encoded subunit 1 there is a linear pathway of biosynthesis where these assembly factors and other structural subunits associate at specific times to regulate specific maturation events. In the absence of some assembly factors, subunit 1 is subject to degradation by the protease Oma1p. In yeast, this results in a deficiency in cytochrome *c* oxidase activity and the inability to grow on substrates requiring intact oxidative phosphorylation. We have performed a mutagenic screen of mitochondrial DNA to identify mutations that can suppress the oxidative phosphorylation deficient phenotype caused by the absence of the assembly factors, COA2 and SHY1. The information gathered from this research will guide us in identifying putative regions required for cytochrome *c* oxidase subunit 1 degradation and also provide more information regarding the proteolytic requirements of the Oma1p protease.

Y3039C *MTG3*, a putative GTPase that regulates mitochondrial ribosome function in *Saccharomyces cerevisiae* . *U. Mehra*, Y. Verma, S. Jakar, K. Datta. University of Delhi, South Campus, New Delhi, INDIA.

Mitochondria are made up of proteins encoded by the nuclear genome as well as its own genome, thus requiring both cytosolic as well as its own translation apparatus for its biogenesis. The proper biogeneis of mitochodria is critical as 1 in 5,000 humans suffers from a mitochondrial disease and a number of these diseases are due to defects in the mitochondrial translation apparatus. Importance of mitochondrial translation apparatus can be estimated with the fact that approximately 25% of the mitochondrial proteome is involved in the establishment and the maintenance of the mitochondrial protein synthesis apparatus and mitochondrial DNA. Mitochondria utilize dedicated ribosome molecules that are encoded by a set of nuclear genes distinct from those coding for its cytosolic counterpart. Mitochondrial ribosomes have reduced RNA content and a higher protein content giving rise to numerous mitochondrial specific proteins conserved from lower eukaryotes to humans. Ribosome biogenesis is a multi-step process aided by assembly factors including GTPases which are thought to utilize energy released upon GTP hydrolysis to promote its biogenesis activity. Mtg3p, is a nuclear encoded mitochondrial protein that is conserved from yeast to humans and is a member of YawG/YlqF family of circularly permuted GTPases. Deletion of *MTG3* leads to defects in utilization of glycerol as the sole carbon source and accumulate 15S rRNA precursors. We have shown Mtg3p associates with the 37S small subunit of the mitochondrial ribosome in a salt dependent manner. Cells harboring *mtg3ts* mutants have aberrant large to small ribosomal subunit ratio consistent with a role in mitochondrial ribosome biogenesis. Our studies also indicate that Mtg3p requires guanine nucleotide binding as well as hydrolysis to carry out

its *in vivo* function at a late step during mitochondrial ribosome biogenesis perhaps just prior to association with mRNA to form 74S monosome. In addition, we have isolated spontaneous suppressors for *Δmtg3ρ*$^{+}$ and have determined them to be due to second-site mutations in the mitochondrial genome. We will be presenting our studies aimed at identifying the molecular complexes that Mtg3p is associated with, domains of Mtg3p that are essential for its *in vivo* function and the downstream partners/processes that it likely controls.

Y3040A Mitochondrial genome large scale deletions in Saccharomyces cerevisiae natural population. *T. Nguyen*, J. DeJesus, H. Fiumera. Binghamton University, Binghamton, NY.

Mitochondrial DNA (mtDNA) large-scaled deletions have been reported in a broad spectrum of human health conditions. Petite-postive yeast species, *Saccharomyces cerevisiae*, offers a powerful model to study mtDNA loss, which can be monitored by quantifying the frequency of spontaneously-formed respiratory-deficient (i.e "petite") colonies in a population. The natural genetic variants contributing to mtDNA loss in yeast presently remain in unmapped territory. By investigating the petite frequency of a subset of haploid derivatives of *S. cerevisiae* natural isolates, we learned that petite frequency is a variable and heritable trait. We also employed a novel collection of yeast strains harboring unique mito-nuclear genome combinations to determine that petite frequency is influenced by both nuclear and mitochondrial genetic variants, and by mt-n epistatic interactions to a lesser extent. In pursuit of unravelling the mechanism of mtDNA loss in yeast, we also looked into the rate of homologous recombination, which has been proposed to be the predominant mechanism for generating mtDNA deletions in yeast. Our preliminary data suggested that petite frequency positively correlated with growth rate, which leads us to hypothesize that fast growing yeast strains require higher mtDNA replication, hence higher rate of homologous recombination in mtDNA. We sought to investigate if high petite frequency can be explained by high rate of mtDNA recombination. Using yeast strains with diverged nuclear genomes paired with a mitochondrial background containing an *ARG8*^{m} auxotrophic reporter gene, we found that the rate of homologous recombination, specifically the rate of mtDNA deletion mediated via direct repeats at the *COX2* locus, is not correlated with the petite frequency in these yeast strains. Simultaneously, we are developing strategies to map the genetic basis underpinning petite frequency, in hopes of better understanding the mechanism behind spontaneous mtDNA loss in *S. cerevisiae*.

Y3041B The Influence of Mitochondrial Morphology on Mitochondrial DNA Stability. *Rey Sia*[1], Christopher Prevost[1,2], Nicole Ashman[1], Christina Seger[1], Kathryn Wershing[1], Elaine Sia[2]. 1) The College at Brockport, State Univ New York, Brockport, NY; 2) University of Rochester, Rochester, NY.

Mitochondria are dynamic organelles that fuse and divide. These changes alter the number and distribution of mitochondrial structures throughout the cell in response to developmental and metabolic cues. Copies of the mitochondrial DNA (mtDNA) and associated proteins are packaged into structures called nucleoids. The replication and distribution of nucleoids is coordinately regulated with mitochondrial division, and alterations to proteins involved in mitochondrial fission and fusion affect the maintenance of functional mtDNA. The proteins that drive the mechanics of mitochondrial fission and fusion have been studied, however, knowledge of the connection to mtDNA integrity is limited. We observed that growth of yeast in different carbon sources affects the frequency of spontaneous petites, independent of whether the sugar is fermentable or non-fermentable. In the cells in which the mitochondrial function is lost, analysis of mtDNA in these yeast reveals that they have undergone large-scale deletions of the mtDNA. When the wild-type yeast strain is grown in dextrose or fructose, the frequency of spontaneous petites is significantly higher than when the same cells are grown in raffinose or galactose. This increase in genome stability is dependent on proteins involved in mitochondrial fission, and components of the ERMES complex, which forms junctions between the ER and mitochondria. This observation provides us with a simple experimental system in which to interrogate the connections between metabolic signals, mitochondrial dynamics, and genome maintenance.

Y3042C Nuclear to mitochondrial translocation of cyclin C promotes stress-induced fission and programmed cell death. *Daniel Smethurst*, Katrina Cooper, Randy Strich. Rowan School of Osteopathic Medicine, Stratford, NJ.

The decision to undergo programmed cell death (PCD) is controlled by a complex interaction between nuclear and mitochondrial signals. The mitochondria are highly dynamic organelles that constantly undergo fission and fusion. However, one of the earliest PCD events is a dramatic shift in mitochondrial morphology toward fission. We have identified the transcription factor cyclin C as the biochemical trigger for stress-induced mitochondrial hyper-fragmentation in yeast (Cooper et al., 2014 Dev. Cell) and mammalian (Wang et al., 2015, MCB) cells. In response to PCD stimuli such as oxidative stress, cyclin C, but not its cognate kinase Cdk8, is released from the nucleus then associates with the fission machinery. Loss of cyclin C prevents mitochondrial fission while its ectopic introduction into the cytoplasm induces complete fragmentation in the absence of stress. The Cdk8 module subunit Med13 anchors cyclin C in the nucleus, and is degraded in response to oxidative stress. Deletion of *MED13* results in mitochondrial hyperfragmentation in unstressed cells. Recent studies have found that mitochondrial fission occurs at junctions between the mitochondria and the endoplasmic reticulum (ER). We have found that the number of these junctions increases upon stress in a cyclin C-dependent manner. The results suggest that cyclin C plays a role in establishing and/or maintaining these stress-enhanced mitochondrial-ER junctions. In yeast, continuous cytoplasmic cyclin C causes constitutive mitochondrial fragmentation, stress hypersensitivity and loss of mtDNA integrity. Conversely, loss of cyclin C function protects cells from oxidative stress. The dual role for cyclin C couples the derepression of stress response genes with a mitochondrial fission program that can lead to cell death. In addition, these roles for cyclin C have been conserved from yeast to mammals.

Y3044B Mitochondria as signaling organelles in aging. *V. Titorenko*, A. Leonov, A. Arlia-Ciommo, Y. Medkour. Concordia Univ, Montreal, PQ, CA.

We employ the yeast *Saccharomyces cerevisiae* as a model to study mechanisms of cellular aging in multicellular eukaryotes. Our high-throughput chemical genetic screen identified lithocholic bile acid (LCA) as a geroprotector which significantly slows yeast chronological aging. Using a combination of subcellular fractionation and mass spectrometry-based quantitative analyses, we revealed that exogenously added LCA enters yeast cells, is sorted to mitochondria, resides mainly in the inner mitochondrial membrane, and also associates with the outer mitochondrial membrane. We found that LCA elicits an age-related remodeling of phospholipid synthesis and movement within both mitochondrial membranes, thereby causing substantial changes in mitochondrial membrane lipidome and triggering major changes in mitochondrial size, number, and morphology. In synergy, these changes in the membrane lipidome and morphology of mitochondria alter the age-related chronology of mitochondrial respiration, membrane potential, ATP synthesis, and reactive oxygen species homeostasis. Our data revealed that the LCA-driven alterations in the age-related dynamics of these mitochondrial processes extend yeast longevity. Thus, mitochondrial membrane lipidome plays an essential role in defining yeast longevity. Using quantitative mass spectrometry, we demonstrated that LCA also alters the age-related dynamics of changes in levels of many mitochondrial proteins, as well as numerous proteins in cellular locations outside of mitochondria. These proteins belong to two regulons, each modulated by a different mitochondrial dysfunction. Proteins constituting these regulons 1) can be divided into several "clusters";, each of which denotes a distinct type of partial mitochondrial dysfunction that elicits a different signaling pathway mediated by a discrete set of transcription factors; 2) exhibit three different patterns of the age-related dynamics of changes in their cellular levels; and 3) are encoded by genes whose expression is regulated by the transcription factors Rtg1p/Rtg2p/Rtg3p, Sfp1p, Aft1p, Yap1p, Msn2p/Msn4p, Skn7p and Hog1p, each of which is essential for longevity extension by LCA. Our findings suggest that LCA-driven changes in mitochondrial lipidome alter mitochondrial proteome and functionality, thereby enabling mitochondria to operate as signaling organelles that orchestrate an establishment of an anti-aging transcriptional program for many longevity-defining nuclear genes. We propose a model for how such LCA-driven changes early and late in life of chronologically aging yeast cause a stepwise development of an anti-aging cellular pattern and its maintenance throughout lifespan.

Y3045C [*PSI*$^+$] formation: Differentiating the role of the retromer complex from vacuole fusion. *B. Wisniewski*, A. Manogaran. Marquette University, Milwaukee, WI.

Prions are misfolded proteins that can aggregate into infectious amyloid fibrils. In yeast, misfolding of the translation termination protein, Sup35p, leads to the formation of a prion called [*PSI*$^+$]. Fusion of Sup35p to GFP (Sup35p-GFP) leads to the appearance of fluorescent intracellular rings: a hallmark of [*PSI*$^+$]. Our lab previously found that strains lacking the open reading frame YOR069W have both reduced ring and [*PSI*$^+$] formation frequencies compared to wild type. Deletion of YOR069W leads to the removal of two genes: *VPS5* and *VAM10* (YOR068C). *VPS5* encodes the retromer complex involved in the recycling of endosome vesicles to the late Golgi and *VAM10* encodes a protein involved in vacuolar fusion. We set out to identify whether *VPS5* or *VAM10* is responsible for the reduced prion formation seen in YOR069W deletion strains. A plasmid containing YOR069W and surrounding regulatory sequences was subjected to site-directed mutagenesis. The start codon of *VPS5* was mutated to arginine or the start codon of *VAM10* was mutated to isoleucine without disrupting the amino acid sequence of the opposite open reading frame. Mutated plasmids were introduced into the YOR069W deletion background and tested for the ability to form Sup35p-GFP rings. Wild type control plasmids rescued the low ring formation phenotype. However, each individual gene disruption plasmid showed the same low ring formation frequency as the double deletion. These data suggest a synergistic effect of the seemingly unrelated *VPS5* and *VAM10* genes in prion formation, and furthermore imply that the endosome recycling pathway and vacuolar fusion pathway both contribute to prion formation.

Y3046A Arl1 and Ypt6 are involved in autophagy in *Saccharomyces cerevisiae*. *Shu Yang*, Anne Rosenwald. Georgetown University, Washington, DC.

Autophagy is a cellular degradation process that sequesters organelles or proteins into a double-membrane structure called the autophagosome, which then fuses with the lysosome or vacuole for hydrolysis. Factors that control membrane traffic are also essential for each step of autophagy. Here we demonstrate that two small GTP-binding proteins in *Saccharomyces cerevisiae,* Arl1 and Ypt6, which belong to the Arf/Arl/Sar protein family and the Rab family, respectively, and control endosome – *trans*-Golgi traffic, are also necessary for starvation-induced autophagy under high temperature stress. Using established autophagy-specific assays we found cells lacking either *ARL1* or *YPT6,* which exhibit synthetic lethality with one another, were unable to undergo autophagy at an elevated temperature, although autophagy proceeds normally at normal growth temperature; specifically strains lacking one or the other of these genes are unable to construct the autophagosome because these two proteins are required for proper traffic of Atg9 to the phagophore assembly site at the restrictive temperature. Using degron technology to construct an inducible *arl1Δ ypt6Δ* double mutant, we demonstrated that cells lacking both genes show defects in starvation-inducted autophagy at the permissive temperature. Our data show that these two membrane traffic regulators have novel roles in autophagy.

Y3047B Cohesin Binding and Function at a Model Euchromatic Gene. *M. Borrie*, H. Joshi, J. Campor, M. Gartenberg. Rutgers University, Piscataway, NJ.

Cohesin is a toroidal-shaped protein complex that binds chromatin by encircling either one or two DNA duplexes. The complex joins distant DNA sites together to mediate sister chromatid cohesion, double-strand break repair and the regulation of gene expression. In yeast, cohesin often associates with the bodies of inactive genes. However, transcriptional activation mobilizes the complex, causing loss from gene bodies and a corresponding increase in binding at intergenic regions between convergently oriented genes. It is not clear whether cohesin mobilization occurs by sliding complexes along DNA or by displacing complexes from DNA entirely. Here, *URA3* was used as a model system to study how

cohesin arrives at a eukaryotic gene and what happens to the complex when transcription ensues. Site-specific recombination was used to excise a pair of DNA circles from sister chromatids that cohesed when *URA3* was present on the circles. Deletion analysis identified a region of the *URA3* promoter that included a poly(dA-dT) segment that was required for cohesion. The cohesin loading complex Scc2/4 is known to bind poly(dA-dT) regions, suggesting that this *URA3* promoter element may be a loading site for cohesin that accumulates on the gene. The DNA circle assay was also used to study how cohesive complexes are mobilized by transcription. Induction of transcription after circularization did not diminish cohesion. However, induction of transcription before circularization (when the template was effectively linear) caused loss of cohesion. Insertion of a convergent gene downstream of *URA3* prevented transcription-driven loss of cohesion. Collectively, these data are consistent with transcription mobilizing cohesin complexes by sliding along DNA.

Y3048C Ubiquilin/Dsk2 promotes inclusion body formation and lysosome-mediated disposal of mutated Huntingtin. *K. Chuang*[1], F. Liang[2], R. Higgins[1], Y. Wang[1]. 1) Florida State University, Tallahassee, FL; 2) Yale University, New Haven, CT.

Ubiquilin proteins contain an ubiquitin-like domain (UBL) and ubiquitin-associated (UBA) domain that interact with the proteasome and ubiquitinated substrates, respectively. Previous works have established the link of ubiquilin proteins to several neurodegenerative diseases, but their molecular function remains elusive. Here, we used misfolded Huntingtin exon I containing 103 polyglutamine expansion (Htt103QP) as a misfolded protein substrate to investigate the function of ubiquilin proteins. We found that yeast ubiquilin mutant (*dsk2*Δ) is sensitive to Htt103QP overexpression and shows defects in formation of Htt103QP inclusion body, a structure containing protein aggregates. Surprisingly, proteasome-dependent Htt103QP degradation is normal in *dsk2*Δ mutant, but *dsk2*Δ mutant showed a defect in the delivery of Htt103QP into vacuoles (lysosomes) in yeast, indicating that ubiquilin proteins facilitate lysosome-dependent clearance of misfolded proteins by promoting inclusion body formation. Our further evidence indicates that the UBL domain of Dsk2 contributes to its functional specificity of ubiquilin/Dsk2. Importantly, the defect of inclusion body formation in in *dsk2* mutants is rescued by human ubiquilin 1 or 2, suggesting functional conservation of ubiquilin proteins.

Y3049A Genetic selection coupled to next-generation sequencing reveals structural requirements for tail-anchor targeting to mitochondria. *Cory D. Dunn*, Abdurrahman Keskin, Emel Akdoğan. Department of Molecular Biology and Genetics, Koç University, Sariyer, Istanbul, TR.

Proteins localized to mitochondria by a carboxyl-terminal tail anchor (TA) play important roles in apoptosis, mitochondrial dynamics, and mitochondrial protein import. In order to reveal structural characteristics of TAs that may be important for mitochondrial targeting, we focused our attention upon the TA of the *Saccharomyces cerevisiae* Fis1 protein. We generated a library of Fis1p TA variants fused to the transcription factor Gal4p. By selecting for mutations within the TA permitting Gal4p to translocate to the nucleus and activate transcription, we were able to enrich for TA variants within our mutant pool which led to decreased membrane insertion. Next-generation sequencing allowed quantification of each TA variant in our mutant library both before and after selection, and high-throughput results were confirmed by microscopy-based and functional analysis of individual, reconstructed Fis1 TA mutants. Consistent with the prediction that the Fis1 TA is alpha-helical, prolines within the TA generally prevented membrane insertion. Charged residues within the hydrophobic core of the Fis1 TA were also able to perturb membrane insertion. Surprisingly, positively charged residues were much more acceptable at several positions within the Fis1 TA than negatively charged residues, providing strong, *in vivo* evidence that lysine and arginine can "snorkel," or partition the nonpolar portion of their side chains into the hydrophobic region of the lipid bilayer while placing the terminal charge near the polar interface of the membrane. In further experiments, we found that lengthening or shortening the Fis1 TA by up to three amino acids did not alter its behavior or localization, arguing against a model in which TA length directs insertion into specific organelles. Finally, our data affirm previous results indicating that the charged carboxyl-terminus of Fis1p is important for localization and insertion at the mitochondrial outer membrane. Our work provides the first high-resolution analysis of an organelle targeting sequence to be generated by deep mutational scanning.

Y3050B Membrane trafficking underlies aging and rejuvenation. *Kiersten A. Henderson*[1], Jason V. Rogers[2], Patricia Gordon[1], Netty Lim[1], Daniel E. Gottschling[1,2]. 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Calico Life Sciences, LLC, South San Francisco, CA.

Replicative aging is asymmetric – mother cells age but produce daughter cells with full lifespans. We recently identified that mother-daughter asymmetry of the plasma membrane H+-ATPase, Pma1, underlies age asymmetry (Henderson et al., eLife, 2014). We found that Pma1 accumulates at the plasma membrane of mother cells during aging, but that daughter cells have very low levels of Pma1.

Pma1 is the major regulator of cytosolic pH (Serrano et al., MCB, 1986). By accumulating at the plasma membrane of mother cells during aging, Pma1 causes an increase in cytosolic pH that limits lifespan by antagonizing vacuolar function. However, nascent daughter cells (buds) and newborn daughters have very low levels of Pma1. Thus, the asymmetric distribution of Pma1 allows daughter cells to achieve normal cytosolic pH and vacuole function, and they are born young.

Because mother-daughter asymmetry of Pma1 underlies aging and rejuvenation, we sought to identify how the spatial distribution of Pma1 is achieved. We performed high-throughput microscopy screens and identified mutants that disrupted Pma1 asymmetry (i.e. increased levels of Pma1 at the bud plasma membrane). We found that disrupting endocytosis, endocytic trafficking, and sorting at the late golgi increases the level of Pma1 in nascent daughter cells.

Because these processes contribute to Pma1 asymmetry, this suggests that they are spatially regulated to occur differently between mother cells and buds and/or cell cycle regulated. We are currently dissecting how these processes are regulated to establish Pma1 asymmetry and we will determine how each process influences asymmetry of cytosolic pH, vacuole function, and lifespan.

Y3051C Clearance of mutated huntingtin protein via K63-linked ubiquitination in yeast cells. *R. Higgins*[1], F. Liang[2], K. Chuang[1], Y. Wang[1]. 1) Florida State University, Tallahassee, FL; 2) Yale University, New Haven, CT.

Misfolded proteins that evade chaperone-mediated refolding and proteasome-dependent degradation can form aggregates in cells. These protein aggregates are cytotoxic and are linked to several neurodegenerative diseases, including Alzheimer's and Huntington's diseases. However, the aggregates can further form a cytoprotective structure termed an aggresome (inclusion body), and studies in higher eukaryotes indicate that K63-linked ubiquitination promotes aggresome formation for some substrates. Polyglutamine (polyQ) expansion within Huntingtin protein contributes to the development of Huntington's disease and expression of mutated Huntingtin with 103 polyQ (Htt103QP) leads to aggresome formation in both mammalian and yeast cells. Using yeast as a model organism, we demonstrate that Htt103QP is degraded primarily through the proteasome prior to aggresome formation, while vacuole (lysosome)-dependent autophagy pathway is responsible for Htt103QP clearance once an aggresome is formed. We further show that K63-linked ubiquitination promotes aggresome formation of Htt103QP, as abolishment of K63 ubiquitination in yeast cells compromises aggresome formation. The observation that mutation of the three lysine (K) residues in the N-terminus of Htt103QP resulted in aggresome formation defect indicates that ubiquitination of Htt103QP itself contributes to aggresome formation. In addition, we found that the E3 ubiquitin ligase Rsp5 (Nedd4) promotes K63-linked ubiquitination of Htt103QP and is required for aggresome formation. Therefore, we can use yeast as a model organism to study the clearance process for misfolded proteins linked to neurodegenerative diseases.

Y3052A A SUMO-targeted ubiquitin ligase reduces the toxicity and transcriptional activity of a poly-Q expanded protein. *O. Kerscher*[1], G. Dreissnack-Sclar[3], R. Levy-Myers[1], N. Pasupala[2]. 1) The College of William & Mary, Williamsburg, VA; 2) The Johns Hopkins School of Medicine, Baltimore, MD; 3) Emory University, Atlanta, GA.

SUMO-targeted ubiquitin ligases (STUbLs) play an important role in the homeostasis of SUMO-modified proteins and SUMO-dependent signaling. Eukaryotic cells that lack STUbLs are hypersensitive to DNA damage, and accumulate gross chromosomal rearrangements and high-molecular weight adducts of SUMO-modified proteins. In this study we show that budding yeast STUbLs (Slx5 and Slx8) also modulate the aggregation, toxicity, and transcriptional properties of poly-glutamine (poly-Q) expanded huntingtin (Htt), the causative agent of Huntington's Disease (HD). We demonstrate that expression of an aggregation-prone Htt construct with 103 glutamine residues (103Q), but not the non-expanded form (25Q), results in severe growth defects in slx5Δ and slx8Δ cells. Concomitantly, an extra copy of SLX5 reduces the accumulation of 103Q aggregates in the cytosol of wild type cells while overexpression of SUMO led to diffuse nuclear staining of Htt. This nuclear enrichment of Htt prompted us to assess the effect of STUbLs on the transcriptional properties of 25Q, 55Q and 97Q. Expression of 25Q, 55Q and 97Q fused to the Gal4 activation domain (AD) resulted in reporter gene auto-activation. Remarkably, the auto-activation of Htt constructs was abolished by expression of Slx5 fused to the Gal4 DNA-binding domain (Slx5-BD) but not an Slx5 SIM mutant (BD-Slx5sim) that fails to interact non-covalently with SUMO. These data suggest a novel role for STUbLs in the recently described proteolysis-independent stripping of transcription factors that also involves Cdc48 and its co-factors.

Y3053B Mechanism of protein quality control at the inner nuclear membrane in budding yeast. *B. Koch*, R. Abblett, H. G. Yu. Florida State University, Tallahassee, FL.

The nucleus of a eukaryotic cell is enclosed by a double-membrane structure, termed the nuclear envelope, which separates the nucleoplasm from the cytoplasm. The outer and inner membranes of the nuclear envelope have individualized functions within the cell. The outer nuclear membrane is continuous with the endoplasmic reticulum membrane, whereas the inner nuclear membrane acts as the intermediate connecting the outer nuclear membrane to the nucleoplasm. Proteins within the inner nuclear membrane (INM) interact with the telomeres, the nuclear lamina and the nuclear pore complex, and play important roles in chromosome movement and nuclear migration. Defective INM proteins can lead to genetic diseases, such as Emory driefuss muscular dystrophy. In order to maintain protein homeostasis at the INM, a specific protein quality control mechanism must be present to remove dysfunctional proteins from the nuclear envelope. Recent work has shown that some INM proteins are degraded through the Asi-E3 ubiquitin ligase pathway in budding yeast. However other INM proteins, including the SUN-domain protein Mps3, are independent of the Asi pathway for degradation. We report here a novel protein quality control mechanism at the INM that is dependent on protein cleavage. Using the expression levels of Mps3 as a genetic tool, we found that noncleavable Mps3 forms aggregates at the nuclear periphery and is lethal in vegetative cells. Genetic analysis demonstrates there is an interaction between the INM proteins and vacuole components, which leads us to propose that certain INM proteins are targeted by the autophagy pathway. Because the SUN-domain and other INM proteins are conserved from yeast to higher eukaryotes, a similar autophagic regulation of INM proteins may take place in mammalian cells.

Y3054C Dissecting pathways underlying asymmetric plasma membrane protein retention. *J. V. Rogers*[1], K. A. Henderson[2], D. E. Gottschling[1,2]. 1) Calico Labs, San Francisco, CA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

Saccharomyces cerevisiae divides asymmetrically to generate a rejuvenated daughter cell and an aged mother. Identifying the underlying asymmetric cellular components could uncover new aging factors and will inform general models for how cellular asymmetries are generated. A recent biochemical screen in our laboratory identified three proteins that are long-lived and asymmetrically retained for ~18 cell divisions in the plasma membrane of mother cells: Pma1, Mrh1, and Sur7. To uncover the mechanism underlying these proteins' asymmetric localization, we have performed a high-throughput microscopy screen to discover genes that, when deleted, cause these asymmetric plasma membrane proteins to appear symmetric during budding.

We found that Pma1 asymmetry requires endocytic, vacuolar, and golgi-to-endosome trafficking whereas Mrh1 and Sur7 do not. Sur7

asymmetry is uniquely lost in a subset of cell wall and vesicular trafficking mutants (*e.g., gas1*Δ, *ecm33*Δ, and *pho88*Δ). A third set of mutants broadly affecting vesicle trafficking pathways (*e.g., cdc50*Δ, *drs2*Δ, *rcy1*Δ, and *swa2*Δ) accumulated cytoplasmic puncta containing Sur7, Mrh1, and Pma1 during budding, but only led to increased plasma membrane symmetry for Pma1. In contrast to Pma1 and Sur7, no mutants were identified that resulted in a strongly symmetric plasma membrane localization of Mrh1. Pulse-chase and photoconversion experiments have demonstrated that all identified mutants affect asymmetry at the step of new synthesis; that is, these mutants aberrantly express and localize new protein to the early bud at a time when, in wild-type, protein localization is repressed. A mechanistic analysis of these mutants and their role in plasma membrane protein asymmetry is ongoing and will be presented.

Y3055A Protein quality control that regulates unassembled ribosomal proteins. *M. Sung*, R. Deshaies. California Institute of Technology, Pasadena, CA.

Assembly of ribosomes accounts for a large fraction of total RNA and protein synthesis in yeast. All ribosomal subunits are present in assembled ribosomes at 1:1 stoichiometry, but it is anticipated that in synthesizing the ~80 proteins that form a ribosome, modest variations in expression must inevitably occur with some subunits expressed in modest excess over others. Ribosomal proteins produced in slight excess will not be able to assemble and are presumably degraded by a quality-control pathway(s) that senses unusable proteins. Given the major contribution of ribosome assembly to total cellular biosynthesis, such a quality-control pathway is likely to play a significant role in protein homeostasis. However, the responsible quality-control mechanisms remain poorly characterized. Here, we demonstrate that overexpression of multiple proteins of the small and large yeast ribosomal subunits is suppressed. Our studies reveal that ribosomal proteins that fail to assemble into ribosomes are rapidly distinguished from their assembled counterparts and are ubiquitinated and degraded by proteasome within the nuclear compartment.

Y3056B Multiple signaling pathways control the *S. cerevisiae* gene expression response to hypoxia. *Erica Avery*, Samuel Macleon, Gurmannat Kalra, Nawshad Hossian, Olivia Wojtowicz, Stephen Willis, Nasrine Bendjilali, Mark Hickman. Rowan University, Glassboro, NJ.

Many organisms, including humans and the yeast *S. cerevisiae*, experience hypoxia, or low oxygen. To adapt to this new environment, cells employ signaling pathways to mount a global change in gene expression. In *S. cerevisiae*, previous studies have focused on the mechanisms of individual pathways but how multiple pathways together mount a large hypoxic response is not well understood. Here, we attempt to identify and characterize all signaling pathways that contribute to the hypoxic response. We propose at least seven signaling pathways composed of 19 genes that mediate the response, with many genes conserved in humans. To study the role of each signaling gene, we have created single deletion strains. To study each pathway, we have created "pathway" mutant strains that contain deletions of all the genes in a pathway. Studying these strains will help delineate the contribution of each pathway and also reveal redundancy in the signaling network. Surprisingly, all of the strains can grow during hypoxia, suggesting that the signaling genes may be important for long-term survival or fitness in hypoxia. To test the role of the signaling genes and pathways in the gene expression response, each strain was subjected to an anaerobic environment for 5, 10, 30, 60, 120, 180, and 240 minutes. RNA was extracted from the cells and mRNA level was determined by RNA-Seq and RT-qPCR analyses. First, we described the wildtype hypoxic response by employing three statistical tests to identify the genes that exhibited a robust time-dependent response. The first test employs DESeq to identify the genes that fit a quadratic when comparing expression to time. The second uses autocorrelation as a measure of the smoothness of a gene's response, and the third compares the means of early vs. late time points to isolate large expression changes over time. A total of 715 genes were identified including almost all of the expected oxygen-regulated genes as well as previously undiscovered genes. Additionally, we found that the hypoxic response is distinct from the environmental stress response and appears to represent a transition to a new growth state. By observing the gene expression response in some of the mutants, we have found that multiple signaling pathways are indeed responsible and that there is redundancy in the response network. This work contributes to our understanding of a large cellular response that involves the coordination of several signaling pathways.

Y3057C Exploring the role of tyrosine phosphorylation in regulating Yck1/2 activity in the glucose sensing pathway. *T. Biswas*, M. Johnston. University of Colorado Denver, Aurora, CO.

Glucose triggers the SRR (Snf3-Rgt2/Rgt1, or Sensor-Receptor/Repressor) glucose sensing pathway, which induces expression of hexose transporters (*HXT*). Extracellular glucose binds to the Snf3 and Rgt2 glucose sensors, leading to Yck1/2-mediated phosphorylation of the Mth1 and Std1 corepressors, resulting in their degradation, which relieves repression of the *HXT* genes. Most signaling pathways are modulated by an interplay of protein kinases and phosphatases. To identify protein phosphatases involved in glucose sensing we tested catalytic and regulatory subunits of protein phosphatases for their ability to induce *HXT1* expression when overexpressed. Over-expression of PTP1 (phosphotyrosine phosphatase) and PPS1 (protein phosphatase S phase; dual-specificity phosphatase) increased *HXT1* expression in cells growing on glucose. A plausible target for a phosphatase in the glucose signaling pathway is Yck1/2, whose Casein kinase 1 orthlogs in mammals and *S. pombe* are inhibited by autophosphorylation and activated by protein phosphatases. Since our results suggest a role for Tyr dephosphorylation, we tested if Yck2 activity is regulated by phosphorylation/dephosphorylation at Tyrosine residues. Conserved Tyr resudues were mutated to Phe (dephosphorylated form) or Glu (phosphomimetic). Interestingly, Tyr76>Glu showed a significant decrease in activity. Our study suggests a role for Ptp1 in regulation of glucose sensing pathway.

Y3058A Multiple targets on the Gln3 transcription activator are cumulatively required for control of its cytoplasmic sequestration. *T. G. Cooper*[1], R. Rai[2], J. J. Tate[3]. 1) University of Tennessee Health Science Center, Memphis, TN; 2) University of Tennessee Health Science Center, Memphis, TN; 3) University of Tennessee Health Science Center, Memphis, TN.

A remarkable characteristic of nutritional homeostatic mechanisms is the breadth of metabolite concentrations to which they respond and resolution of those responses; adequate but rarely excessive. Two general ways of achieving such exquisite control are known: stoichiometric mechanisms where increasing metabolite concentrations elicit proportionally increasing responses, and the actions of multiple independent metabolic signals that cumulatively generate appropriately measured responses. Intracellular localization of the nitrogen-responsive transcription activator, Gln3 responds to four distinct nitrogen environments: nitrogen limitation or short-term starvation, i.e. nitrogen catabolite repression (NCR), long-term starvation, glutamine starvation and rapamycin inhibition of mTorC1. We have previously identified unique sites in Gln3 required for rapamycin-responsiveness and Gln3-mTor1 interaction. Alteration of the latter results in loss of about 50% of cytoplasmic Gln3 sequestration. However, except for the Ure2-binding domain, no evidence exists for a Gln3 site responsible for the remaining cytoplasmic Gln3-Myc^{13} sequestration in nitrogen excess. Here we identify a serine/threonine-rich (Gln3$_{477\text{-}493}$) region required for effective cytoplasmic Gln3-Myc^{13} sequestration in excess nitrogen. Substitutions of alanine but not aspartate for serines in this peptide partially abolish cytoplasmic Gln3 sequestration. Importantly, these alterations have no effect on Gln3-Myc^{13}'s responses to rapamycin, methionine sulfoximine or limiting nitrogen. However, cytoplasmic Gln3-Myc^{13} sequestration is additively and almost completely abolished when mutations in the Gln3-Tor1 interaction site are combined with those in Gln3$_{477\text{-}493}$ cytoplasmic sequestration site. These findings clearly demonstrate that multiple individual regulatory pathways cumulatively control cytoplasmic Gln3 sequestration. Supported by NIH grant GM-35642-26.

Y3059B The ergosterol biosynthesis pathway is required for optimal induction of the unfolded protein response after phenol stress. *G. Edwalds-Gilbert*, A. Baris, O. Smith, A. Hilborn, A. Marks, K. Tanguay, V. Yu. Claremont McKenna, Pitzer, and Scripps Colleges, Claremont, CA.

Phenolic compounds exist in abundance in the environment as the product of natural and synthetic processes and can be harmful, stress-inducing exotoxins. Bisphenol-A (BPA), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are phenol-based compounds found in polycarbonate plastics and food preservatives and therefore have a particularly high degree of human exposure. Exposure to these compounds has been associated with numerous diseases, yet their mechanisms of action are not well understood. A quantitative evaluation of growth of wild type and haploid deletion strains was used to identify the genes that modulate response to phenol stress induced by BPA, BHA, and BHT in Saccharomyces cerevisiae. Exposure to phenol stress in wild type yeast induces the unfolded protein response (UPR). The UPR is a conserved cellular process that responds to stressors that cause unfolded proteins to accumulate in the endoplasmic reticulum, eventually inducing cell death. In yeast, one portion of the UPR is activated when unfolded proteins bind to inositol-requiring enzyme 1 protein (Ire1p). Ire1p then oligomerizes, activating HAC1 pre-mRNA splicing; Hac1 is a transcription factor that promotes expression of chaperone proteins that refold or degrade unfolded proteins. Ire1-GFP showed clustering after treatment with BHT for 2 hours. Ergosterol is similar to cholesterol in humans and maintains membrane fluidity; mutants with deleted ergosterol genes do not produce ergosterol and were identified in the deletion mutant screen as possible modulators of phenol stress response. This suggests that like the UPR, ergosterol biosynthesis is affected by phenols and reduced membrane fluidity may potentially inhibit Ire1p movement and oligomerization. To test this hypothesis, we treated ergosterol pathway deletion mutants *Δerg 2*, *Δerg6* and *Δerg24* with BPA, BHA, and BHT during 12 hour time courses. We measured HAC1 pre-mRNA splicing using RT-PCR. Strains deleted for genes encoding proteins in the ergosterol biosynthesis pathway had growth rates that were below wild type growth rates when exposed to BPA, BHA, and BHT and showed lower levels of UPR induction after phenol stress. These data demonstrate an important pathway through which common phenols act in yeast, which may inform research on human exposure to these chemicals.

Y3060C The scaffold protein Bem1 connects active Cdc42$_{GTP}$ to different effector proteins in a cell cycle specific manner. *S. Grinhagens*, L. Rieger, N. Johnsson. Ulm University, Ulm, Baden-Württemberg, DE.

Saccharomyces cerevisiae is a generally accepted model organism to study the establishment and maintenance of cell polarity. In order to polarize, the yeast cells have to accumulate the GTPase Cdc42 in its active GTP bound form at the growth tip of the cell. The scaffold protein Bem1p performs an important function in this process by simultaneously binding to Cdc42$_{GTP}$, its GEF Cdc24, and certain effector proteins of Cdc42$_{GTP}$. We show that Bem1p can channel the flow of Cdc42$_{GTP}$ from Cdc24 to its effectors through a SH3 domain-based interaction network. Channelling becomes essential once the concentration of Cdc42$_{GTP}$ is artificially reduced. We further show that the composition of this network changes during the cell cycle. During bud growth Bem1p links Cdc42 and Cdc24 to PAK kinases and effectors of exocytosis and the actin cytoskeleton. Upon entry into mitosis and during cytokinesis Bem1 becomes actively isolated from these effectors. This behaviour of Bem1 reflects its changing localization during different stages of the cell cycle. Using isolated domains and mutants thereof we try to understand the localization of Bem1 to these different compartments.

Y3061A Altered expression levels of *HKR1*, which encodes a transmembrane signaling mucin, confer resistance to HM-1 killer toxin on *Saccharomyces cerevisiae*. *S. Kasahara*, U. Suzuki. Miyagi Univ, Sendai, Miyagi, JP.

The yeast *Williopsis saturnus* var. *mrakii* (syn. *Hansenula mrakii*) produces a proteinous killer toxin called HM-1, which has a strong cytocidal effect on sensitive yeasts such as *Saccharomyces cerevisiae*. *HKR1* (*Hansenula mrakii* killer toxin resistant gene 1) was originally identified in the genome of *S. cerevisiae* as a gene whose overexpression overcame the effect of HM-1. The gene product Hkr1p is a large cell surface protein composed of 1,802 amino acids. It contains a signal peptide sequence at the N-terminus, Ser/Thr-rich mucin-like repetitive sequences and a putative transmembrane domain. Also the calcium binding EF hand motif and the leucine zipper are found in its cytoplasmic tail and the extracellular domain is presumed to be highly glycosylated. It is hence categorized as a transmembrane signaling mucin. Recently it has been reported that Hkr1p and another transmembrane signaling mucin Msb2p act as putative osmosensors in the high osmolarity glycerol (HOG) signaling pathway.

We here report that only short portions of *HKR1* endow HM-1 resistance to *S. cerevisiae* cells. Moreover, even when the reverse strand of *HKR1* was transcribed (presumably the expression of the gene was suppressed by the antisense RNA), *S. cerevisiae* cells acquired resistance to HM-1. Since mutations at the positions of internal ATGs which could act as translation initiation codons in *HKR1* did not affect the resistance to HM-1, we speculated that the acquisition of HM-1 resistance by partial *HKR1* expression was independent from the translation event, that is, overexpression of Hkr1p was not the case. Also we speculate that the expression of *HKR1* was downregulated rather than overexpressed due to transgenic cosuppression when the gene was driven by the *GAL1* promoter, then the accumulation of the gene product Hkr1p declined finally. From these observations, we postulate that when the expression level of *HKR1* was altered, especially downregulated, the cells acquired resistance to HM-1. Quantifying studies for Hkr1p accumulation using antibodies are anticipated. We also observed budding patterns of those transformants expressing partial *HKR1*. The cells of the haploid *S. cerevisiae* A451 strain typically select budding sites axially in a unipolar pattern, but bipolar and randomized budding patterns were observed more frequently under the existence of HM-1. We then found that expression levels of *HKR1* affected bud site selection of *S. cerevisiae* as well.

These observations will provide critical information to understand the mechanism of the cytocidal effect of HM-1 and the function of Hkr1p in the cellular signaling of *S. cerevisiae* in the context of HM-1 resistance.

Y3062B Multiple MAPK cascades regulate the transcription of *IME1*, the master transcriptional activator of meiosis in *Saccharomyces cerevisiae*. *Y. Kassir*, S. Kahana-Edwin, M. Stark. Technion Inst, Haifa, IL.

Entry into meiosis in *S. cerevisiae* depends on the expression and activity of a master regulator, Ime1. The transcription of *IME1* is regulated by all the meiotic signals through an atypical large and complex 5' region. In this report we focus on a distinct *cis*-acting regulatory element UASru, whose activity is regulated by multiple signals. A glucose signal inhibits UASru activity through the cAMP/protein kinase A pathway and the transcription factors (TF), Com2 and Sko1. A nitrogen source represses UASru activity through the TF Sum1. Our results demonstrate that all the known MAPK cascades that operate in vegetative cultures directly affects UASru function: The Hog1 MAPK transmits a high osmolarity signal to UASru through the Sko1 TF; The Mpk1 MAPK transmits an elevated temperature signal through the TFs Swi4/Mpk1 and Swi4/Mlp1; Fus3 and Kss1 MAPKs transmit novel nutrient signal to UASru through the Ste12/Ste12 and Ste12/Tec1 TFs, respectively. This signal is specific to UASru and does not affect the mating and filamentation response elements that are regulated by these MAPK and TFs. Moreover, UASru does not respond to the mating and filamentation signals. Thus, the three optional developmental pathways available to yeast, namely, meiosis, filamentation and mating are regulated by the same MAPKs in a specific manner. A hypothesis aimed to explain how specificity is achieved will be discussed.

Y3063C The Transmission Interface in Yeast Pleiotropic Drug Resistance (PDR) Pumps Controls Substrate Specificity by Linking ATP Hydrolysis to Drug Extrusion. *Karl Kuchler*[1], Narakorn Khunweeraphong[1], Thomas Stockner[2], Cornelia Klein[1]. 1) Medical University Vienna, Vienna, AT; 2) Medical University of Vienna, Institute of Pharmacology, Vienna, AT.

ABC transporters constitute a ubiquitous superfamily of membrane pumps containing an evolutionary conserved ATP-binding cassette domain. They mediate energy-driven efflux of a great variety of substrates, including amino acids, ions, sugars, as well as synthetic and naturally occurring xenobiotics or toxins. A subset of yeast pumps plays a major role in the so-called pleiotropic drug resistance (PDR) phenomenon, where overexpressed ABC transporters such as *S. cerevisiae* Pdr5 or Snq2 confer resistance to a vast variety of drugs. This phenomenon resembles anti-tumor resistance in cancer cells, which is mediated by proteins such as P-glycoprotein, BCRP or MRP. However, very little is known about the molecular mechanism of function of yeast PDR transporters, mainly due to a lack of structural information. To gain insight into the principles underlying transport through yeast ABC proteins, we have initiated a structural modelling approach yielding improved homology models of yeast PDR transporters. The models enabled the prediction of the structural organization of putative transmembrane regions and their connection to the dimeric nucleotide binding domains (NBDs). Remarkably, we have identified residues in the transmission interface, which are critical for both function and formation of the NBD dimer, which is essential for ATP hydrolysis and substrate translocation. A mutational analysis of the Pdr5 and Snq2 transporters identifies further residues critical for ATP-binding, hydrolysis as well as drug substrate specificity, and defines the molecular basis of substrate specificity in the context the interplay of NBD dimerization and ATP consumption. We will present modeling data along with mutational validation, and discuss how homology modeling can facilitate genetic structure-function analysis to reveal possible mechanisms of yeast PDR transporters.

This work was supported through the SFB035-P20 project from the Austrian Science Foundation to KK.

Y3064A Boi1 and Boi2 contribute to Secretory Pathway Polarization. *J. Kustermann*, Y. Wu, N. Johnsson. Ulm University, Ulm, Baden-Württemberg, DE.

Asymmetric cell division is essential for the generation of cell diversity and forms the basis for rejunvenation during cellular aging. For decades, the model organism *Saccharomyces cerevisiae* (*S. c.*) has been studied to elucidate the protein networks that enable asymmetric cell division and connect cell polarity establishment to intracellular transport processes. In our work we decipher a new pathway in which the annilin-related yeast proteins Boi1 and Boi2 link Cdc42, the master regulator of cell polarity establishment in *S. c.* to exocytosis. Boi1 and Boi2 are scaffold proteins of similar structure and partially redundant functions that both bind Cdc42. *BOI1* and *BOI2* exhibit synthetic lethality and a knock-down of *BOI1* in a *BOI2* deletion strain causes a severe accumulation of post-golgi vesicles in the daughter cell. In accordance with this observation, I found physical and genetic interactions between Boi1/2 and members of the vesicle fusion machinery. Furthermore, I provide evidence that Boi1 and Boi2 are on top of an interaction cascade to polarize the fusion of post-golgi vesicles to the tip of the cell.

Accordingly, I propose a model in which Boi1 and Boi2 serve as direct adapters between essential core components of the cell polarity and the vesicle fusion machinery and thereby contribute to the polarization of the secretory pathway.

Y3065B The NaCl-activated signaling network responsible for protein phosphorylation in yeast reveals potential decision points in the growth-versus-stress decision. *M. MacGilvray*[1], E. Shishkova[2], D. Chasman[3], J. Coon[2], A. Gasch[1]. 1) Laboratory of Genetics, University of Wisconsin - Madison; 2) Department of Chemistry, University of Wisconsin - Madison; 3) Wisconsin Institute for Discovery, University of Wisconsin - Madison.

Cells respond to stressful conditions using a complex, multi-faceted approach that includes changes in gene expression, protein abundance, and post-translational modification. Coupled to these changes is arrest of growth, suggesting that these are competing cellular processes. Although the affects of transcript changes in response to stress are well characterized in *Saccharomyces cerevisiae*, we have only a rudimentary understanding of the upstream regulatory network and post-translational protein modifications that regulate stress response. To gain a better understanding of the regulatory network that controls stress response, including how stress and growth response pathways are connected, we developed an experimental and computational workflow to infer stress-activated signaling networks using protein-protein interactions and phosphorylation changes. Using a integer linear programming approach, we combined high throughput protein interaction datasets with quantitative mass spectrometry performed on the lab strain, BY4741, and derivative mutants of the cell cycle protein Cdc14, the MAPK Hog1, and the phosphodiesterase Pde2, before and five minutes after NaCl exposure. The resulting network implicated new regulators in the yeast stress response, revealed a complex, hierarchical organization of kinases and phosphatases, and identified potential decision points in the growth-versus-stress decision. Interestingly, our network is enriched for yeast genes whose human orthologs are linked to diseases, such as cancer, that can be caused by defects in kinase regulation. Through identification of novel pathway and regulator connections, including those involved in stress response and growth, we will improve our understanding of both yeast physiology and human disease causing mechanisms.

Y3066C Identification of Interacting Partners of the Yeast Trans-Membrane Stress Sensor Protein Mtl1p as a Model for Drug Discovery in Fungi. *N. Martínez-Matías*[1], E. Santiago-Cartagena[1], I. Stagljar[2], J. Rodríguez-Medina[1]. 1) University of Puerto Rico, Medical Sciences Campus, San Juan, PR; 2) 1. Donnelly Centre, Department of Biochemistry, and Department of Molecular Genetics, University of Toronto, Ontario M5S 3E1 Canada.

Statement of the purpose – Cell wall and membrane–embedded proteins comprise a very special class of molecular components. They are usually involved in processes such as adhesion, signal transduction, and metabolism. Moreover, some of them have even been associated to disease. It is for this reason that these proteins, and also their cytosolic interacting partners, constitute major targets in drug discovery for pharmacotherapy. Despite being often difficult to study because of their hydrophobicity, methods have been developed to study membrane proteins and their interactions *in vivo*. In *Saccharomyces cerevisiae,* the membrane sensor Mtl1p has been shown to have a role in the cellular response to oxidative stress induced by exposure to H_2O_2, and to glucose starvation. The purpose of our research is the identification of protein partners whose interactions with the cytosolic domain of Mtl1p are relevant to its function in order to test them as targets for therapeutic drug discovery in pathogenic fungi. **Methods** – Our method is based in the *integrated Membrane Yeast Two Hybrid* (iMYTH) system for the detection of interactions between two proteins of interest. The membrane protein Mtl1p was employed as the *bait*, while all cytosolic and some membrane protein partners of Mtl1p that were captured by this assay (referred to as the *prey*) were subjected to a confirmatory test. **Summary of results** – We have preliminarily identified 25 novel prey proteins that interacted with the cytosolic domain of the Mtl1p bait and were classified as potentially relevant to its function. **Conclusions** – Our results demonstrate that novel interactions with cytosolic partners of trans-membrane proteins can be identified using this experimental approach. **Acknowledgments** - This research is supported by the University of Puerto Rico, Medical Sciences Campus, and NIH awards RCMI G12MD007600, RISE R25GM061838, and INBRE P20GM103475.

Y3067A Exploration of stress-induced genetic interactions in *Saccharomyces cerevisiae*. *V. Messier*[1], E. Koch[2], M. Costanzo[1], C. Myers[2], C. Boone[1], B. Andrews[1]. 1) Donelly Center, Toronto, Ontario, CA; 2) University of Minnesota, USA.

Our group has developed and exhaustively screened genetic interactions (GIs) at a genome-wide scale under standard growth conditions [1]. The Synthetic Genetic Array (SGA) methodology has also been used to explore how genetic interactions change in response to DNA damage and other stress conditions (e.g. [2, 3]), but a systematic exploration of the condition-sensitivity of genetic interactions has not been undertaken. We are building a conditional GI reference dataset, using high density single and double mutant arrays grown on agar plate. We aim at probing cellular responses to a panel of chemical-induced stress targeted at essential functions such as DNA and membrane integrity, transcription, translation and protein turnover, nutrient quality and sensing. As a proof of principle, we crossed ~200 condition-sensitive mutant query strains with a mini-array of 1200 highly informative mutant bait strains and performed a SGA analysis in six different growth conditions. Surprisingly, we observed genes that previously failed to be characterized by SGA under standard growth conditions displaying conditional GIs for specific functions, revealing their purpose in stress-induced cells. For example, the gene URM1 encoding an ubiquitin-like protein shared GIs with genes annotated for chromatin remodeling and signal transduction, both functions being suggested for urmylation in cells [4, 5]. Moreover, genes implicated in condition stress-response demonstrate GIs enrich for expected and unexpected functions, the latest represent new functions or functional switch under specific stress conditions. For instance, the target of rapamycin (TOR) kinase (TOR1, TOR2) and regulators shared GIs enrich for ribosome biogenesis (10^{-8}), chromatin remodeling (10^{-6}), chromosome segregation (10^{-5}) and DNA replication (10^{-5}). Chromosome missegregation and chromatin remodeling were described as a target in rapamycin treated yeast cells [6, 7]. The regulation of DNA replication by rapamycin-induced stress is novel and requires further validation. We, therefore, assemble data supporting a role of TOR activity in DNA replication under rapamycin growth.

[1] Costanzo et al. 2016 *Science*, under revision; [2]Bandyopadhyay et al. 2010 *Science* 300, 1385-9; [3] Martin et al. 2015 *Nat Genet.* 47, 410-4; [4] Haarer et al. 2013 G3 3, 553-61; [5] Goehring et al.2003 *MBOC* 14, 4329-4; [6] Worley et al. 2016 *G3* 6, 463-74; [7] Choi et al. 2000 *Cur biol.* 10, 861-4.

Y3068B The Yeast Kinase Ksp1 Regulates Cellular Stress Response and mRNP Dynamics. *Nebibe Mutlu*, Daniel T. Sheidy, Philip C. Andrews, Anuj Kumar. University of Michigan, Ann Arbor, MI.

The cellular stress response in yeast and metazoans is complex, encompassing precisely regulated signaling pathways that coordinate processes such as nutrient sensing and transcriptional/translational regulation. The *S. cerevisiae* kinase Ksp1 plays a central role in this process. *KSP1* was first identified in a screen for high-copy suppressors of a mutation in the nucleotide exchange factor *SRM1*. More recently, we and others have identified *KSP1* as a gene required for wild-type yeast pseudohyphal growth, likely through its association with the Target of Rapamycin Complex (TORC1). Ksp1 negatively regulates autophagy via TORC1, and its localization changes during pseudohyphal growth. In particular, we have found that Ksp1 kinase activity is required for the transition to a pseudohyphal growth form in competent strains of *S. cerevisiae*. The signaling network of Ksp1, however, has not been studied directly on a proteome-wide scale in a filamentous background. To address this, we undertook an analysis of Ksp1 signaling through quantitative phosphoproteomics, identifying proteins differentially phosphorylated in a catalytically inactive kinase-dead Ksp1 mutant under conditions of nitrogen and glucose stress. Gene Ontology term enrichment analysis of the set of proteins differentially phosphorylated upon loss of Ksp1 kinase activity identifies a statistically significant set of proteins associated with mRNA-protein (mRNP) granule formation. mRNPs, encompassing P-bodies and stress granules, present an additional form of stress response thought to regulate mRNA translation. We find that Ksp1 localizes to mRNP granules in a filamentous background, in agreement with recent studies of mRNPs in a non-filamentous yeast strain. Moreover, the kinase activity of Ksp1 is required for wild-type localization of several mRNP component proteins/regulators upon growth to a high cell density, including Pbp1 and the P21-activated kinase ortholog Ste20. We further find that Ksp1 kinase activity regulates the localization of Kog1, a subunit of TORC1. Collectively, these results suggest a function for Ksp1 in coordinating TORC1-signaling and the regulation of mRNP dynamics, likely through phosphorylation of key effectors. Ongoing studies are aimed at identifying the mechanism through which this signaling is coordinated.

Y3069C Investigation of the nuclear translocation and degradation of Mth1. *J. Pierce*, M. Johnston. University of Colorado, Aurora, CO.

The rapid response of *Saccharomyces cerevisiae* to glucose is regulated by several pathways. The Snf3/Rgt2-Rgt1 (SRR) pathway detects extracellular glucose and regulates induction of *HXT* gene expression, allowing the cells quickly to import glucose. In the absence of glucose, Mth1 acts as a co-repressor with the Rgt1 DNA-binding protein to repress *HXT* gene expression. Upon addition of glucose, Mth1 exits the nucleus, interacts with the glucose sensors, where it is phosphorylated by the casein kinases Yck1/2. Mth1 is consequently ubiquitinated and then degraded, relieving repression of *HXT* gene expression. We determined by a heterokaryon assay that shuttling of Mth1 between the nucleus and the cytoplasm is not regulated by glucose. Inhibiting shuttling by adding an NLS to Mth1 prevented derepression of *HXT1* expression. Adding an NES to Mth1 prevents its degradation but does not interfere with the induction of *HXT1* expression. These results suggest that Mth1 is degraded in the nucleus and must be exported from the nucleus for *HXT* expression to be induced. Intrinsic NLS and NES sequences must be present in Mth1 to permit its shuttling between the nucleus and the cytoplasm. We located one NLS through truncation and internal deletion experiments. Previous studies identified eight serine residues in conserved Yck1/2 consensus phosphorylation sequences in Mth1 required for its degradation. We found that phosphorylation of only four of these eight serine residues, most importantly Ser133, are necessary for degradation of Mth1. Therefore, nuclear translocation and phosphorylation of Mth1 are required for its degradation and the ability of *Saccharomyces cerevisiae* to rapidly respond to glucose.

Y3070A Novel interacting protein partners of Wsc1p and Mid2p identified by iMYTH and TAP-MS. *E. Santiago-Cartagena*[1], M. Babu[2], I. Staglar[3], J. R. Rodriguez-Medina[1]. 1) University of Puerto Rico-Medical Sciences Campus, San Juan, PR; 2) Research and Innovation Center, University of Regina, Saskatchewan, Canada; 3) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Canada.

The PKC1-Cell Wall Integrity Pathway (PKC1-CWIP) is essential for survival and maintenance of cell wall integrity in pathogen and model yeast cells. In *S. cerevisiae*, the main protein sensors of the PKC1-CWIP, Wsc1 and Mid2, are responsible for the activation of the pathway in the presence of cell wall stress. Therefore, Wsc1p and Mid2p are an attractive target for antifungal drugs because they contribute to cell viability. The purpose of this research is to validate the sensor-interacting proteins previously identified by integrated Membrane Yeast Two-Hybrid (iMYTH) using Tandem Affinity Purification coupled to Mass Spectrometry (TAP-MS). To validate the iMYTH interactions, Wsc1-TAP and Mid2-TAP bait strains were co-precipitated by affinity to calmodulin sepharose beads and compared with a wild-type BY4741 strain under normal growth conditions. Results showed that Wsc1p and Mid2p baits were recovered. One protein, encoded by RPL40A gene was confirmed as a Mid2p interactor. Twenty new interactors were identified for Wsc1p and 23 new interactors for Mid2p in the TAP-MS. Ndr1p was the only interactor found to be previously annotated in the BioGRID. In conclusion, the Mid2-Rpl40a interaction was validated by both iMYTH and TAP-MS. The novel Wsc1p and Mid2p-interacting proteins identified come from a range of different biological processes, including stress response, protein synthesis and cell wall organization.This research was supported by University of Puerto Rico-Medical Sciences Campus, University of Toronto, University of Regina and NIH grants G12MD007600, U54MD007587, P20GM103475 and R25GM061838.

Y3071B Spatial control of translation repression and polarized growth by conserved NDR kinase Orb6 and RNA-binding protein Sts5. *Illyce Suarez*[1], David J. Wiley[1], Maitreyi Das[1,3], Marbelys Rodriguez Pino[1], Chuan Chen[1], Tetsuya Goshima[6], Kazunori Kume[5], Dai Hirata[5], Takashi

Toda[4,5], Fulvia Verde[1,2]. 1) University of Miami Miller School of Medicine, Miami, FL, FL; 2) Marine Biological Laboratory, Woods Hole, MA, USA; 3) Department of Biochemistry and Cellular and Molecular Biology. The University of Tennessee, Knoxville, TN, USA; 4) The Francis Crick Institute, Lincoln's Inn Fields Laboratory, 44 Lincoln's Inn Fields, London WC2A 3LY, United Kingdom; 5) Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi-Hiroshima 739-8530, Japan; 6) National Research Institute of Brewing, 3-7-1 Kagamiyama, Higashi-Hiroshima 739-0046, Japan.

Recent work has highlighted the role of RNA-binding proteins in the formation of ribonucleoprotein (RNP) granules by phase transition, but the mechanisms and regulation of this process are not fully understood. Here, we describe a novel function of the conserved NDR kinase Orb6 in inhibiting the localization of mRNA-binding protein Sts5 into RNPs. In fission yeast, Orb6 kinase has an established function in cell morphogenesis through the spatial control of Cdc42 GTPase. In this work, we show that regulation of Sts5 represents a genetically separable mechanism by which Orb6 kinase promotes polarized cell growth.

We find that Orb6 kinase inhibits the formation of Sts5 puncta that often colocalizate with processing (P) bodies by promoting Sts5 interaction with 14-3-3 protein Rad24. In addition, Orb6 kinase prevents degradation of Sts5-bound mRNAs, many of which encode proteins that have been shown to be essential for polarized cell growth. The condensation of Sts5 into cytoplasmic puncta is spatially and temporally controlled by Orb6 kinase, and interfering with this regulatory mechanism disrupts the pattern of polarized cell growth. Our work thus uncovers a role for Orb6 kinase in the spatial control of translational repression during normal cell morphogenesis.

Y3072C Ty1 integrase interacts with RNA polymerase III-specific subunits to promote insertion of Ty1 elements into the *Saccharomyces cerevisiae* genome. *S. T. Cheung*[1,2], L. Ma[2], P. H. W. Chan[3], H. Hu[4], T. Mayor[1,3], H. Chen[4], V. Measday[1,2]. 1) 1Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada; 2) Wine Research Centre, University of British Columbia, Vancouver, BC, Canada; 3) Centre for High-Throughput Biology, University of British Columbia, Vancouver, BC, Canada; 4) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan 115.

The Ty1 retrotransposon belongs to a family of long terminal repeat elements in the *Saccharomyces cerevisiae* genome that replicates through an mRNA intermediate and is subsequently reverse transcribed into cDNA for genome insertion mediated by Ty1-encoded integrase (IN). Like many eukaryotic retroviruses, Ty1 insertions avoid the coding regions of the genome in order to prevent lethality of the host and propagate. Ty1 elements insert within a 1-2 kb region upstream of actively transcribed RNA Polymerase (Pol) III genes but the host factors that mediate Ty1 element insertion remained elusive for decades. We purified Ty1IN from yeast cell lysates using anti Ty1IN antibody-coupled magnetic beads followed by mass spectrometry analysis. The Ty1IN co-purified proteins were enriched with peptides corresponding to multiple RNA Pol III subunits. To further investigate the interaction between Ty1IN and Pol III subunits, we purified Pol III using GFP-Trap Immunoprecipitation (IP). Co-IP experiments with multiple GFP-tagged Pol III subunits and Ty1IN showed that Ty1IN interacts with most Pol III-specific subunits, but not with shared Pol subunits. However, since RNA Pol III is a stable complex and salt washes after GFP-Trap purification may not eliminate indirect protein interactions, we conducted an *in vitro* binding assay with purified Pol III-specific subunits and Ty1IN produced in *E. coli.* We demonstrated that the Pol III complex purified from yeast and Rpc31, Rpc34 and Rpc53 purified from *E. coli* interact with Ty1IN *in vitro*. To further characterize the Pol III IN-binding domain we used a strain carrying an N-terminal truncation mutant of Rpc53, *rpc53Δ2-280* and found that Ty1 element insertion upstream of an integration hotpot, *SUF16*, a glycine tRNA gene, was ablated. Yet, the overall transposition frequency of the *rpc53Δ2-280* mutant strain was reduced by only ~25%, suggesting possible retargeting of Ty1 element insertions in the genome. Furthermore, we found that the interaction between Ty1IN and Rpc37, but not other Pol III subunits, is disrupted in the *rpc53Δ2-280* strain. This data suggests that Ty1IN may bind to multiple subunits of Pol III to mediate Ty1 integration independently. Together, we reported that Ty1IN interacts with RNA Pol III through the Rpc53/Rpc37 subcomplex, and plausibly via the Rpc82/Rpc34/Rpc31 subcomplex, to mediate insertion of Ty1 elements upstream of Pol III-transcribed genes. Since the structure of Ty1IN and the mechanism of genome integration are highly conserved among retrotransposons and eukaryotic retroviruses such as HIV-1, our study provides important insights into how INs seize host proteins for targeted genome insertion.

Y3073A The Shu complex promotes error-free tolerance of alkylation-induced base-excision repair products. *B. W. Herken*[1], S. K. Godin[1], Z. Zhang[1,2], J. W. Westmoreland[3], A. G. Lee[1], M. J. Mihalevic[1], Z. Yu[1,2], R. W. Sobol[1,4], M. A. Resnick[3], K. A. Bernstein[1]. 1) University of Pittsburgh School of Medicine, Pittsburgh, PA; 2) Tsinghua University School of Medicine, Beijing, China; 3) National Institute of Environmental Health Sciences, Research Triangle Park, NC; 4) University of South Alabama Mitchell Cancer Institute, Mobile, AL.

The budding yeast Shu complex is an obligate heterotetramer that promotes error-free homologous recombination by facilitating the assembly of Rad51 filaments on single-stranded DNA after the resection of a double-strand break. The Shu complex is composed of two Rad51 paralogs, Psy3 and Csm2, a third protein Shu1, and a SWIM domain containing protein called Shu2. Orthologs of the Shu complex are conserved among eukaryotes, and mutations in human Rad51 paralogs are associated with cancer predisposition and Fanconi anemia. We have been characterizing a novel function for the Shu complex in the repair of DNA alkylation damage during post-replicative repair (PRR). Here we uncover the interplay between the Shu complex members and base excision repair (BER) machinery upon MMS-induced alkylation damage, providing evidence for the Shu complex's role in bypassing certain BER intermediates. Specifically, cells that lack the BER glycosylase *MAG1*, or the apurinic/apyrimidinic (AP) endonucleases *APN1* and *APN2,* preferentially utilize HR to repair MMS damage. The increased HR observed in *mag1Δ* and *apn1Δ apn2Δ* cells is Shu complex dependent. Furthermore, mutation of the translesion polymerase *REV3*, which is involved in error-prone PRR, further sensitizes these cells to MMS in a *shuΔ* background. Our work illustrates a role for the Shu complex in the promotion of error-free DNA damage resolution in a replicative context and provides further evidence for its importance in maintaining genomic stability.

Y3074B The role of the MRX complex in chromosome mobility and homology search. *F. Joseph*, R. Rothstein. Columbia University Medical Center, New York, NY.

Homologous recombination (HR) is the process used by DNA sequences to recover missing genetic information at the site of a double stand break (DSB). An essential aspect of HR that remains poorly understood is the mechanism of homology search. To study this aspect of HR in diploid yeast cells, a fluorescent chromosome tagging system was used to track chromosome mobility of the two homologs in response to DSBs. We previously found that yeast cells dramatically increase their chromosome mobility in response to DSBs. This response has two components: the first is a large "local" increase in the mobility of the cut chromosome; the second is a smaller "global" increase in mobility at other undamaged sites in the genome (including the homolog). The MRX complex, consisting of Mre11, Rad50, and Xrs2 proteins, plays multiple roles in HR; such as processing broken DNA ends and initiating DNA damage checkpoint signaling. Because of its multiple roles, we hypothesized that MRX would play a role in regulating chromosome mobility. To test this hypothesis we deleted Mre11, the protein-scaffolding component of the complex in cells with our chromosome tagging system. Chromosome mobility was assayed both before and after DNA damage. We find that when a site-specific DSB is induced with I-*Sce*I at the tagged locus in an *mre11Δ* strain, the local mobility of that site is greatly delayed. However, when the same mutant cells are treated with 40 Gy of ionizing radiation (IR), producing ~ 4 random DSBs in the genome, mobility of the tagged locus displays a normal global mobility response. This global mobility response can be blocked by inhibition of the PI3K checkpoint kinases using caffeine. In addition, we find that IR treatment of *mre11Δ* cells results in an increase in the number of both Rad51 and Rad52 foci that is indistinguishable from wild type cells. In contrast, accumulation of Rad51 and Rad52 foci after a site-specific DSB is delayed compared to a wild type strain. We propose that a single DSB requires resection initiation by the MRX complex to generate checkpoint dependent mobility, whereas, IR treatment rapidly results in checkpoint dependent DNA mobility due to the accumulation of multiple processed ends created by alternative means. Finally, we observe that pairing between tagged homologous chromosomes after induction of a site specific DSB is a delayed in a *mre11Δ* strain. This delay correlates with the observed delay in local mobility, supporting a model in which local mobility is necessary for pairing of homologous chromosomes.

Y3075C Unsolicited rDNA copy number variants frequently occur in yeast deletion collections and transformations. *Elizabeth X. Kwan*[1], Xiaobin S. Wang[1,2], Haley M. Amemiya[1], M. K. Raghuraman[1], Bonita J. Brewer[1]. 1) University of Washington, Seattle, WA; 2) Columbia University, New York, NY.

Ribosomal DNA (rDNA) is present in many copies in eukaryotic organisms and has been recently implicated in the regulation of gene expression, responses to replication stress, telomeric silencing, and aging. The budding yeast *S. cerevisiae* maintains 150 rDNA copies in a tandem array on chromosome XII. Instability of the yeast rDNA array is well documented, but the rDNA locus typically returns to wildtype copy numbers after perturbation, suggesting internal regulation of copy number maintenance. We sought to identify determinants of yeast rDNA copy number by performing a screen using the Yeast Knock-Out (YKO) single gene deletion collection. We also wanted to assess the relationship between rDNA number and yeast replicative lifespan and therefore examined the rDNA sizes of 434 YKO strains, 221 of which were reported to have extended replicative lifespans. We found no correlation between yeast longevity and rDNA copy number, but did identify 64 YKO candidates with significantly changed rDNA copy numbers. However, in the process of validating candidates using *de novo* gene deletions and tetrad analysis, we found that the altered rDNA phenotypes in the candidate YKO strains were not associated with the deleted genes. Instead, we found that rDNA copy number variants (CNVs) appeared at random within multiple transformants from each *de novo* gene deletion. Further examination revealed that cells taken through standard transformation protocols generated rDNA CNVs and that exposure to lithium acetate alone increased the rate of observed rDNA CNVs. Since lithium acetate transformation is commonly used in yeast strain construction, unanticipated rDNA CNVs could be potential passenger mutations that may have repercussions in the increasing number of reported cellular processes in which rDNA copy number matters.

Y3076A The Saccharomyces Genome Database Variant Viewer. *Olivia W. Lang*, Travis K. Sheppard, Benjamin C. Hitz, Stacia R. Engel, Giltae Song, J. Michael Cherry, The SGD Project. Stanford University, Palo Alto, CA.

The complete genome sequence of the budding yeast *S. cerevisiae*, and its annotation, is by the Saccharomyces Genome Database (SGD;http://www.yeastgenome.org), as a public resource for researchers. Until recently, the genome sequence displayed at SGD has been derived solely from the S288C strain background. We have now incorporated 11 additional *S. cerevisiae* genomes into SGD, as well as providing their annotation. We have also developed a new Variant Viewer to further support the community's analysis of these new data. Variant Viewer allows visualization and comparison of sequences from multiple strains. Users can quickly scan the entire genome, or a subset of genes, for overviews of both slight and significant sequence differences. SGD's Variant Viewer also includes a more detailed comparison of sequence differences between strains that highlights insertions, deletions, and SNPs that is available for both viewing and download. The Variant Viewer offers yet another way to explore the genomic sequence data available at SGD, as part of our continuing mission to educate students, enable bench researchers and facilitate scientific discovery. This work is supported by a grant from the NHGRI (U41 HG001315).

Y3077B The yeast Ty1 retrotransposon requires Nuclear Pore Complex subunits for transcription and genomic integration. *S. Manhas*, L. Ma, V. Measday. University of British Columbia, Vancouver, BC, CA.

Background: Nuclear pore complexes (NPCs) are molecular gateways that orchestrate cargo between the cytoplasm and nucleus and regulate cellular processes such as, gene expression. We utilized the Ty1 retrotransposon of *Saccharomyces cerevisiae* (*S. cerevisiae*) to study retroviral integration because Ty1 and retroviruses, including human immunodeficiency virus (HIV-1), have similar replication cycles and structurally and functionally conserved integrase (IN) enzymes required for genomic insertion of retrotransposons and retroviruses. Ty1 IN interacts with

subunits of RNA polymerase III to mediate insertion upstream of RNA Polymerase III transcribed genes such as tRNA genes. Since yeast tRNA genes are recruited to NPCs, we examined the role of NPC subunits, called nucleoporins (Nups), in Ty1 transposition.
Methods: We tested a panel of 19 Nup mutant yeast strains for Ty1 mobility defects by monitoring the formation of HIS+ colonies in Nup mutant strains carrying a plasmid with a *HIS3* marked Ty1 element. To determine if the defects in Ty1 mobility in Nup mutants were due to differences in the availability of Ty1 transposition intermediates, we quantified Ty1 mRNA levels in these yeast strains by quantitative PCR (qPCR), Ty1 cDNA levels by southern blotting, and Ty1 Gag protein levels by western blotting. We also examined Ty1 nuclear localization by fluorescence microscopy and tRNA gene expression by qPCR. We monitored endogenous Ty1 insertion events in our panel of Nup mutant strains by PCR to analyze Ty1 integration levels upstream of the glycine tRNA gene *SUF16*, which is a hot spot for Ty1 targeting. Because the *SUF16* locus is located within the pericentromere of chromosome III, which may have different targeting requirements than other chromosome regions, we also measured Ty1 insertion upstream of the serine *SUP61* tRNA gene which is located on the right arm of chromosome III.
Results: Of the 19 mutants tested here, 11 (*nup120Δ, sec13-1, nup170Δ, nup53Δ, nup188Δ, nup192-15, nup60Δ, mlp1Δ, mlp2Δ, nup159-1, pom34Δ*) Nup mutant yeast strains had Ty1 mobility defects and altered Ty1 element insertion upstream of the *SUF16* and *SUP61* loci. Interestingly, but unexpectedly, we discovered that Nup mutant yeast strains that had wild type levels of Ty1 mobility (*nup84Δ, nup133Δ, ndc1-4, nup42Δ, nup100Δ, pom152Δ, nup2Δ*), also had impaired Ty1 element insertion upstream of *SUF16* and *SUP61*. The *nup60Δ* strain had strikingly impaired levels of Ty1 mRNA resulting in minimal Ty1 Gag protein, and cDNA production whereas the remaining Nup mutant yeast strains had minimal defects in Ty1 transcription, translation, cDNA production or Ty1-IN localization. Taken together, our data suggests that the NPC may function as part of both the Ty1 element transcription and integration machinery. .

Y3078C A systematic appraisal of Cdc13's domain organization. *S. Y. Mersaoui*, R. J. Wellinger. Université de Sherbrooke, Sherbrooke, Québec, CA.

Budding yeast Cdc13p is essential and a key protein involved in telomere maintenance and genome stability. Genetic and high-resolution structural studies of several of its fragments reveal an architectural organisation with five distinct domains , which include four OB-fold domains and one recruitment domain. However, many details on the *in vivo* roles of those domains and their potential inter-domain interactions remained elusive. In this work we constructed tagged versions of the Cdc13 protein and then generated five alleles, where each one contains a single deletion of a proposed domain. To study the roles of each domain in telomere maintenance, we took advantage of *cdc13-independent* cells. Although viable, these strains "Adapt" and inactivate check-point signalling to bypass the accumulation of telomeric DNA damage. The results show:

1- The first 80 amino acids of the OB1 domain contribute to stabilization of the Cdc13 protein. *cdc13Δ*-cells with this mutated protein show a strong capping defect. However this capping defect can be rescued when this mutated protein is co-expressed with the hypomorphic *cdc13-1* allele. This result suggests a physical interaction and therefore supports a model where Cdc13 occurs as homodimer at the telomere. **2-** Co-IP experiments, using two tagged-versions of Cdc13 strongly argue for this homodimer model. Surprisingly, the hypomorphic *cdc13-1* allele doesn't display any sign of dimerization defect at restrictive temperatures. Our data rather support the idea that Cdc13 dimerization requires at least two interfaces where the DBD domain contains the core dimerization interface. **3-** The third domain (OB2) is essential for cell viability. *cdc13-ob2Δ* is unable to bind telomeric DNA *in vivo* and results in a capping deficiency in both *cdc13Δ* and *cdc13-1* (*ts*) strains. This mutated protein still behaves as a dimer, indicating that OB2 is not involved in dimer formation. However, immunofluorescence analysis shows a mis-localized protein residing in the cytoplasm. Re-inserting a heterologous NLS peptide in place of OB2 restores nuclear localization, *cdc13-independent* capping and suppresses the *ts* phenotype of *cdc13-1* mutant strain. Moreover, expression of the *cdc13-ob2Δ-NLS* results in hyper elongated telomere, suggesting an additional function. **4-** *Cdc13-independent* cells are sensitive to damage induced by genotoxic drugs. Remarkably, ectopic expression of Cdc13 cells restores a normal resistance to those drugs. This observation suggests an unexpected role of Cdc13 in DNA damage response at telomeres. Results obtained with the same deletions as described above, we conclude that this sensitivity to genotoxic drugs in the *cdc13Δ* cells may be due to the eroded state of the telomeres in these cells.

Y3079A Investigating genome instability induction mechanisms in yeast *DIS3* mutants. *K. Milbury*[1,2], P. Stirling[1,2,3]. 1) Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, British Columbia, CA; 2) Genome Science & Technology, University of British Columbia, Vancouver, British Columbia, CA; 3) Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, CA.

Chromosome instability (CIN) is characterized by an increased rate of the unequal distribution of DNA between daughter cells. These large changes in chromosome structure or number can occur due to both mitotic defects leading to aneuploidy and DNA damage-induced chromosome rearrangements. Previous large-scale screens for CIN genes identified *DIS3*, which codes for a catalytic component of the core RNA exosome complex, as a novel CIN gene in the yeast *Saccharomyces cerevisiae*. Presumed reduction-of-function mutations in the human *DIS3* orthologue have been identified in roughly 11% of multiple myeloma (MM) cases. We sought to determine the mechanism of CIN in *DIS3* mutants and to recapitulate MM-associated point mutations at conserved sites in yeast cells, in order to understand potential connections of CIN to MM. We have found that MM-associated *DIS3* mutations induce DNA:RNA hybrid accumulation and increased rate of CIN, although immunofluorescence analysis of DNA damage foci revealed no increase in double-strand breaks. Microarray analysis of one MM mutant has additionally demonstrated downregulation of cell cycle components, consistent with the potential for mitotic defects. Further, genetic interaction profiling by synthetic genetic array indicates MM-associated *DIS3* mutations synthetically interact with rRNA processing proteins, as well as a host of mitotic regulators and metabolic pathways. Together, these results demonstrate extensive phenotypic consequences of MM-associated point mutations in *DIS3*, and support a model for CIN in *DIS3* mutants involving defects in cell cycle processes.

Y3080B Diverse Sites of Chromosome Breakage in Retrotransposon Overdose Yeast Strains. *L. Z. Scheifele*[1], C. M. Lanzillotta[1], C. McLeod[1], S. Minikel[1], N. Monteleone[1], B. Yang[1], S. J. Wheelan[2]. 1) Department of Biology, Loyola University Maryland, Baltimore, MD; 2) Department of Oncology, Division of Biostatistics and Bioinformatics, Center for Computational Genomics, Johns Hopkins School of Medicine, Baltimore, MD.

Fragile sites are frequent sites of chromosomal rearrangements. Human cells contain numerous fragile sites that differ remarkably in their propensity for instability (Glover 1984), yet we do not understand why a subset of fragile sites is more prone to instability and to rearrangement than the other fragile sites. We have used retrotransposon overdose (RO) yeast cells as a model system for fragile site stability; retrotransposon pairs are known to serve as sites of instability (Lemoine 2005), and RO strains contain many more single retrotransposon elements and retrotransposon pairs in a variety of genomic contexts. RO strains show an elevated level of instability under the same conditions as human fragile sites such as replication stress. When RO strains are subjected to treatment with methylmethanesulfonate (MMS) in combination with a temperature-sensitive polymerase-α allele (*pol1-17*) or deletion of the replication checkpoint protein *MEC1*, there is an increased incidence of chromosome rearrangement in RO strains relative to wild-type. While RO strains displayed a diverse complement of chromosome aberrations, a subset were recurrent among independent isolates, suggesting that particular chromosome loci were more susceptible to chromosome breakage than others. Genome sequencing of strains containing chromosome aberrations revealed that many aberrations were coincident with Ty1 and Ty2 retrotransposons, with particular retrotransposon elements more likely than others either to be present at rearrangement breakpoints or to be deleted follwing replication stress. When comparing the sites of rearrangement induced in *pol1-17* cells and *mec1* cells, there were few common chromosome aberrations, suggesting that different mechanisms of replication stress may induce rearrangement at distinct sites. The location of chromosome aberrations was also examined following experimental lab evolution. As expected, RO strains displayed both a greater number and a greater diversity of chromosome rearrangements following lab evolution. Due to the destabilizing effect of retrotransposons, we expected frequent loss of Ty elements following evolution, yet we discovered both new insertions of Ty elements as well as deletions; these deletions were just as likely to occur at single retrotransposon elements as at retrotransposon pairs. These data suggest that the arrangement of retrotransposons as pairs is itself insufficient to make a locus unstable, but rather that additional factors govern the varying stability of these repetitive regions.

Y3081C Rad51 regulates the global mobility response to double-strand breaks. *Michael Smith*, Rodney Rothstein. Columbia University Medical Center, Department of Genetics and Development, New York, NY 10032.

In *Saccharomyces cerevisiae*, chromosomal mobility increases after the formation of a double-strand break (DSB). Cut loci expand their radius of exploration substantially in a process termed local mobility. In addition, there is an increase in global mobility following a DSB, in which even undamaged loci expand their radius of exploration. Here, we examine the role of the RecA-like recombinase Rad51 in regulating the mobility of chromosomes in diploid budding yeast cells after DSB formation. We find that global mobility is dependent on *RAD51* throughout the cell cycle, and that Rad51 regulates mobility in G1 as well as S phase chromosomes. This regulation depends on a functional Walker A ATPase domain, but does not require dsDNA binding or strand exchange activities of Rad51 protein. Although some ssDNA binding is essential for global mobility, extensive filament formation is dispensable, as *rad52Δ* cells are still capable of promoting increased movement. Finally, we find that DNA damage checkpoint induction is both necessary and sufficient to induce global mobility in diploid cells. Interestingly, this induction, even in undamaged cells, is dependent upon the presence of Rad51. We propose that Rad51 serves as an important damage sensor that signals to the DNA damage checkpoint machinery to promote mobility.

Y3082A How telomeres are maintained: the role of Ku-mediated telomerase recruitment. *David C. Zappulla*, Evan P. Hass. Johns Hopkins University, Baltimore, MD.

The ends of linear eukaryotic chromosomes shorten with each cell cycle due to the end-replication problem, ultimately leading to senescence and death. Telomerase countervails this deficiency by synthesizing telomeric repeats by reverse transcribing a portion of its RNA subunit, thus providing cell proliferation potential, critical to aging and cancer. It has become apparent that telomerase provides telomere-length homeostasis by preferentially extending the shortest ends. However, it is not clear how this selective telomerase action is achieved. One major mechanism by which telomerase is regulated is its recruitment to telomeres. In *S. cerevisiae*, two subunits of the RNA-protein enzyme have been shown to be involved in recruitment: Est1 recruits telomerase to the telomeric end-binding protein Cdc13, whereas Ku recruitment was also known to occur, but it was not clear how. We have determined that telomerase recruitment by Ku requires the interacting protein, Sir4, which is an integral part of telomeric silent chromatin. Epistasis analysis puts Ku, Sir4 and the Ku-binding site of telomerase RNA, TLC1, in the same pathway. Additionally, we were able to bypass Ku in telomerase recruitment and telomere length regulation by tethering telomerase RNA directly to Sir4. Having established the Ku-Sir4 recruitment pathway, we then evaluated its role in telomere length regulation. Introducing multiple Ku-binding sites in telomerase RNA caused telomere hyperlengthening, demonstrating the acute sensitivity of telomere length to the Ku-mediated telomerase recruitment pathway. This phenotype reminded us of the runaway-telomeres of *rif1*Δ and *rif2*Δ mutants and since Rif proteins also directly compete with Sir4 for binding to the telomeric double-stranded DNA binding protein Rap1, we tested if hyperelongated telomeres in *rif*Δ mutants require Ku function in telomerase. We found that nearly wild-type telomere length returns to *rif*Δ cells upon deletion of the Ku-binding hairpin in TLC1. These results suggest that direct interplay between Rif and Sir4 proteins along the distal portions of chromosomes is key to sensing telomere length and, in turn, appropriately recruiting telomerase preferentially to the shortest telomeres.

Y3083B Combinatorial histone readout by the dual PHD domains of Rco1 mediates Rpd3S chromatin recruitment and the maintenance of transcriptional fidelity. *Julia DiFiore*[1], Stephen McDaniel[1], Jennifer Fligor[2], Chun Ruan[3], Haochen Cui[3], Joseph Bridgers[4], Angela Guo[4], Bing Li[3], Brian Strahl[1,4]. 1) Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Northwestern

University Feinberg School of Medicine, Chicago, IL; 3) UT Southwestern Medical Center, Dallas, TX; 4) Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC.

The PHD domain is found in many chromatin-associated proteins and functions to recruit effector proteins to chromatin through its ability to bind both methylated and unmethylated histone residues. Here, we show that the dual PHD domains of Rco1 – a member of the Rpd3S histone deacetylase complex recruited to transcribing genes – operate in a combinatorial manner in targeting the Rpd3S complex to histone H3 in chromatin. While mutations in either the first or second PHD domain allow for Rpd3S complex formation, the assembled complexes from these mutants cannot recognize nucleosomes or function to maintain chromatin structure and prevent cryptic transcriptional initiation from within transcribed regions. Taken together, our findings establish a critical role of combinatorial readout in maintaining chromatin organization and in enforcing the transcriptional fidelity of genes.

Y3084C Defects in the nucleosome entry-exit site impair transcription termination. *A. Elizabeth Hildreth*, Karen M. Arndt. University of Pittsburgh, Pittsburgh, PA.

In eukaryotes, the chromatin template acts as a restrictive barrier to RNA polymerase II (Pol II), the molecular machine that transcribes protein coding and some noncoding RNAs. Chromatin consists of repeating nucleosomes, which contain approximately 147 bp of DNA surrounding an octamer of histone proteins H2A, H2B, H3, and H4. Transcription is controlled by factors that remove or modify these nucleosome barriers, allowing Pol II to contact otherwise occluded DNA. The mechanisms by which chromatin is modified are well understood in regard to transcription initiation and elongation. Despite a few studies showing that transcription-coupled histone modifications and select chromatin remodelers are important for proper termination, little else is known about the role of chromatin at this final termination step. We are investigating the role of chromatin in transcription termination using *Saccharomyces cerevisiae* as a model. To screen for histone H3 and H4 residues required for proper termination, we have made use of a plasmid library encoding histones with all possible alanine substitutions and a well-characterized, growth-based termination reporter. Our unbiased screen has identified ten residues in H3 and H4, which when mutant, cause defects in termination. Interestingly, many of these residues reside in or near the DNA entry-exit site of the nucleosome. This protein surface, including portions of histones H3 and H2A, is responsible for coordinating the first 30 bp of DNA, thus regulating the stability of the protein-DNA complex. Analysis of strains harboring these mutant histones has revealed transcriptional read-through at endogenous genes, altered nucleosome occupancy at candidate loci, and defects in the placement of a transcription-coupled histone modification previously implicated in termination and repression of cryptic transcription. Research in other labs has shown that conditions that speed up the rate of transcription elongation cause termination factors that associate with Pol II to effectively miss the appropriate termination window. We have begun assessing the elongation rates of Pol II in mutant yeast strains to determine whether altered transcriptional rates contribute to the observed termination defects. Additionally, we have begun screening H2A residues within the DNA entry-exit site for similar defects. Together, these data implicate the DNA entry-exit site as an important player in proper termination of transcription.

Y3085A Controlling methylation during cell fate determination. *M. J. Law*, M. A. Finger. Rowan University-GSBS Stratford, NJ.

Cell fate decisions require integrating extracellular signaling cues into tightly regulated transcription programs. Communication between the RNA polymerase II holoenzyme complex and dynamic post-translational histone modifications is central for transcription. The RNA pol II holoenzyme is composed of two evolutionarily conserved yet functionally distinct complexes; the core mediator and the CDK8 submodule. In the budding yeast *Saccharomyces cerevisiae,* the CDK8 submodule, composed of cyclin C, Cdk8p, Med12p, and Med13p, plays important roles in mediating transcription of stress responsive and developmental genes. Histone Lysine methylation is a key regulator of transcription during cell differentiation. Histone H3Lys4 methylation ($H3K4^{Me}$), regulated by the opposing activities of the Set1p methyltransferase and the Jhd2p demethylase, is important for all phases of transcription. While much work has been done to investigate how $H3K4^{Me}$ regulates transcription, less is understood about how Set1p and Jhd2p are controlled during cell fate determination.

Work in my lab has uncovered an unexpected genetic interaction between the CDK8 submodule, the $H3K4^{Me}$ regulators, and yeast cell fates. When deprived of nutrition, diploid yeast can enter one of two partially overlapping differentiation pathways, meiosis or pseudohyphal growth (PH). We have recently published that both *CNC1*/*CDK8* and *JHD2* are required to inhibit PH while yeast are cultured in rich conditions. This study also revealed that Cnc1p/Cdk8p repress locus-specific $H3K4^{3Me}$ independently of *JHD2*, indicating that Cnc1p/Cdk8p can regulate transcription during differentiation by controlling $H3K4^{Me}$ levels.

To identify loci subject to *CNC1*-dependent $H3K4^{3Me}$ controls, we employed ChIP-seq. These experiments identified significant enrichment for $H3K4^{3Me}$ in metabolic promoters, which is consistent with a model in which *CNC1*/*CDK8* control $H3K4^{Me}$ of genes important for cell fate decisions. We further explored how *CNC1*-mediated $H3K4^{Me}$ controls are integrated into cell fate decisions by measuring $H3K4^{1Me, 2Me, 3Me}$ in rich and nutrient deprived growth conditions. These experiments revealed an important role for *CNC1*/*CDK8* inhibiting $H3K4^{Me}$ during nutrient deprivation, supporting a key function during cell fate determination. We determined that these methylation controls had a direct impact on target gene transcription using RT-qPCR. Finally, we queried whether *CNC1*/*CDK8* inhibit Set1p catalysis or recruitment by performing ChIP-qPCR directed towards Set1p or $H3K4^{3Me}$. These experiments found that Set1p recruitment is inhibited by *CDK8* as cells are poised to differentiate. These data support a model in which *CNC1*/*CDK8* control cell fate decisions by restricting Set1p recruitment in response to developmental cues.

Y3086B Histone H3K4 demethylase *JHD2* antagonizes the function of highly conserved histone chaperones FACT and Spt6 through the Rpd3S histone deacetylase complex. *K. Lee*, M. Meneghini. University of Toronto, Toronto, Ontario, CA.

Mammals encode a highly conserved family of histone H3 lysine 4 (H3K4) demethylases known as the JARIDs that have important roles in

regulating gene expression and controlling the balance between pluripotency and differentiation in stem cells. Gaining mechanistic and physiological insight on the consequences of JARID-mediated H3K4 demethylation has been challenging due the high level of redundancy in the mammalian JARID gene family. Methylated histones function as scaffolds for the recruitment of activating or repressive chromatin factors. The recruitment of specific factors is likely modulated by JARID-mediated H3K4 demethylation. We study Jhd2, the single JARID family H3K4 demethylase, in budding yeast *Saccharomyces cerevisiae*, using this simple model to investigate the consequences of JARID-mediated H3K4 demethylation. Genetic interrogation of *JHD2* revealed that temperature sensitive growth defects exhibited by mutants of the highly conserved histone chaperones Spt6 and FACT are rescued by *JHD2* deletion. Rescue is specific to loss of *JHD2* enzymatic activity and requires H3K4 trimethylation. *JHD2* deletion does not directly affect FACT protein stability or genomic localization, but genetic epistasis suggests that *JHD2* deletion rescues Spt6 and FACT temperature sensitivity indirectly through the modulation of the small Rpd3 histone deacetylase complex. In our working model, Jhd2-mediated H3K4 demethylation functions to regulate Rpd3S recruitment through its plant homeodomain containing subunit Rco1.

Y3087C Nutrient availability impacts chronological lifespan and Sir-based silencing in S. cerevisiae. *D. McCleary*, J. Rine. University of California at Berkeley, Berkeley, CA.

Sir2 is an NAD+-dependent protein deacetylase necessary for the formation of heterochromatin in the budding yeast Saccharomyces cerevisiae. It has been implicated in a number of important processes including the regulation of yeast aging. Sir2 function is also known to be influenced by a variety of nutrients, including nitrogen, phosphorus, and carbon source. Even though Sir2 regulates aging and can respond to changes in nutrient availability, it is unknown whether Sir2 is involved in the well-documented and evolutionarily conserved extension of lifespan resulting from calorie restriction. Past studies have produced conflicting results, possibly due to experimental constraints that require the use of unbuffered minimal media for all experiments.

We have now developed an assay whereby yeast chronological aging can be studied in any media, allowing for Sir2's aging role to be better understood. While Sir2 doesn't seem to be involved in calorie restriction-mediated lifespan extension in minimal media, it does seem to be necessary for full calorie restriction-mediated lifespan extension in complex media. We have also shown for the first time that increasing glucose concentration alters Sir2-mediated heterochromatic silencing at the mating type locus HML, and that the directionality of this effect is opposite on minimal and complex media. Deletion of SIR2 also leads to opposite aging phenotypes during growth on minimal and complex media, leading to the exciting hypothesis that the stability of Sir2-based heterochromatic silencing at HML or some other Sir2 deacetylation target links glucose availability to aging dynamics.

Y3088A The histone prolyl isomerases Fpr3 and Fpr4 regulate nucleolar chromatin architecture. *Christopher J. Nelson*, Neda Savic. University of Victoria, Victoria, BC, CA.

The structure of chromatin occludes much of the underlying DNA sequence. Accordingly, processes that impinge on DNA sequence, including the transcription of RNAs and the recombination, repair and replication of DNA, use histone chaperones and histone post-translational modifiers to overcome this barrier. Fpr3 and Fpr4 are yeast histone chaperones that also contain C-terminal FKBP histone prolyl isomerase domains. How these nuclear enzymes are used to regulate chromatin *in vivo* has not been well resolved.

Using reporter genes integrated throughout the yeast genome, as well as RNA-Seq, we find that Fpr3 and Fpr4 co-operate to establish a defined transcriptionally-silent chromatin domain within the rDNA locus. To obtain a global view of Fpr3 and Fpr4 function, we performed modified synthetic genetic interaction screens to query which biological processes are sensitive to these enzymes. These experiments revealed a synthetic lethality between Fpr4 and components of the RNA exosome; a complex that functions to degrade aberrant RNA transcripts. Together our results demonstrate that nuclear FKBPs and the RNA exosome co-operate to down-regulate RNAs generated from rDNA spacers. They also suggest that nuclear prolyl isomerases are particularly important at organizing rDNA chromatin. Finally, the effects of some chromatin modifiers on RNA expression may be masked by RNA exosome action.

Y3089B Chromatin regulation of pericentromic non-coding RNA in *Saccharomyces cerevisiae* and its effect on chromosome stability. *A. Ravi Shankar*, J. Gallagher. West Virginia University, Morgantown, WV.

Non-coding RNAs in higher eukaryotes are known to play important roles in genome stability, whereas in *Saccharomyces cerevisiae* any explicit roles are yet to be deciphered. Centromeres in eukaryotes are broadly classified as regional or point centromeres. *S. cerevisiae* contain point centromeres that do not exhibit centromeric silencing as regional centromeres do, but have specialized chromatin essential for function. In yeast, essential genes are often found near the centromere. Hence, non-coding RNA found near the centromere may be responsible for maintaining centromere function and prevent spreading of silencing chromatin. The minimal point centromeres are 125 base pairs in length with three conserved elements (CDE) and contain the centromere specific histone H3 variant, Cse4. Of the 16 yeast chromosomes almost half of them were found to have pericentromeric Stable Unannotated Transcripts (pSUTs) which were all found to be transcribed away from the CDEIII side of the yeast centromere. SUTs were discovered using genome-wide tiling arrays covering the entire yeast genome.
These pericentromeric noncoding RNAs were found to be regulated by Sir1 and Cac1, which are important for heterochromatic silencing at other regions of the genome. Studies on the interaction time of Sir1 at the centromere, and the cell cycle stage in which this takes place, will help fill the gap in knowledge regarding the silencing mechanisms involved. The Nonsense Mediated Decay pathway was mostly responsible for the degradation of these pericentromeric transcripts in the cytoplasm. Absence of pSUTs increases sensitivity to chemicals such as hydroxyurea when combined with *sir1, cac1* double mutants or with an NMD mutant suggesting that the pSUT itself may play a role in DNA

replication through centromeric DNA. The extent of conservation of this region within the *Saccharomyces* clade would emphasize its importance. Further studies will address the function of the pSUTs on chromosome stability and determine requirements for transcription.

Y3090C Interactions between variant histone H2A.Z and linker histone H1 in budding yeast. *J. Riggs*, J. Huang, P. Chen, L. Winston, M. Sica, S. Holmes. Wesleyan University, Middletown, CT.

Loss of variant histone H2A.Z in budding yeast cells leads to alterations in Sir protein-dependent transcriptional silencing, possibly due to impaired function of chromatin boundaries. We observed that deletion of *HHO1*, coding for linker histone H1, could suppress silencing defects seen in *htz1Δ* strains. We subsequently observed that loss of H1 also ameliorates the growth defects seen in strains lacking H2A.Z. *HTZ1* is synthetically lethal with several dozen yeast genes; these genes have widely varying functions. To examine the specificity of the H1-H2A.Z interaction we examined whether loss of H1 can restore viability to synthetically lethal *htz1Δ ykoΔ* strains. Preliminary evidence suggests that loss of H1 specifically rescues the viability or slow growth of strains combining *HTZ1* deletions with deletions of genes involved in histone deacetylation or ubiquitination. Finally, we have used chromatin immunoprecipitation to establish that strains lacking H2A.Z show reduced association of histone H1 with chromatin. Overall our experiments suggest that loss of H2A.Z triggers an H1-dependent disruption of normal chromatin structure.

Y3091A Association of CAF-1 and Rrm3p with paused replication forks. *Hollie Rowlands*, Brandon Wyse, Roxanne Oshidari, Ashley Cheng, Piriththiv Dhavarasa, Krassimir Yankulov. University of Guelph, Guelph, Ontario, CA.

Genes positioned close to telomeres are either fully active or fully silenced and infrequently convert between these two states, a phenomenon referred to as Telomere Position Effect. The mechanisms of epigenetic conversions at the telomeres, and in general, are not well understood. Recently we have shown that the destruction of Chromatin Assembly Factor – I (CAF-I) in *S.cerevisiae* reduces the frequency of such telomeric conversions. CAF-I is a histone chaperone which rebuilds nucleosomes from old and new histones immediately after the passage of the replication forks. It is believed that its nucleosome assembly activity is central to the transmission of epigenetic marks to the chromatin of daughter cells and that it acts as a key factor in the preservation of epigenetic states.

In this poster we show that the deletion of *RRM3*, a DNA helicase that releases paused replication forks, has a similar effect on the frequency of epigenetic conversions. We present our analyses on the occupancy of CAF-1 and Rrm3p at replication forks that are stably paused *in vivo* by the association of the Fob1p protein. We correlate these Chromatin Immuno-Precipitation (ChIP) analyses with analysis of the interactions of Rrm3p and CAF-1 with the fork clamp Pol30p (PCNA, Proliferating Cell Nuclear Antigen).

We propose that CAF-I, albeit being involved on the faithful transmission of epigenetic marks, is needed for epigenetic conversions when replication forks stall.

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Y3092B Invasion of a heterochromatic locus during homologous recombination disrupts its transcriptional silencing. *Kathryn Sieverman*, Jasper Rine. Department of Molecular and Cell Biology, California Institute for Quantitative Biosciences, University of California at Berkeley, Berkeley, CA.

Eukaryotes from yeast to humans employ heterochromatin, a compact chromatin structure, to stably silence gene expression and ultimately influence cellular identity and genomic integrity. In the budding yeast *Saccharomyces cerevisiae*, the Silent Information Regulator (SIR) proteins impart heterochromatic silencing at the silent mating-type loci *HML* and *HMR*. Silencing at *HML* and *HMR* leads to a 1000-fold reduction in transcription over the expressed state, is stably inherited from mother to daughter cell, and is critical for maintaining haploid identity. Our lab recently developed a novel assay to capture the dynamics of heterochromatic silencing in budding yeast, which we have named the Cre-Reported Altered States of Heterochromatin, or CRASH, assay. We placed the *cre* recombinase gene at the *HML* locus to monitor heterochromatin stability. Lapses in silencing at *HML* allow Cre to excise a loxP-flanked RFP cassette, subsequently activating GFP expression and revealing for the first time rare and transient silencing losses in wild-type cells. In this study, we exploited the sensitivity of the CRASH assay to understand how homologous recombination within the silent *HML* locus impacts its ability to remain transcriptionally silent. Recombination within heterochromatin requires access to an otherwise occlusive chromatin structure, yet whether this invasive process also disrupts silencing is unknown. We introduced into cells equipped with the CRASH assay a double-strand break that induces homology-directed repair from *HML*. Interestingly, our results demonstrate that homologous recombination can occur in a heterochromatic donor without an obligate loss of silencing. However, it appears that many cells that template recombination at *HML* lose transcriptional silencing in the process. The reasons for these two distinct outcomes are under active investigation.

Y3093C Replication factors function in Cohesion and Condensation. *R. V. Skibbens*, Donglai Shen, Caitlin Zuilkoski. Lehigh University, Bethlehem, PA.

To ensure accurate chromosome segregation, the yeast genome undergoes dramatic structural changes that include DNA replication, sister chromatid cohesion and chromosome condensation. Numerous studies reveal that DNA replication factors impact cohesion establishment, but the molecular mechanism through which this regulation occurs and the extent that replication factors similarly regulate chromosome condensation remains unknown. Here, we investigate two DNA replication factors that regulate temporally distinct steps in cohesion. Chl1 is a DEAH-box DNA helicase that functions early during DNA replication to promote sister chromatid cohesion. The extent to which Chl1 promotes condensation, however, remains unknown. To address this deficit, we tested for Chl1-dependent condensation at both the rDNA locus and along the chromosome arm. Using both Net1-GFP and a modified FISH procedure to visualize rDNA locus structure, the results show that *chl1*

deletion strain exhibit significant levels of decondensation, relative to wildtype cells. We also tested for condensation defects along the chromosome arm using a two GFP assay kindly provided by Dr. Frank Uhlmann. Compared to wildtype cells, *chl1* mutant cells exhibited a significant increase in inter-GFP distances. These results reveal that Chl1 plays a critical role in condensation - extending the role of Chl1 beyond cohesion and identifying Chl1 as an early regulator of chromatin structure. PCNA is a sliding clamp that promotes processive DNA replication and interacts with the cohesion establishment factor Ctf7/Eco1. Previous results revealed that overexpression of PCNA rescues the conditional growth defects in *ctf7/eco1* mutant cells. The molecular basis of this rescue, however, remains unknown. We thus tested whether PCNA overexpression rescues either the cohesion or condensation defects present in *ctf7/eco1* mutant cells. The results reveal that PCNA overexpression rescues the cohesion defects, but not the condensation defects, of *ctf7/eco1* mutants. These results support prior findings from our lab that PCNA rescues the cohesion, but not the condensation, defects in *pds5* mutant cells. Thus, PCNA plays a critical role in regulating a unique and distinct aspect of cohesin biology. In combination with other findings from our lab, these results support a new model in which cohesin is deposited onto each sister chromatid early during DNA replication and that this deposition relies on Chl1 DNA helicase-dependent alterations of DNA structure. Later during DNA replication, and possibly signaled through completion of Okazaki maturation, PCNA promotes cohesin-dependent establishment of sister chromatin cohesion but not chromosome condensation. Thus, cohesin functions (and possibly structures) are separable and likely spatially regulated throughout the genome. .

Y3094A Nucleosomes Are Essential for Proper Regulation of a Multigated Promoter in *Saccharomyces cerevisiae*. *Robert Yarrington*, Jenna Goodrum, David Stillman. University of Utah, Salt Lake City, UT.

We have previously shown that transcription factor binding at *HO* follows a temporal cascade, with SBF bound at the left half of URS2 (URS2-L) serving to relay a signal from upstream chromatin changes at URS1 to the SBF bound at the right-half of URS2 (URS2-R) that ultimately activate gene expression. We show here that proper nucleosomal context of the URS2 promoter region is essential for this complex transcriptional regulation. Chromatin at the yeast *HO* promoter is highly repressive and numerous coactivators are required for expression. We modified the *HO* promoter with segments from the well-studied *CLN2* nucleosome-depleted region (NDR), creating chimeric promoters differing in nucleosome occupancy but with binding sites for the same activator, SBF. Nucleosome depletion resulted in substantial increases in both factor binding and gene expression and allowed activation from a much longer distance, presumably by allowing coactivators recruited at the upstream URS1 region to act further downstream. Nucleosome depletion also affected sequential activation of the *HO* promoter, resulting in promoters no longer requiring both URS1 and URS2-L, as either regulatory region alone is now sufficient to promote binding of the SBF factor to URS2-R. Furthermore, nucleosome depletion at URS2 altered the timing of *HO* expression and bypassed the regulation that normally restricts expression to mother cells. Our results reveal insight into how nucleosomes can create a requirement for ordered recruitment of factors to facilitate complex transcriptional regulation.

Y3095B The interactions between Pol30p (PCNA, Proliferating Cell Nuclear Antigen), Chromatin Assembly Factor -1 and Rrm3p: the role of the CDC28 and CDC7 protein kinases. *P. Dhavarasa*, B. Wyse, H. Rowlands, L. Fedak, M. Guilleman, K. Yankulov. University of Guelph, Guelph, Ontario, CA.

The interactions between Pol30p (PCNA, Proliferating Cell Nuclear Antigen), Chromatin Assembly Factor -1 and Rrm3p: the role of the CDC28 and CDC7 protein kinases

Piriththiv Dhavarasa, Brandon Wyse, Hollie Rowlands, Lauren Fedak, Mathew Guilleman and Krassimir Yankulov

Department of Molecular and Cellular Biology, University of Guelph, Ontario

The destruction of Chromatin Assembly Factor -1 (CAF-1) or the DNA helicase *RRM3* affect the frequency of epigenetic conversions at the telomeres of *S.cerevisiae*. CAF-I is a histone chaperone, which reassembles nucleosomes from old and new histones immediately after the passage of the replication fork. *RRM3* is a helicase that relieves replication forks that have paused at sites of tight protein binding or active transcription. Both Rrm3p and CAF-1 interact with Pol30p, the yeast homologue of the Proliferating Cell Nuclear Antigen (PCNA). These interactions are mediated by a dedicated conserved PCNA-Interacting-Peptide (PIP) consensus sequence present on both the largest subunit of CAF-1 (Cac1p) and on Rrm3p. Interestingly, there are many other proteins that interact with PCNA via PIPs thus suggesting an intriguing interplay of PCNA-binding factors at the replication fork. At the same time, the interaction between CAF-1 and PCNA is in part regulated by two Cyclin-Dependent Kinases, *CDC28* and *CDC7*.In an attempt to mechanistically decipher the effects of CAF-1 and Rrm3p on epigenetic conversions, in this poster we address two important issues:

The possibility of competition and/or cooperating between Pol30p, Cac1p, Rrm3p and several other PCNA-binding proteins.

The extent to which protein kinases regulate the stability of CAF-I and its association with PCNA.

We will show evidence from extensive competitive and non-competitive two-hybrid assays with *POL30*, *CAC1* and *RRM3*. The effect of mutations on multiple phosphorylation sites of Cac1p on the stability of CAF-I and its ability to associate with Pol30p in cell extracts will also be presented. Finally, we will show our analysis with recombinant proteins on the competition between Cac1p and Rrm3 for their interaction with Pol30p.

Y3096C Interaction between the HAT Gcn5 and the phosphatase PP2A-Rts1 at the yeast centromere. *Masha Evpak*, Emily Petty, Angela Nicholson, Lorraine Pillus. University of California, San Diego, La Jolla, CA.

DNA is wrapped around histone proteins, making up the fundamental unit of chromatin—the nucleosome. This organization is used to regulate diverse cellular functions and efficiently store the genetic code. The dynamic addition and removal of molecules on histones is catalyzed by various enzymes, including histone acetyltransferases—which add acetyl-CoA—and phosphatases—which remove phosphate

groups. These post-translational modifications represent a mode of regulating cellular processes. The enzyme Gcn5 is a conserved H3/H2B histone acetyltransferase with multiple established roles and a proposed role in centromeric function. The protein phosphatase 2A (PP2A) has a conserved regulatory subunit, Rts1, which regulates diverse processes including cell size, septin dynamics during cell division, and chromosomal biorientation. Our lab recently described a genetic interaction between *GCN5* and *RTS1*, whereby overexpression of *RTS1* rescues *gcn5* mutant phenotypes, and simultaneous loss of both genes is lethal. Due to reported connections of both Gcn5 and Rts1 to the centromere, we sought to determine whether the centromeric histone Cse4 is involved in the relationship between these two enzymes. We performed a screen of phosphorylatable and acetylatable Cse4 residues to determine whether any Cse4 residues are actively modified in the *GCN5/RTS1* relationship. We identified three Cse4 residues that are critical for the suppression of *gcn5Δ* phenotypes by *RTS1* overexpression. Mutating each of these identified residues to alanine interferes with rescue of *gcn5Δ* temperature sensitivity, DNA damage sensitivity, and cell cycle progression. Further studies will be conducted to determine how these Cse4 residues are used in the centromeric function of these conserved enzymes. Both Rts1 and Gcn5 regulate diverse processes and are frequently implicated in disorders including cancer and neurodegeneration, and understanding their role at the centromere will help elucidate their function in cellular regulation.

Y3097A Set6: A novel lysine methyltransferase in *Saccharomyces cerevisiae*. *D. Jaiswal*, Erin Green. University of Maryland Baltimore County, Baltimore, MD.

Histone methyl marks orchestrate programming of the genome, regulating gene expression programs that control growth, survival and differentiation in response to changing environmental cues. Thus, the discovery and functional characterization of new histone methylation marks holds great promise in expanding our understanding of how chromatin dynamics influence genomic programs. Characterization of evolutionarily conserved histone methylation sites in *Saccharomces cereviaise* has provided essential insight into the function of post translation modification in diverse eukaryotic systems, including humans. Taking advantage of the versatile molecular genetic tools available in yeast, we have investigated the biochemical and biological roles of the novel candidate histone methyltransferase, Set6, whose function is not known. Set6 contains a SET domain, a common signature of chromatin regulatory proteins that catalyzes lysine methylation. Here, we use genetic assays to show that Set6 participates in cell cycle regulation, and may specifically be important for progression through S phase. We further use biochemical approaches to demonstrate that Set6 is likely an active methyltransferase that may primarily target non-histone proteins. Overall, we hypothesize that Set6 is a key regulator of cell cycle progression in budding yeast that acts via its methylation activity on non-histone proteins.

Y3098B Fission yeasts DNA strands chirality *mat1*-switching mechanism explains development of diverse organisms. *A. J. S. Klar*. NCI-Frederick Cancer Res Fac, Frederick, MD.

The somatic selective chromatid-specific Imprinting mechanism produces sister cells with different cell-types in evolutionarily diverse fission yeasts (reviewed in[1]). Motivated by our yeast work that discovered this monochromatid gene expression phenomenon and the selective chromatid segregation phenomenon we discovered in mouse cells[2], we had proposed that DNA strands chirality can may be converted into a/symmetric gene expression of sister cells to provide a general mechanism for eukaryotic development. This mechanism has indeed explained the mathematics of developmental mutant phenotypes: (I) visceral organs asymmetry development in mice and of (II) neuronal left/right asymmetry in nematode[3,4]; (III) etiologies of human brain hemispheric laterality to cause mirror hand movements disorder in *rad51/RAD51* persons[5]; (IV) psychoses disorders by chromosome 11 translocations[6]; (V) the two-colored wing spots pigmentation of the *Bruchus* beetle piebald mutant[7]; and (VI) the split hand-/foot malformation by the chromosome 2 rearrangements[8]. So, just as mechanisms of cell cycle, cell biology, meiosis, recombination etc. are conserved from yeast to metazoans, DNA strand-based developmental mechanism has been conserved in diverse organisms. Results of *mat1* imprinting in yeast and newer support for this mechanism for human limb development will be presented. Note: the conventional morphogen model of development has been withdrawn[9].

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Y3099C NADPH levels control sirtuin-dependent heterochromatin stability in *Saccharomyces cerevisiae*. *A. Plaza-Jennings*, R. Janke, J. Rine. University of California Berkeley, Berkeley, CA.

Many chromatin-modifying enzymes use common metabolites as cofactors, but the connections between central metabolism and epigenetics remain poorly explored. The link between NAD^+ levels and sirtuin function has been well established, but recent work for our lab has uncovered an unanticipated depth in the links between metabolism and epigenetics. We found that disruption of an early step in the pentose phosphate pathway affected heterochromatin stability in a spatially heterogeneous manner within yeast colonies. This spatial heterogeneity in silencing likely reflects spatial heterogeneity in metabolism within yeast colonies. The pentose phosphate pathway is a major source of both five carbon sugars and NADPH. Here, we present evidence that it is the cellular NADPH levels that affect the stability of heterochromatin at the yeast silent mating locus *HML*.

Y3100A Loss of gene silencing is not a feature of yeast aging. *Gavin Schlissel*, Jasper Rine. UC Berkeley, Berkeley, CA.

Yeast replicative aging involves myriad changes to the basic biology of the yeast cell. Previously it was reported that replicatively old yeast mother cells lose their ability to silence genes at *HML* and *HMR* loci, suggesting that the SIR complex becomes non-functional in old cells. Here

we performed pedigree analysis using a recently developed and exquisitely sensitive silencing reporter, in combination with a custom microfluidic device, to characterize age-associated loss of silencing. In hundreds of yeast pedigrees, we found no evidence that silencing loss at *HML* and *HMR* is related to yeast aging. Furthermore, when we quantified RNA from purified populations of replicatively old cells, we found that old cells' gene expression does not reflect silencing loss at *HML* and *HMR*. Although silencing was maintained in old cells, we successfully replicated previous experiments that showed that old *Mat**a*** cells fail to respond to α-factor. This result was originally interpreted to mean that silencing is lost in old cells; however, we found that this effect depends on the α-factor concentration, and that old *Mat**a*** cells respond efficiently to large doses of mating pheromone.

Y3101B Linking the critical functions of two essential NuA4 acetyltransferase subunits. *Naomi Searle*[1], Ana Lilia Torres Machorro[2], Lorraine Pillus[1]. 1) UC San Diego, La Jolla, CA; 2) National Institute for Respiratory Diseases, Mexico City, Mexico.

The eukaryotic genome is packaged into chromatin, which is composed of a nucleosome unit containing DNA wrapped around a histone octamer. Chromatin components are subject to various modes of post-translational regulation that have many established roles, including functions in recombination, DNA damage repair, and transcription. Acetylation is a key post-translational modification that is mediated by the opposing enzymatic activities of lysine acetyltransferases (HATs) and deacetylases (HDACs). HATs often exist in large multimeric complexes, such as NuA4 and SAGA, both of which are conserved across species. In humans, the essential catalytic subunit of NuA4, Tip60, along with additional essential subunits such as EPC1/2, are associated with multiple carcinomas. We recently reported that the requirement for *ESA1,* the *S. cerevisiae* ortholog of Tip60, could be bypassed by concurrent loss of Rpd3L, an opposing HDAC complex. This bypass is promoted by establishing a relatively balanced cellular acetylation state.

Here, we report on the analysis of the remaining essential components of NuA4, including Arp4, Act1, Swc4, Tra1, and Epl1. The bypass state is characterized by a reduction of cellular fitness, including sensitivity to high temperature and DNA damage, low histone H4 acetylation, and cell cycle defects. These phenotypes were selectively suppressed by loss of additional deacetylases such as *HDA1*. Whereas Esa1 and several other NuA4 subunits are well-described, there are subunits that remain to be fully characterized. Specifically, Epl1, the ortholog of human EPC1/2, although essential, is poorly understood *in vivo* across species, and is a focal point of interest in this study. Taking advantage of the bypass suppression, we present new functional studies of Epl1 in *S. cerevisiae* with the aim of better understanding the essential role of this crucial NuA4 subunit. Due to the conservation of Epl1 between budding yeast and humans, many of our basic findings in *S. cerevisiae* can be translated to human cellular function and biology.

Y3102C Epigenetic conversions at the telomeres of *S.cerevisiae* – links to DNA replication. *K. Yankulov*. Univ Guelph, Guelph, ON, CA.

The continuity of gene expression programs is in part maintained by the faithful transmission of exiting chromatin structures from the parental cells to the daughter cells. Replication-coupled processes ensure that the epigenetic marks on the old histones (and on DNA in metazoan organisms) are faithfully reproduced after the synthesis of the new strands of DNA and the deposition of new histones. At the same time, changes in chromatin structure are necessary during metazoan development and in the adaptation of *S.cerevisiae* and many pathogens to the changing or host organism environment. It is not clear if the same machinery is involved in these epigenetic changes between active and silent states of the genes. In reality, we know very little about how epigenetic conversions take place.

Recently we have developed an assay for the quantitative assessment of the frequency of epigenetic conversions at the telomeres of *S.cerevisiae*. We have documented that the destruction of Chromatin Assembly Factor -1 (CAF-1) or the DNA helicase *RRM3* substantially reduce the frequency of such conversions. CAF-I is a histone chaperone, which reassembles nucleosomes from old and new histones immediately after the passage of the replication forks. *RRM3* is a helicase that relives replication forks that have paused at sites of tight protein binding or active transcription.

Current models suggest that both Rrm3p and CAF-1 are recruited to replication forks via an interaction with the Proliferating Cell Nuclear Antigen (PCNA, *POL30*) and that this interaction is regulated by the DBF4-Dependent Kinase, DDK. I will present evidence that the Cyclin Dependent kinase CDK (Cdc28p) rather than DDK is a key regulator of the association of CAF-I with chromatin, while DDK affect its stability and its affinity to PCNA. Two separate posters will present details on these findings.

I will propose the model that CAF-I, albeit being involved on the faithful transmission of epigenetic marks, is also needed for epigenetic conversions at paused replication forks.

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Y3103A A closer look on telomerase RNA biogenesis –Tlc1's "lasting" story. *E. Bajon*, Y. Vasianovich, N. Laterreur, R. Wellinger. Université de Sherbrooke, Sherbrooke, Québec, CA.

The telomerase holoenzyme acts as a reverse transcriptase to elongate shortened, non-functional telomeres. This enzyme is composed of several protein subunits and assembled onto a scaffolding non-coding RNA, called Tlc1 in budding yeast. Like some other protein subunits (Est1, Est2, Est3, Pop1), Tlc1 is essential for telomerase activity and telomere maintenance *in vivo*. However, the pathways and mechanisms leading to an assembled and functional telomerase complex are not understood. Telomerase biogenesis has been studied via genetics and biochemistry, but real time *in vivo* data are by and large missing. As a readout for telomerase biogenesis, Tlc1 is a target of choice, as it is the limiting subunit for telomerase activity in Saccharomyces cerevisiae (its expression was measured at 25-30 RNAs/cell). Previous work showed that Tlc1 can shuttle between nucleus and cytoplasm, although no canonical ncRNA maturation is known to happen in the cytoplasm in yeast. Our lab previously used the MS2-GFP system to study Tlc1 dynamics and recruitment to telomeres *in vivo*, and the results revealed telomere-associated clusters of telomerase, called T-Recs, correlated with telomere elongation (Gallardo et al.; Mol Cell 2011).

In order to gain more detailed insights into Tlc1 ncRNA biogenesis, we are using a combination of the MS2-GFP and the Cre-Lox systems. The endogenous TLC1 locus was floxed so that the expressed Tlc1 untagged. The MS2 tag can be inserted at will via and inducible Cre-EBD protein. The new TLC1-MS2 locus gives rise to Tlc1-MS2 RNAs, which can be visualized via the MS2-GFP fusion protein. These newly transcribed, GFP-bound Tlc1 RNAs can thus be followed from the transcription site to the final target at telomeres by time-lapse microscopy in living cells. It is also possible to IP these RNAs in order to decipher how the telomerase complex assembles. This construct, named TLC1-MS2-IN, thus has the potential to allow studies on the first biogenesis steps of Tlc1 and telomerase. A similar construct, dubbed TLC1-MS2-OUT, allows for the inducible removal of the MS2 tag. This second construct allows studies of Tlc1 degradation, half-life, and telomerase nucleocytoplasmic recycling. Preliminary Southern- and Northern-blots results with this latter setup show that there is a lag between the genomic recombination and the differential RNA expression, suggesting that Tlc1 is a stable RNA that can last for the duration of at least one cell cycle.

This study, apart from giving insight into Tlc1 biogenesis and telomerase assembly, could thus help better define ncRNA maturation or degradation pathways in budding yeast.

Y3104B Yeast RNA 3'-end processing factors promote RNA Polymerase II ubiquitination and degradation following UV-type DNA damage. *Jason N. Kuehner*[1], Jay Kaufman[1], Claire Moore[2]. 1) Emmanuel College, Boston, MA; 2) Tufts University School of Medicine, Boston, MA.

Genomic information is vital for the survival and reproduction of all living cells. The integrity of DNA is frequently challenged by damaging agents like ultraviolet (UV) radiation, which can ultimately lead to mutation and disease. The DNA Damage Response (DDR) is at the front lines of genomic defense, acting to promote recognition and repair of DNA lesions. A critical consequence of the DDR is a transient decrease in the levels of RNA Polymerase II (Pol II) and mRNA, during which time the cell can repair transcribed regions of the genome. UV irradiation of human cells reduces mRNA at least in part by inhibiting RNA 3' processing, and processing factors likewise promote Pol II ubiquitination and degradation. These data indicate that 3' processing factors provide an important link to the DDR, but the scope and mechanism of these interactions are as yet unknown.

Our labs have previously shown that UV-induced inhibition of RNA processing is a conserved response in yeast, and UV-type damage induces genome-wide variation in poly(A) sites. We sought to identify additional functional links between 3' processing factors and the DDR in the yeast model system in order to better characterize the mechanism of coordination. We have observed that yeast 3' processing mutants are sensitive to UV-type DNA damaging agents. Yeast 3' processing mutants exhibit unique genetic interactions with nucleotide excision repair mutants but not with factors in homologous recombination or post-replication repair pathways. Yeast 3' processing mutants also impair the ubiquitination and degradation of Pol II following DNA damage. In ongoing experiments we are testing the role of 3' processing factors in the recruitment of ubiquitination and DNA repair machineries following UV damage. Overall these results suggest that 3' processing factors promote DNA repair by removing Pol II stalled at UV-type DNA lesions, a functional interaction that is conserved between yeast and human cells.

Y3105C mRNAs accumulate near transcription sites, nuclear pore complexes, or within the nucleolus when RNA processing is disrupted. *B. Paul*, B. Montpetit. Department of Cell Biology, University of Alberta, Edmonton, Alberta, CA.

Many proteins interact with a newly synthesized RNA to mediate biogenesis and maturation. These temporally and spatially regulated interactions facilitate individual processing events and direct each RNA to a specific fate (e.g. nuclear export or decay). To further characterize mRNA biogenesis and export, we performed a fluorescent in situ hybridization (FISH) screen of >1000 essential gene mutants in *Saccharomyces cerevisiae* that resulted in the identification of 29 mutants that exhibited poly(A)-RNA accumulation in the nucleus. Gene ontology places the majority of the genes into three biological processes: RNA export from nucleus, nuclear RNA catabolic processes, and chromosome segregation.

It is known that defects in the processing of various non-coding RNAs (e.g. rRNA, snRNA, snoRNA, and tRNA) can lead to the stabilization of polyadenylated RNA species; therefore, mutants may accumulate poly(A)-RNA due to disruptions in RNA biogenesis that are independent of mRNA. To identify mutants that specifically alter mRNA biogenesis and export, single-molecule FISH (smFISH) assays were performed on all 29 mutants using probes against three different mRNAs (e.g. *GFA1*, *ACT1* and *CCW12*). Based on transcript localization and abundance, we identified defects in mRNA processing and export in 26 of 29 mutants.

By employing smFISH and markers for various subdomains of the nucleus (e.g. Ndc1-GFP for the nuclear envelope, *ITS1* for the nucleolus, and a LacO/LacI-GFP marked gene locus), we could further subdivide mutants into three classes based on the accumulation of poly(A)-RNA and mRNA within discrete regions of the nucleus. This included: (1) mRNAs and mRNA binding proteins becoming enriched in the nucleolus when nucleocytopalsmic transport, rRNA biogenesis, or RNA processing and surveillance was disrupted, (2) the buildup of mRNAs near transcription sites in 3' end processing and chromosome segregation mutants and (3) transcripts being enriched near nuclear pore complexes when components of the mRNA export machinery were mutated. These data demonstrate that alterations in a broad set of nuclear processes leads to the accumulation and possible retention of mRNAs at discrete locations within the nucleus. This may reflect common failure points in mRNA export and/or active mechanisms to protect the cell against cellular stress and dysfunction.

Y3106A A Genetic Screen of the Yeast Kinome Reveals Gin4p Regulation of mRNPs. *Eric E. P. Cosky*, Nebibe Mutlu, Anuj Kumar. University of Michigan, Ann Arbor, MI, USA.

Eukaryotes from yeast to metazoans respond to cellular stressors through a precisely coordinated interplay between regulatory and signal transduction pathways controlling gene expression. The regulation of mRNA translation is an important aspect of the eukaryotic gene expression program, and the assembly of cytoplasmic mRNA-protein (mRNP) complexes is presumably a significant mechanism by which post-transcriptional control is achieved. Among eukaryotes, mRNPs are well conserved and form in response to numerous stressors, such as heat

shock, osmotic or oxidative stress, and starvation. In yeast, processing bodies and stress granules are among the most well-studied mRNPs, but their exact functions and regulation remain to be fully resolved.

Previous studies have identified that Snf1p (AMPK) phosphorylation activates the RNA processing Xrn1p exonuclease and Ste20p (PAK) phosphorylation of Dcp2p affects both its decay of certain mRNAs and its localization to p-bodies. However, the extent to which kinase signaling pathways regulate mRNP activity is likely much broader. Under the hypothesis that kinase phosphorylation events regulate mRNP dynamics, we performed a genetic screen on homozygous yeast mutants deleted for kinase genes. For this screen, mRNPs were initially visualized by live-cell imaging using a *PGK1* reporter mRNA containing binding sites in its 3'-UTR for the U1A-GFP fusion protein. Plasmids encoding the modified *PGK1* gene and U1A-GFP were transformed into 114 yeast strains, each deleted for a single kinase gene. This kinomic screen identified eight deletion mutants exhibiting mRNP localization phenotypes upon acute glucose deprivation. These mRNP phenotypes were validated using an integrated fusion of mCherry to sequence encoding the mRNP marker protein Dcp2p.

In particular, a homozygous strain deleted for *GIN4* exhibited a striking loss of mRNPs. Under conditions of heat shock, osmotic or oxidative stress, and starvation, mRNPs form in wild type but not *gin4 Δ/Δ* cells. Interestingly, Gin4p is involved in bud growth and septin ring assembly with several kinase-dependent and independent functions. When a plasmid bearing *GIN4* and its native promoter is re-introduced, wild type levels of mRNPs are restored in *gin4 Δ/Δ*. The kinase dead $Gin4^{K48A}$ mutant displays wild type numbers of mRNPs, which suggests that a Gin4p kinase-independent function is modulating mRNP activity. Notably, inhibition of bud growth and septin ring assembly does not perturb wild type levels of mRNPs. Therefore, our data imply a novel Gin4p kinase-independent function affecting mRNP formation, which we are actively investigating further.

Y3107B Molecular genetic tools for manipulation of the oleaginous red yeast *Rhodotorula toruloides*. *S. J. Aves*, A. M. B. Johns, J. Love. University of Exeter, Exeter, UK.

Rhodotorula toruloides (*Rhodosporidium toruloides*) is a red, basidiomycete yeast. When grown under nitrogen limited conditions it can accumulate lipid to over 70 % of its dry biomass making it of interest for production of biofuels as well as having other potential uses in biotechnology. In order to be able to fully investigate and exploit this organism, genetic tools for manipulation of this yeast are required.

The *R. toruloides* genome has a high genomic GC content which can cause cloning problems, making *in vitro* genetic manipulations difficult. To overcome this, plasmid vectors have been constructed for *Agrobacterium tumefaciens*-mediated transformation of *R. toruloides*, incorporating elements for yeast *in vivo* assembly in *Saccharomyces cerevisiae*. These vectors have been developed to facilitate easy exchange of promoters and genes to be expressed, and can be used with either antibiotic or auxotrophic selection.

To permit controlled expression of genes in *R. toruloides*, we have identified four homologous inducible promoters. Their properties and basic structure have been characterized by fusion to a codon-optimized EGFP reporter gene, measuring fluorescence by flow cytometry. Each promoter can give tight regulation with induction times of between 4 and 16 hours. Because each promoter uses different induction and repression conditions, this provides a toolset of regulatable promoters such that for any given experiment there should be suitable promoter(s) for which conditions should not affect the results.

We have developed a suite of molecular genetic tools for recombinant DNA technology and controlled gene expression which will facilitate molecular genetic investigation and biotechnological exploitation of oleaginous red yeast.

Y3108C Analysis of the pseudokinase domain of the SAGA and NuA4 component Tra1. *M. Berg*, J. Karagiannis, C. Brandl. University of Western Ontario, London, Canada.

Tra1 is a 433 kDa component of the *Saccharomyces cerevisiae* SAGA and NuA4 transcriptional co-activator complexes. Tra1 interacts with transcriptional activators to recruit the complexes to specific promoters. As a PIKK (phosphoinositide three-kinase-related kinase) family member, Tra1 is characterized by a C-terminal phosphoinositide 3-kinase (PI3K) domain. Unlike other PIKK family members, Tra1 lacks kinase activity. The PI3K domain is still essential and mutations in this domain result in altered gene expression. To investigate the structure and function of Tra1's PI3K domain, we are characterizing an allele we call $tra1_{Q3}$ where three conserved arginine residues within the PI3K domain are mutated to glutamine. This allele results in slow growth under stress and decreased transcription activation. The protein mislocalizes to the cytoplasm and has decreased association with SAGA and NuA4. Pull downs of the mutant complexes also show altered interactions with SAGA-interacting proteins. Suppressors of $tra1_{Q3}$ fall into the cleft of the kinase domain when mapped onto the known mTOR PI3K structure. Based on the reduced nuclear localization and failure to associate with SAGA and NuA4 demonstrated by this mutant, we believe the PI3K domain of Tra1 is important for the biosynthesis of Tra1 into SAGA and NuA4.

Y3109A Role of chromatin modulators during polymerase switch for ribosomal RNA synthesis in *Saccharomyces cerevisiae*. *KUSHAL BHATT*, Heather Conrad-Webb. TEXAS WOMAN'S UNIVERSITY, DENTON, TX.

Ribosomal RNA synthesis consumes major portion of the cellular resources necessitating its strict regulation. Under non-stress conditions RNA polymerase I (Pol I) synthesizes a 35S precursor rRNA, but during stress (nitrogen deprivation) synthesis also occurs by RNA polymerase II (Pol II) from a cryptic site upstream of Pol I binding site. This polymerase switch has been observed in higher eukaryotes leading to the hypothesis that the switch serves as back up mechanism for rRNA synthesis during stress allowing the survival of the cell. Pol II rRNA synthesis may be followed using an rDNA-LacZ reporter construct. Nitrogen deprivation leads to a three-fold increase in the b-galactosidase activity. The corepressor Ssn6-Tup1 is known to repress transcription, when recruited to target promoters by repressor proteins. Absence of this corepressor leads to an increase in enzyme activity under normal and stress conditions. Furthermore, the absence of repressor proteins Sko1, Nrg1, Sut1 that are known to recruit Ssn6-Tup1 also showed an increase in b-galactosidase activity. Hmo1, a High Mobility Group protein, binds

to rDNA to regulate pol I rRNA synthesis. As expected b-galactosidase activity increases significantly in the absence of Hmo1 in both non-stress and stress conditions . We hypothesize that stress signaling pathways will signal these repressors to inhibit their silencing function. Repressor complexes will be investigated during normal growth and stress for secondary modifications and their binding to rDNA. These studies will help dissect the regulation of rRNA synthesis and cell survival mechanisms under stress conditions.

Y3110B Investigating a novel function of snRNP assembly factor Prp24 in regulating H2B monoubiquitylation. *Katie S. Bolling*, A. Gregory Matera, Brian D. Strahl. Curriculum in Genetics & Molecular Biology, University of North Carolina, Chapel Hill, NC.

Investigating a novel function of snRNP assembly factor Prp24 in regulating H2B monoubiquitylation

A key step in regulation of eukaryotic gene expression is the removal of introns through splicing. While it has been well-established that the majority of splicing occurs co-transcriptionally, many questions remain regarding the coupling of these processes. It has been shown that altering rates of transcription leads to changes in splicing efficiency; however, the mechanism underlying these effects is not well understood. Post-translational modifications (PTMs) of histone proteins are a key regulator of chromatin structure, and several histone PTMs have been associated with transcription and splicing. One such PTM is mono-ubiquitylation of H2B (H2Bub1) at a conserved lysine residue (K123 in S. cerevisiae). A very tight correlation between H2Bub1 levels and active transcription has been demonstrated, and recent studies have linked H2Bub1 to splicing as well. In S. cerevisiae, loss of H2Bub1, either by deletion of the E3 ubiquitin ligase which places the mark (Bre1) or by mutation of the ubiquitylated residue, leads to an increase in intron retention and synthetic growth defects with splicing factor mutants. Additionally, studies in human cells have shown that knockdown of a conserved small nuclear ribonucleoprotein (snRNP) recycling factor, SART3, lead to an increase in H2Bub1. Our investigations have shown this effect is conserved in S. cerevisiae, and is mediated by a highly conserved residue in the first RNA Recognition Motif (RRM) of Prp24, the SART3 ortholog. Interestingly, although RRM1 does not bind snRNAs, mutation of this residue causes significant growth defects and severe temperature sensitivity. Mutations of other RRMs in Prp24, known to affect its ability to bind the snRNA U6, do not affect H2Bub1. These results suggest that Prp24 has a previously uncharacterized role in regulation of H2Bub1. Future experiments will reveal if Prp24's influence on H2Bub1 is connected to its association with splicing factors. Additional studies will test for genetic and physical interactions between Prp24 and Bre1, as well as deubiquitylating enzymes Ubp8 and Ubp10. Finally, investigation of Prp24's effects on transcription elongation rate, splicing, and the cell cycle will uncover potential indirect influences on H2Bub1 levels. Together, the results of this investigation will shed light on a novel, likely conserved role of Prp24 in regulating H2Bub1 and contribute new insight into the link between chromatin, transcription, and splicing.

Y3111C Regulation of *S. cerevisiae* in response to 4NQO by the polymorphic transcription factor, Yrr1. *J. Gallagher*, X. Rong-Mullins. West Virginia Univ, Morgantown, WV.

As we enter the era of whole-genome sequencing, cataloging genetic variation between individuals in a species falls short in understanding the functional impact of this variation on an individual's phenotype. There is variation of tolerance among different *S. cerevisiae* strains to the DNA damaging drug, 4-nitroquilione 1-oxide (4NQO). A clinical yeast isolate, YJM789, is very tolerant to 4NQO while S288c, a laboratory yeast strain, is sensitive. The genetic variation in 4NQO response is due Yrr1, a polymorphic transcription factor that shift the cellular response in resistance to 4NQO by changing rates of respiration which changes the metabolism of 4NQO. In ChIP-seq and RNA-seq we find that variation in transcription of genes required for response to 4NQO are poorly correlated with variation in Yrr1 binding upstream of these genes. *SNQ2* encodes an ABC transporter which is strongly transcriptional induced in response to 4NQO with no change in Yrr1 binding. How the variation in transcription factors change RNA pol II transcription is under investigation. All the Yrr1 polymorphisms are outside the Zn-finger DNA binding domain and cluster at the C-terminus. By comparing the conservation of Yrr1 across yeast strain, a one residue in the C-terminus stood out as a potential phosphorylation site in $Yrr1^{YJM789}$ and a second one changes the charge near a second phosphorylation site. Converting that site to a phosphomimic amino acid in the 4NQO-sensitive allele of $Yrr1^{S96}$ confers 4NQO resistance similar to $Yrr1^{YJM789}$. Yet how these single amino acid differences change the transcriptional outcome is unclear. We have used transcriptome, mutational analysis and protein-protein interactions to understand how these polymorphisms change the function of Yrr1.

Y3112A Pdc2 and Thi3 in *Candida glabrata* regulate both amino acid and thiamine starvation and mediate the switch of biosynthetic capacity in response to starvation. *C. L. Iosue*, J. Nahas, D. Sens-Castet, E. Lang, D. D. Wykoff. Villanova Univ, Villanova, PA.

Transcription factor specificity is different for some signal transduction pathways in *Saccharomyces cerevisiae* and *Candida glabrata*. For the thiamine signal transduction (THI) pathway there are a number of differences between the two species at the level of signaling and transcription. By comparing the THI pathways in the two species, we have uncovered a novel molecular switch that alters the expression of thiamine and amino acid biosynthesis genes. In *S. cerevisiae*, *PDC2* encodes a transcription factor that activates the transcription of both thiamine biosynthetic genes and glycolytic genes. Proper regulation of thiamine responsive genes requires the additional regulators Thi2 and Thi3. *C. glabrata* is missing *THI2*; however, *C. glabrata* is still capable of inducing THI genes, indicating a difference in transcriptional requirements. Deletion of *ScPDC2* appears lethal in *S. cerevisiae* because cells are unable to utilize glucose effectively. In *C. glabrata*, there is no apparent growth defect in a *Cgpdc2*Δ strain, suggesting that Pdc2 regulates different genes between the two species. RNA-seq experiments with wild-type, *Cgpdc2*Δ, and *Cgthi3*Δ strains indicate that *Cg*Pdc2 regulates both thiamine and amino acid biosynthesis genes and not pyruvate decarboxylase genes. Interestingly, in wild-type *C. glabrata*, amino acid biosynthesis genes are upregulated in thiamine-replete conditions and ~10-fold repressed during thiamine starvation. This repression is lost in a *Cgthi3*Δ, suggesting *Cg*Thi3 plays a role in recruiting *Cg*Pdc2 to THI promoters in thiamine-limited conditions. We are exploring the altered regulation of genes by *Cg*Pdc2 by identifying the cis element(s) that *Cg*Pdc2 binds using promoter-YFP fusions with flow cytometry as well as *in vitro* EMSA experiments. We are also performing cross-

complementation studies to determine whether *Cg*Pdc2 is capable of rescuing the lethality of the *Scpdc2*Δ strain. This work should uncover a novel mechanism by which *C. glabrata* cells partition energy to either synthesize amino acids or synthesize thiamine, using the occupancy of promoters by *Cg*Pdc2 as the switch.

Y3113B Understanding the molecular interactions mediating transcriptional and demethylase activities of Gis1. *P. Konduri*, T. Wang, S. Lal, J. Comer, L. Zhang. University of Texas at Dallas, Richardson, TX.

Heme plays versatile and fascinating roles in controlling fundamental biological processes. Heme serves as a signaling molecule for oxygen levels in yeast, as heme function is entwined to molecular oxygen. Heme and oxygen regulate the expression of many genes in eukaryotes by modulating activity of regulatory proteins. In yeast, Gis1 is a DNA-binding transcriptional regulator belonging to the JHDM3/JMJD2 subfamily of demethylases. It is highly homologous to the mammalian JmjC domain containing protein JMJD2b, which plays an important role in histone demethylation, oxygen regulation and hormonal signaling. This study aims to dissect the molecular interactions regulating Gis1 activity by determining the effect of heme, oxygen and Gis1 interacting proteins on regulation of transcriptional activity and demethylase activity of Gis1. Notably, recent experiments in our lab showed that Gis1 senses changes in oxygen and heme levels, and its transcriptional activity is controlled stringently by heme concentration. Gis1 is a unique transcriptional regulator containing 894 amino acid residues with multiple domains and complex biological functions. Our biochemical studies indicate that heme binds directly to Gis1 and JMJD2b proteins. We also identified via mass spectrometric analysis that Gis1 interacts with different set of proteins under conditions of hypoxia, low heme and high heme. Together, our results show that Gis1 represents a novel class of complex transcriptional regulators mediating heme signaling.

Y3114C Understanding heme regulation of JmjC domain containing transcription factor Gis1. *S. Lal*, C. Konduri, T. Wang, J. Comer, L. Zhang. The University of Texas at Dallas, Richardson, TX.

Heme is an important signaling molecule that mediates the effect of oxygen on several cellular processes. In yeast, heme serves as a secondary messenger of oxygen as production of heme requires oxygen. Gis1 is a transcription factor containing the highly conserved JmjC domain. JmjC domain containing proteins belong to JHDM3/JMJD2 subfamily (lysine-specific) of histone demethylases which can function as oxygen sensors. Studies in our lab has shown that Gis1 is regulated by oxygen and heme. Protein localization studies showed that under hypoxia Gis1 is retained in the cytoplasm and oxygen is required for the nuclear localization of Gis1. Interestingly, a putative heme-responsive cysteine-proline sequence (CP motif) is present in the JmjC domain. Upon further investigation, we found that Gis1 binds heme directly, and its transcriptional activity is regulated by heme levels in the cell. Further work is being carried out to pinpoint the domain(s) mediating the effect of heme on the transcriptional activity of Gis1. Additionally Gis1 has a paralog, Rph1 which also contains the JmjC domain. Gis1 and Rph1 are 34% similar, and their zinc finger domains are almost identical. Rph1 is a repressor of autophagy-related genes in nutrient-rich conditions, which is independent of its histone demethylase activity. It is also a repressor of DNA repair gene *PHR1*. We are investigating if Rph1 is also regulated by heme.

Y3115A Investigating the role of the *S. cerevisiae* Paf1 complex in global regulation of transcription. *Alex R. Lederer*[1], Mitchell Ellison[1], Travis Mavrich[1], Lawrence E. Heisler[2], Marinella Gebbia[2], Corey Nislow[3], Karen M. Arndt[1]. 1) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260; 2) Terrance Donnelly Centre and Banting & Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada; 3) Department of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada.

In eukaryotes, the organization of DNA into a highly compact chromatin structure presents an obstacle for RNA polymerase during transcription. Consequently, organisms have evolved molecular machines that alter the chromatin template through a variety of mechanisms. This machinery includes the conserved Polymerase-Associated Factor 1 Complex (Paf1C), which associates with RNA polymerase II, promotes specific chromatin modifications, and regulates expression of certain genes. Past work has shown that Paf1 plays an important role in mediating transcription of genes across diverse pathways of cellular biology; however, its effects on transcription of noncoding DNA have not been thoroughly investigated. Genome-wide studies have revealed extensive noncoding DNA transcription across the yeast genome. Some of this noncoding transcription impacts the expression of neighboring genes, while giving rise to unstable transcripts that face rapid degradation by the TRAMP complex and its poly-A polymerase subunit, Trf4. To investigate the impact of the Paf1C on the greater transcriptome, whole genome tiling array studies were performed on RNA prepared from *paf1Δ*, *trf4Δ*, *paf1Δtrf4Δ*, and wild-type strains. Using differential expression analysis, we have identified classes of protein-coding genes whose expression is strongly dependent on Paf1. Notably, we observed decreased expression of phosphate signaling genes and increased expression of iron transport genes in *paf1Δ* cells, as validated by northern blot analysis and reverse transcription quantitative polymerase chain reaction (RT-qPCR). We have also confirmed previous findings that *paf1Δ* cells have severe transcription termination defects at snoRNA genes, which encode a class of noncoding RNAs involved in ribosome assembly. Finally, preliminary analysis of *paf1Δtrf4Δ* and *trf4Δ* cells has provided new insights on transcription of other noncoding RNAs, including CUTs, and antisense transcription. These findings confirm that Paf1 is a major participant in regulation of the global transcriptome. Moving forward, we aim to identify the specific mechanisms by which Paf1 regulates these diverse gene classes and further elucidate the impact of these defects in the cell.

Y3116B Magnification of negative allelic effects by environmental stress renders yeast segregants unable to grow at 37°C on ethanol. *T. Matsui*, I. Ehrenreich. U. of Southern California, Los Angeles, CA.

The budding yeast *Saccharomyces cerevisiae* is a valuable model system for characterizing the genetic and molecular mechanisms that enable fungal growth at human body temperature (37°C). However, most work on this topic has been performed on glucose, the organism's preferred

carbon source. In contrast, much less is known about the mechanisms that enable growth at 37°C on non-preferred carbon sources, such as ethanol. To provide new insights into this problem, we comprehensively dissected the genetic basis of inability to grow at 37°C on ethanol (37e) in a cross of the BY4716 (BY) lab strain and the YJM789 (YJM) clinical isolate. In total, we detected 15 loci, with roughly half of the causal alleles contributed by each cross parent. We then cloned and measured the quantitative growth effects of four loci that, in an otherwise YJM genetic background, result in a broad spectrum of growth levels at 37e. Based on this work, we resolved three of these loci to a kinase (*IKS1*), a component of the vacuolar protein sorting machinery (*VPS70*), and a stress granule-associated RNA binding protein (*YGR250C*). We found that these loci mainly contribute to inability to grow at 37e through large additive effects. However, the effects of these four loci are not specific to 37e, as each locus also shows a significant, but appreciably weaker growth effect on ethanol at standard culturing temperature (30°C) and on glucose at 37°C. This latter finding suggests that inability to grow at 37e is caused by additive genetic variants that show effects that are intensified in a more stressful environment.

Y3117C High-resolution phenotypic landscape of the RNA Polymerase II trigger loop. *Chenxi Qiu*, Olivia Erinne, Ping Cui, Huiyan Jin, Alvin Tang, Nandhini Mutukrishnan, Craig Kaplan. Texas A&M University, College Station, TX.

The conserved trigger loop (TL) lies in the RNA polymerase II (Pol II) active site, multi-tasking in substrate selection, catalysis, and translocation. To dissect TL function at individual-residue resolution, we quantitatively phenotyped nearly all possible TL single substituted mutants *en masse* in *Saccharomyces cerevisiae*. Three major mutants classes were revealed by clustering of growth phenotypes linked to transcription defects or various stresses. TL phenotypic classes have distinct distributions within the TL, and distinguish previously characterized catalytically fast and slow mutants. Substitutions in residues A1076, M1079, T1080, and L1101 support the proposed function of an intra-TL hydrophobic pocket in stabilizing the open TL conformation. Disruption of this pocket confers phenotypes consistent with hyper-activation of Pol II. In addition, allele-specific suppression/exacerbation between newly characterized TL mutants and mutants in the TL adjacent domains is consistent with the contribution of the TL adjacent funnel and bridge helices to TL dynamics. Our phenotyping platform, which is readily applicable to other yeast proteins, enables incorporation of structural and phenotypic data to dissect the Pol II active site with high functional resolution.

Y3118A Comprehensive analysis of the *SUL1* promoter of *Saccharomyces cerevisiae* . *Matthew S. Rich*[1], Celia Payen[1], Alan F. Rubin[4], Giang T. Ong[1], Monica R. Sanchez[1], Nozomu Yachie[5], Maitreya J. Dunham[1], Stanley Fields[1,2,3]. 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Department of Medicine, University of Washington, Seattle WA; 3) Howard Hughes Medical Institute, University of Washington, Seattle, WA; 4) Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; 5) Research Center for Advanced Science and Technology, University of Tokyo, Tokyo, Japan.

In the yeast *Saccharomyces cerevisiae*, beneficial mutations selected during sulfate limited growth are typically amplifications of the *SUL1* gene which encodes the high affinity sulfate transporter, resulting in fitness increases of >35%. *Cis*-regulatory mutations have not been observed at this locus; however, it is not clear whether this absence is due to a low mutation rate such that these mutations do not arise, or they arise but have limited fitness effects relative to those of amplification. To address this question directly, we assayed the fitness effects of nearly all possible point mutations in a 493 base segment of the gene's promoter through mutagenesis and selection. While most mutations were either neutral or detrimental during sulfate-limited growth, eight mutations increased fitness more than 5% and as much as 9.4%. Combinations of these beneficial mutations increased fitness only up to 11%. Thus, in the case of *SUL1,* promoter mutations could not induce a fitness increase similar to that of gene amplification. Using these data, we identified functionally-important regions of the *SUL1* promoter and analyzed three sites that correspond to potential binding sites for the transcription factors Met32 and Cbf1. Mutations that create new Met32 or Cbf1 binding sites also increased fitness. Some mutations in the untranslated region of the *SUL1* transcript decreased fitness, likely due to the formation of inhibitory upstream open-reading frames. We plan to extend this methodology to study the effect of mutagenesis on other yeast promoters.

Y3119B The role of the Mediator complex in Ty1 retrotransposition in *S. cerevisiae*. *Alicia Salinero*[1], M. Joan Curcio[1,2], Randall Morse[1,2]. 1) University at Albany School of Public Health, Albany, NY; 2) Wadsworth Center, NYSDOH, Albany, NY.

Retrotransposons are mobile genetic elements that replicate via an RNA intermediary. These elements have been known to drive evolutionary rearrangements and contribute to genomic instability. In *Saccharomyces cerevisiae*, the most abundant and active retroelement is Ty1. This 6 kb element encodes Gag, protease, integrase, and reverse transcriptase, and relies on numerous additional host factors to successfully complete all stages of its mobility cycle. One such set of host factors is the 25 subunit Mediator transcriptional co-activator complex. The Mediator complex is organized into the core head, middle, and tail domains, with a transiently associated kinase domain. The tail domain functions mainly as a target of DNA binding transcriptional activator proteins, while the head and middle recruit the RNA polymerase II machinery to the transcription start site. With the exception of the kinase domain, deletion of non-essential subunits from all Mediator domains has a drastic impact on Ty1 mobility. Disruption of the Mediator tail module in *med2Δ*, *med3Δ*, and *med15Δ* yeast decreases Ty1 activity to undetectable levels, while disruption of the head module (*med 18Δ* and *med20Δ*), and middle module (*med1Δ*, *med5Δ*, and *med31Δ*) increase Ty1 mobility substantially. However, these changes in overall Ty1 mobility do not correspond with significant differences in Ty1 transcription or Gag translation. It is only at the level of viral like particle assembly and reverse transcription of Ty1 cDNA that Mediator subunit deletions exhibit any observable alteration to Ty1 mobility. While Mediator affects Ty1 mobility post-translationally, the process depends on the presence of the Ty1 promoter, as determined by measuring the activity of Ty1 driven by a TEF1 promoter. Recent work from the Garfinkel lab has identified a truncated form of the Gag protein (p22) that disrupts VLP formation and prevents reverse transcription of Ty1

cDNA. Translation of p22 relies on increased levels of an internal transcript known as Ty1i. Mediator tail mutant strains show an increase in this Ty1i RNA. Thus, Mediator plays a substantial role in Ty1 mobility by controlling levels of Ty1i mRNA. Further research is underway to determine the mechanism by which this occurs.

This work was supported by NSF grants MCB0949722 and MCB1516839 to RHM and NIH grant GM52072 to MJC.

Y3120C Transcriptional regulation of quiescence state promoting factor Saf1p by MADS box motif protein Rlm1p in *S.cerevisiae*. *Meenu Sharma*, Vijeshwar Verma, Narendra Kumar Bairwa. Shri Mata Vaishno Devi University, Katra, Jammu and Kashmir,India.

Abstract:

RLM1 gene encodes for the MADS box transcription factor and involved in the cell wall integrity checkpoint in *S. cerevisiae*. RLM1p protein is phosphorylated and activated by the MAP-kinase Slt2p. RLM1 has been reported as activating transcription factor during stress. Nutrient limitation stress leads to situation where yeast cells enter into the quiescence state. During this stress the quiescence state promoting factor SAF1 gene product which constitutes the SCF E-3 ligase complex, recruits adenine deaminase factor Aah1p for ubquitination and subsequent degradation by 26S proteasome in *S. cerevisiae*. Here we investigated the association between RLM1 and SAF1 gene at transcriptional level for regulation during stress to understand the mechanism of cell cycle transition from active division to quiescence state in response to stress stimuli. For this we analyzed the expression profiling database (GEO) and yeast stress expression database ((http://www.ystrexdb.com) of *S.cerevisiae* cells for expression status of the RLM1, SAF1 genes in stress conditions. We observed that in variety of stress conditions (drug, pH, temperature, microbial toxin, inorganic compound) the RLM1 and SAF1 was constitutively over expressed at log2FC>1 and adjusted p-value < 0.05 setting in comparison to control cells. Further we analysed the association matrix between SAF1 gene and RLM1 using yeastextract (http://www.yeastract.com/) web tool for the association between genes and transcription factors. The analysis predicted the regulation of SAF1 gene by RLM1 transcription factor during stress conditions. Based on analysis we hypothesized that double knockout of saf1 and rlm1 genes cells may be resistant to stress condition which need to be tested experimentally.

(**Acknowledgement:** The authors acknowledge the support of Department of Biotechnology, GOI).

Y3121A Impairment of cell signalling in *Saccharomyces cerevisiae* as a result of suboptimal *PRPP* synthetase activity. *Eziuche A. Ugbogu*[1], Lilian M. Schweizer[2], Michael Schweizer[3]. 1) Abia State University, Uturu, Abia State, Nigeria; 2) School of Life Sciences, Heriot-Watt University, Edinburgh, UK; 3) School of Life Sciences, Heriot-Watt University, Edinburgh, UK.

PRPP (phosphoribosyl-pyrophosphate) is a key metabolite that plays a central role in many life processes, such as in the *de novo* and salvages biosynthesis of purine and pyrimidine nucleotides and therefore is essential for life. The *Saccharomyces cerevisiae* genome contains five paralogous *PRS* genes which exist as two complexes, one of which is a heterodimer, Prs1/Prs3 and the other, a heterotrimer, Prs2/Prs4/Prs5. Prs1 and Prs5 are distinguished by the presence of one and two NHRs (Non-Homologous Regions), respectively. Physical evidence for the genetically-defined Prs1/Prs3 complex was obtained by showing that in the absence of Prs3, Prs1 is unstable. In addition to interacting with Prs3, Prs1also interacts with Slt2, the MAPK of the Cell Wall Integrity (CWI) pathway. Point mutations in *PRS1* corresponding to missense mutations associated with human neuropathies or in the signature divalent cation- and/or PRPP-binding sites of Prs polypeptides interfere with CWI signalling resulting in temperature and/or caffeine sensitivity. The C-terminally located NHR5-2 of Prs5 is unusual in that it contains three potential phosphorylation sites. Mutation of these amino acid residues impinges on the expression of the transcription factor, Rlm1, an endpoint of the CWI pathway and reduces the temperature-dependent transcription of *FKS2*, the gene encoding the stress-induced catalytic subunit of 1, 3-β-glucan synthase essential for maintaining CWI. The results of our study support the hypothesis that the *PRS* gene family links, as a result of gene duplication and acquisition of NHRs, primary metabolism with CWI signalling in *S. cerevisiae*.

Y3122B Roles of Gis1-interacting Proteins in Heme Regulation of Gis1 Activity. *T. Wang*, P. Konduri, S. Lal, J. Comer, L. Zhang. The University of Texas at Dallas, Richardson, TX.

Heme is a cofactor for many important proteins that act in multiple cellular processes including aerobic respiration, biosynthesis, and small molecule processing. Previously, it has been shown that heme binds to regulatory and signaling proteins such as Hap1 in yeast, and Bach1 and Rev-erbα in mammals and controls their transcriptional activity. Recently, our studies investigated the regulatory role of heme in JmjC-domain-containing protein Gis1. Analysis of *lacZ r*eporter driven by a PDS (post diauxic shift) element showed a significant increase in Gis1 transcriptional activity under high heme concentration. Spectrophotometric analysis demonstrated direct interaction of heme with Gis1 and JmjN/C domain of Gis1 mammalian homologous JMJD2B. Given that a high number of unique interaction partners have been identified for Gis1 previously, it is conceivable that Gis1-interacting proteins can mediate heme regulation of Gis1 activity. By performing pull-down assay followed by mass spectrometry, *in vitro*, we identified a group of proteins that interact with Gis1 under high heme concentration. Knocking out of encoding genes of the identified interacting proteins led to a substantial decrease in heme activation of Gis1. Our studies indicate that Gis1 transcriptional activity is heme dependent, and Gis1-interacting proteins can promote the heme activation of Gis1 activity.

Y3123C A new method for inferring the genetic architecture of expression variation from allele-specific expression experiments. *X. Zhang*, J. Emerson. University of California, Irvine, Irvine, CA.

Allele-specific expression (ASE) reveals the mechanism of expression variation between two differentially expressed alleles. Expression variation can be classified as *trans*-acting when the variation is due to diffusible elements such as transcription factors or *cis*-acting when it is caused by mutations in regulatory sequences such as enhancer elements. Knowing the relative contribution of *cis*- and *trans*-acting variation to total expression variation is crucial in understanding the evolution of gene regulation. We predict that poorly replicated experiments and

experiments employing an unsuitable model underestimate the variance among independent biological replicates, and ignore the effect of hybrid-specific expression due to novel cis-trans interaction, leading to high rates of false positives for inferring differential ASE. We propose a new model which is statistically well-justified both from first principles as well as from empirical work. This method takes advantage of higher replication and appropriate reference genomes.

We introduce an R package which implements our model based on beta-binomial sampling of expression at two alleles. With this package, we test both our model and a previous binomial model using yeast expression data from recent work permitting testing of 48-fold replication. We show that our model estimates *cis*/*trans* expression differences with a type I error rate of approximately 5% when the significance level is set to 0.05. The binomial model underestimates the variance in the data and exhibits a much larger type I error rate of 0.15-0.45. This model will significantly revise conclusions from previous allele-specific expression studies; a large proportion of previous differential expression results are likely false positives, and we probably still do not have a very accurate picture of the nature of allele specific expression even in model systems.

Y3124A Bypassing quality control in 60S Ribosome Biogenesis. *S. Patchett*, Arlen Johnson. The University of Texas at Austin, Austin, TX.

In an actively growing yeast cell, thousands of ribosomes are synthesized per minute. After rapid assembly, these intricate molecular machines must faithfully translate genetic information into all cellular proteins. Quality control mechanisms during ribosome biogenesis are therefore critical to ensure that immature subunits and functionally defective subunits do not enter the translating pool.

During ribosome biogenesis, ribosomal subunits are pre-assembled in the nucleus and exported to the cytoplasm in an inactive state. Before they can engage in protein synthesis, the immature pre-60S subunits must first undergo cytoplasmic maturation. This pathway involves a series of ATPase and GTPase-driven steps that load critical ribosomal proteins, including Rpl10, and remove accessory factors that block ribosome function. These steps are critical for both the final structure of the ribosome and to ensure ribosomal subunits are competent for translation.

The release of Tif6, an accessory factor that blocks 40S joining, and the release of Nmd3, an export adaptor, are the final known steps in 60S maturation. Two proteins, Sdo1 and Efl1, cooperate to release Tif6 from the pre-60S subunit. Intriguingly, Sdo1 binds in the ribosomal P-site and is a structural mimic of tRNA, while Efl1 is closely related to elongation factor eEF2. We have suggested that this “translation-like” binding of Efl1 and Sdo1 is a functional check of the integrity of the 60S peptidyl-transferase center before its first round of translation. Ribosomes that fail this test do not release Tif6 or Nmd3, and are therefore prevented from engaging in translation.

We have shown that ribosomes carrying mutations in *RPL10,* including T-cell leukemia associated *rpl10-R98S,* specifically fail in this quality control step. To understand the consequences of bypassing quality control, extragenic suppressing mutations of *RPL10* mutant alleles were isolated. Importantly, the suppressing mutations bypass the biogenesis defect, but allow mutant ribosomes to engage in translation. Through the characterization of extragenic suppressors, we have uncovered several distinct pathways of bypassing 60S quality control.

Y3125B Early branching *Saccharomyces* for understanding the genetics and evolution of an industrially important genus. *EC. Baker*, W. G. Alexander, D. Peris, Q. Langdon, C. T. Hittinger. University of Wisconsin - Madison, Madison, WI.

In the last several years the relationships among the strains previously referred to as *Saccharomyces bayanus,* have been clarified. This has led to the splitting of these strains into the sister species *Saccharomyces uvarum* (formerly *S. bayanus var. uvarum*) and *Saccharomyces eubayanus* (previously the unknown *S. bayanus*-like contributor to hybrid lager yeasts), and a complex of multi-species hybrids. Hybrids of *S. eubayanus* and *S. uvarum* display more than 10x the spore viability of any other *Saccharomyces* hybrid pair, greatly facilitating genetic studies. Intriguingly, while these species are roughly 10% diverged at the nucleotide level, they are sympatric in nature and, apparently, very physiologically similar. Despite this however, brewing strains of each species (always *Saccharomyces cerevisiae* hybrids or introgressed strains) are strongly allied with different types of fermentations: wines and ciders for *S. uvarum* and lager style beers for *S. eubayanus.* To better understand what separates these industrially important yeasts we have begun exploring the phenotypic and genetic diversity within and between *S. eubayanus* and *S. uvarum*. By comparing the whole-genome sequences of hybrid lager yeasts with their parent species we found that the *S. eubayanus* portion of the lager yeast genomes has experienced pronounced and pervasive relaxation of selective pressure. In addition, we also identified several gene clusters that are likely to be important for *S. eubayanus’* contribution to maltose consumption, which is essential for efficient fermentation. With gene knockout and replacement studies we are further exploring this essential industrial trait. To determine the genetic and phenotypic basis of separation between *S. uvarum and S. eubayanus,* both in nature and in human related fermentations, we are using genome comparisons and analysis of hybrids. Preliminary studies of hybrids indicate possible difference in RNA metabolism, potentially providing insight into the functions of RNA metabolism in ancestral *Saccharomyces* and extant yeast lineages. By studying the diversity contained both within and between these species we not only increase our understanding of this pair of industrially important yeasts, but as an early branching lineage of the *Saccharomyces* they can shed light on the evolution of some peculiar traits, such as the early loss of RNAi, in this important model genus.

Y3126C High-throughput investigation into the evolutionary forces underlying sequence divergence. *Drew T. Doering*, Chris Todd Hittinger. University of Wisconsin-Madison, Madison, WI.

Predicting the relationship between protein sequence and function remains a major challenge in genomics. Homologs that are divergent in sequence often have conserved functions, while rare single nucleotide polymorphisms in a population are the basis of many human diseases. Variation in protein-coding sequence can impact function in various ways, such as by altering folding, enzyme kinetics, binding affinity, or other factors. Here I present a method I am developing to investigate the effects of sequence divergence on the functions of proteins from both

retrospective and prospective viewpoints. This method will enable the characterization of full-length protein variants expressed from the native genomic locus through quantification of variant frequencies in response to competitive growth in selective conditions. Here, using *Saccharomyces* as a model genus, I show how this method will illuminate the evolutionary fitness landscape of a small protein (Atx1) from all *Saccharomyces* species and homologs from other yeasts and multicellular eukaryotes, including humans. Using established genome-editing technologies, I will generate strain libraries that are completely isogenic, except at the *ATX1* locus. I will then conduct pooled competitions of cells expressing *ATX1* variants in selective conditions and monitor the changes in variant frequencies by high-throughput sequencing. Deriving fitness values from these frequency changes enables the comparison of the effect of a given genetic variant on protein function and, ultimately, illuminates the constraints present in the fitness landscape of *ATX1*. Thus far, measuring growth of yeast strains expressing individual Atx1 homologs in selective conditions has revealed differences in fitness, including a general trend toward greater sequence divergence resulting in lower fitness. Notably, *HAH1* (the human homolog of *ATX1*) is able to complement the yeast deletion. Pooled competitive growth assays will further reveal the fitness consequences of genetic variation as cells expressing Atx1 homologs compete for limited resources. Additionally, testing rare *ATX1* variants generated by saturation mutagenesis will ultimately enable the characterization of a fitness landscape for *ATX1*. This technology could be used to study genes implicated in human disease and serve as a platform for diagnosis of heritable diseases that are due to hypomorphic alleles that are present at low frequencies in humans. With the emergence of precision medicine enabled by affordable genome sequencing, physicians could use data generated by this type of study to diagnose a wide variety of genetic diseases using only the patient's genome sequence.

Y3127A The making of biodiversity across the yeast subphylum. *C. T. Hittinger*[1,2], D. A. Opulente[1], A. B. Hulfachor[1], J. DeVirgilio[3], J. Kominek[1], M. Kuang[1], A. Rokas[4], C. P. Kurtzman[3]. 1) Laboratory of Genetics, Genome Center of Wisconsin, Wisconsin Energy Institute, J. F. Crow Institute for the Study of Evolution, University of Wisconsin-Madison, Madison, WI, USA; 2) DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI, USA; 3) Bacterial Foodborne Pathogens and Mycology Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, Peoria, IL, USA; 4) Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA.

Yeasts offer a unique opportunity to understand the genetic mechanisms that generate biodiversity. Their remarkably diverse heterotrophic metabolisms have allowed them to partition nutrients in every major aquatic and terrestrial biome and to inhabit every continent. They have evolved radically different carbon metabolisms and energy management strategies. Most yeasts prefer respiration, but some species, such as the genetic model system *Saccharomyces cerevisiae*, have evolved highly fermentative lifestyles. A handful of clades can accumulate over half of their dry weight as fatty acids, while several species can ferment pentose carbon sources, such as xylose, which is the second most-abundant sugar in plant biomass. The Y1000+ Project (http://y1000plus.org) seeks to determine the genetic basis of yeast metabolic diversity across the subphylum Saccharomycotina. Here we report progress on generating and analyzing functionally annotated genome sequences for all ~1,000 known species of yeasts, as well as comparing their genome contents with rich functional, ecological, and metabolic data. By creating and analyzing the first comprehensive genomic dataset and catalog of metabolic diversity for a high-level taxonomic rank, the Y1000+ Project will illuminate how key genetic innovations and convergent genetic changes have driven diversification across half of billion years of evolution. Comprehensive taxon and genome sampling will allow us to determine how metabolic diversity is encoded in their genomes; the tempo and mode of change; and which phenotypic traits and genetic changes lead to niche expansion, clade diversification, and long-term survival. The genetic features that cause some yeasts to brew beer, others to proliferate on cacti, and still others to blight crops or cause lethal infections are gradually coming into focus.

Y3128B Uncovering rules governing gene replacement between humans and yeast. *Jon M. Laurent*[1], Aashiq H. Kachroo[1], Christopher M. Yellman[1], Austin G. Meyer[1,2], Claus O. Wilke[1,2,3], Edward M. Marcotte[1,2,4]. 1) Center for Systems and Synthetic Biology, Institute of Cellular and Molecular Biology, University of Texas at Austin, Austin, TX; 2) Center for Computational Biology and Bioinformatics, University of Texas at Austin, Austin, TX; 3) Department of Integrative Biology, University of Texas at Austin, Austin, TX; 4) Department of Molecular Biosciences, University of Texas at Austin, Austin, TX.

Owing to its ease of manipulation and rapid growth, the baker's yeast *Saccharomyces cerevisiae* is a popular model organism for studying many aspects of eukaryotic biology. Due to the functional conservation still present between much of the human and yeast proteome, yeast has served an important role in the study of human biology and disease. Inspired by these studies, our lab has been systematically testing which essential yeast proteins are replaceable by their human counterparts, screening for functionality by the human genes' ability to rescue growth in the absence of the yeast proteins. We have found that replacement of genes with 1:1 orthology is governed largely by gene modules, such that pathways or complexes are similarly replaceable or not (Kachroo et al. (2015) *Science*, 348:921-925).

We have now begun to expand our replacement set to include all other essential yeast-human orthologs, covering all ortholog classes (e.g., many:1, 1:many, many:many). We have now tested over 700 pairs of orthologs between the two species. We have observed a variable pattern of replaceability across different ortholog classes, with an obvious bias towards differential replaceability inside gene families, rather than all members of a family being similarly able to replace. In order to determine which properties of co-orthologs can potentially explain this differential replaceability, we have assembled a set of quantitative features of the genes and gene families, including calculated properties of the genes' sequences (e.g., gene and protein lengths, sequence similarities, codon usage) and other properties such as protein interactions, mRNA and protein abundances, and transcription and translation rates. We then quantify how well each feature can predict replaceability, while differentiating between co-orthologs. Further, given our comprehensive results of individual replacements and the rules governing them (e.g. modularity, expression levels), we have now begun to integrate multiple members of human pathways or complexes into the yeast genome, with the goal of building entirely 'humanized' processes.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

These data demonstrate that a substantial portion of conserved yeast and human genes perform much the same roles in both organisms even after >1 billion years of evolution, and provide a direct test of the ortholog-function conjecture across gene familes. Many of the genes that can be replaced have important roles in human disease, including cancer. 'Humanized' strains will simplify drug discovery against human proteins, enable studies of the consequences of human genetic polymorphisms, and empower functional studies of entire human cellular processes in a simplified organism.

Y3129C Evolutionary dynamics of second beneficial mutations via a double-barcoding platform. *F. Li*[1,2], X. Liu[2,3], E. Coutsias[1,2], JR. Blundell[2,3,4], SF. Levy[2,3]. 1) Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY; 2) Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, NY; 3) Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY; 4) Department of Applied Physics, Stanford University, Stanford, CA.

We have previously built an ultra-high resolution barcoding system that can be used to simultaneously track the evolutionary dynamics of millions of yeast lineages in real time. By evolving an initially isogenic barcoded population, we identified and characterized tens of thousands of mutations that provide a benefit to the initial genotype. Describing the mutation rate spectrum of these first beneficial mutations, the fitness effect of each mutation and the rate to that fitness effect, allowed us to predict the evolutionary dynamics of the population for ~80 generations. However, making predictions out to longer times is challenging because the evolutionary dynamics will depend heavily on second beneficial mutations and how these interact with first mutations. With this technology, second mutations are difficult to detect because they are most likely to occur in lineages that have already expanded to large sizes. Here, we develop a new double-barcode lineage tracking system that allows us to measure the fitness effects and occurrence times of thousands of first and second mutations in the same evolving yeast population. This system consists of a double landing-pad capable of accepting two large barcode libraries that can be inserted at different times during an evolution. The addition of second barcodes late in an evolution allows us to break up large lineages containing a first mutation in order to quantitatively study the second mutations that occur on top. Using this system, we are evolving presumably isogenic haploid and diploid yeast libraries containing ~200,000 first barcodes in carbon-limited media at two temperatures. At a time when most cells contain a first beneficial mutation but few contain a second, we will insert the second library of ~2M barcodes and evolve for another 200 generations. Amplicon sequencing of double barcodes will be used to track lineage trajectories following re-barcoding. Mathematical models will be used to estimate the occurrence times and fitness effects of second mutations, and how they depend on the first mutation. This high resolution and large scale evolution experiment will enable us to better understand macroscopic epistasis by systematically characterizing the relationship between first mutations and potential for further adaptation.

Y3130A Quantitative evolutionary dynamics of a large number of yeast segregants. *Xianan Liu*[1,2], Fangfei Li[1,3], Takeshi Matsui[4], Ian Ehrenreich[4], Sasha Levy[1,2]. 1) Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, NY; 2) Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY; 3) Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY; 4) Molecular and Computational Biology Section, Department of Biological Sciences, University of Southern California, Los Angeles, CA.

The distribution of fitness effects (DFE), or beneficial mutation rate spectrum, is the rate at which mutations of each fitness effect occur in a particular genotype. We have previously characterized the DFE in an initially isogenic yeast population using high-resolution lineage tracking and shown that it can be used to predict the evolutionary dynamics of large populations in the lab. In more complex evolutionary scenarios where initial genotypes are heterogeneous, the evolutionary dynamics depends on both the fitness and the DFE of each genotype. Yet, little is known about how the DFE varies across genotype space. Here, we focus on measuring how the DFE depends on the genotype using a novel tandem integration double barcoding system. This system relies on two incompatible loxP variant sites in the genome, which sequentially bring two barcoded plasmid libraries (BC1 and BC2) to the common genomic location. The trajectory of each double barcode lineage can subsequently be monitored by pooled growth and amplicon sequencing. We have generated a large haploid segregant pool from a cross between genetically divergent strains, the lab strain BY4741 and the pathogenic clinical isolate YJM789. We have picked and verified ~200 segregants, each barcoded with a unique BC1, and measured their fitness by pooled competition to find a subset that represent a broad spectrum of initial fitnesses. To measure the DFE of many segregants, we are barcoding each with ~100,000 BC2 barcodes and generating ~5 pools that contain overlapping segregants. The construction of segregant pools are guided by numerical simulations to maximize the number of segregants for which the DFE can be estimated. Serial batch evolution with these pools in three different environments will be used to examine how much the DFE varies between segregants, and if and how much the DFE depends on the initial fitness. Next, we will sequence the genome of each segregant to perform a quantitative trait loci (QTL) analysis for various features of the DFE. This QTL study will test the hypothesis that some genetic loci explain some variance in the DFE and thereby modulate evolvability.

Y3131B Mining *Saccharomyces* diversity and experimental evolution for cellulosic biofuel production. *D. Peris Navarro*[1*], R. V. Moriarty[1], W. G. Alexander[1], K. Sylvester[1], M. Sardi[1], D. Libkind[2], P. Gonçalves[3], J. P. Sampaio[3], Q. M. Wang[1,4], F. Y. Bai[4], J. B. Leducq[5], C. Landry[5], K. Hyma[6], J. Fay[6], T. K. Sato[1], C. T. Hittinger[1]. 1) University of Wisconsin-Madison, Madison, WI, USA; 2) CONICET-UNComahue, Bariloche, Argentina; 3) Universidade Nova de Lisboa, Caparica, Portugal; 4) Chinese Academy of Sciences, Beijing, China; 5) Université Laval, Québec, Canada; 6) Washington University, St. Louis, MO, USA.

In the last decade, the application of new molecular techniques has helped to identify *Saccharomyces* species and unravel their diversity. Indeed we present a poster related to wild and industrial dynamics of *S. eubayanus* lineage (see Langdon Q.). Mining nucleotide variation and the translated effect on different levels of stress tolerance, sugar consumption rates, or compound production, might be the first step to

unravel interesting industrial traits from strains. Here, we screen 510 *Saccharomyces* strains, exploring diversity levels on par with the diversity between human and birds, in AFEX-Corn Stover Hydrolysate (ACSH) and report the performance of the best candidates during microaerobic fermentations in ACSH, YPDX and YPDX + Feruloyl Amide. Our results show a potential application for lineages such as *S. paradoxus* and *S. mikatae* which show tolerance to ACSH conditions, and *S. uvarum* for their xylose consumption rates. In addition, we mimic the process of brewing hybrid domestication in this biofuel media. We studied the performance of 8 artificial hybrids and compared them to their parents. We also subjected the hybrids to experimental evolution during microaerobic fermentations in ACSH for 50 generations. We observed a great potential for hybridization, and developed a new system for generating efficiently hybrids, called HyPr (Hybrid Production). Now, to explore more diversity we are generating new hybrids with chassis strains and selected *Saccharomyces* lineages; together with experimental evolution and whole genome sequencing, we expect to unravel the genetic components underlying interesting traits, which could be integrated in artificial gene networks in chassis strains to improve their performance in industrial conditions.

Y3132C Condition-dependent differentiation and division of labor in clonal *Saccharomyces cerevisiae* biofilms. *B. Regenberg*, K. E. Hanghøj, K. S. Andersen, J. J. Boomsma. Department of Biology, University of Copenhagen, Denmark.

How multicellularity with division of labor between cell types evolved is one of the most fundamental questions in biology, but previous studies have hardly explored single-gene pre-adaptations that may allow differentiation to be realized. We show that differential expression of *FLO11* produces an evolutionary stable division of labor trait in clonal *S. cerevisiae* biofilm colonies on medium with intermediate viscosity. Differentiated Flo11$^{+/-}$ colonies obtain a 4-fold growth advantage over undifferentiated colonies by overgrowing glucose resources instead of depleting them as undifferentiated Flo11^{-} colonies do, and they maintain their differentiated state by switching non-adhesive cells to adhesive cells with predictable probability in the growing edge. Our results demonstrate how differentiation allows fitness advantages by preemptive territory acquisition and robustness against invading free-riding strains. *S. cerevisiae* biofilm thus represents a suitable model for studying the very first evolutionary steps towards clonal multicellularity.

Y3133A Using the experimental evolution of long-lived yeast species for testing evolutionary theories of aging. *V. Titorenko*, A. Gomez-Perez, A. Arlia-Ciommo, Y. Medkour. Concordia Univ, Montreal, PQ, CA.

We use the yeast *Saccharomyces cerevisiae*, a unicellular eukaryote amenable to high-throughput molecular analyses, as a model organism for empirical validation of evolutionary theories of programmed or non-programmed aging and age-related death. We found that lithocholic bile acid (LCA) is a geroprotector which significantly delays the onset and reduces the rate of yeast chronological aging. Unlike mammals, yeasts do not synthesize bile acids. Our analysis of how LCA and other anti-aging compounds (including resveratrol, caffeine, and rapamycin) synthesized and released into the environment by one species of the organisms composing an ecosystem extend longevity of other species within this ecosystem suggests that these interspecies chemical signals may create xenohormetic, hormetic and cytostatic selective forces driving the evolution of longevity regulation mechanisms at the ecosystemic level. To test this hypothesis, we carried out the LCA-driven experimental evolution of long-lived yeast species. Our selection yielded three yeast strains with greatly extended lifespans. As an empirical test of the antagonistic pleiotropy and life history optimization theories of aging, we analyzed the trade-offs between early-life fitness and longevity by measuring the relative fitness of each of the laboratory-evolved long-lived yeast species in a direct competition assay with the parental wild-type strain. The assay was carried under caloric restriction (CR) conditions, which mimic the natural stressful environment of cyclical starvation, as well as under non-CR conditions in a more favorable environment. We found that, if cultured under field-like CR conditions, each of the three long-lived yeast species exhibits reduced fitness. Such a trade-off between early-life fitness and longevity was significantly less pronounced under laboratory-like non-CR conditions. Our studies of the mechanisms underlying the observed trade-off between early-life fitness and longevity revealed that 1) none of the laboratory-evolved long-lived yeast species has reduced fecundity; and 2) the decreased relative fitness of each of these species was not due to a reduction in growth rate early in life. Our monitoring of the age-dependent dynamics of changes in mitochondrial composition, morphology, and function implies that the longevity-associated fitness defect in each of the three laboratory-evolved long-lived yeast species is a dominant genetic trait attributed to specific lifespan-extending alterations in mitochondria. Based on our findings, we propose a hypothesis of the natural selection forces and underlying mechanisms that drive the evolution of yeast longevity and maintain a finite yeast lifespan within ecosystems.

Y3134B Does antifungal drug resistance potentiated by Hsp90 arise from stress-induced mutations? *A. E. Yuan*, D. F. Jarosz. Stanford University, Stanford, CA.

Theory holds that the capacity of a population to evolve in response to a selective pressure is due to the enrichment of pre-existing adaptive variants. That is, successful alleles are not produced by selection itself. This line of thinking has recently been challenged by the observation that some types of selection can induce mutations that confer an adaptive advantage. Although the generality of such findings is controversial, a number of induced mutagenesis systems have now been characterized at the genetic level in bacteria. Much less is known about whether and how such processes might operate in eukaryotic cells. The highly conserved molecular chaperone Hsp90, which assists in the folding of many proteins that are critical for growth and development, has been proposed to broadly influence the capacity of mutations to produce new phenotypes. Because the activity of Hsp90 is stress-regulated, we investigated whether it might provide a means through which the environment could influence acquisition of adaptive phenotypes. Using the classic statistical assay known as the fluctuation test, we observed that a modest change in Hsp90 activity altered the mechanisms by which yeast cells acquire resistance to the commonly prescribed antifungal drug fluconazole. The distribution of resistant variants we obtained suggested that fluconazole exposure itself likely induced the acquisition of resistant phenotypes. This process strongly depended upon Hsp90 activity; induced resistance was not observed in cells with reduced Hsp90

activity. Furthermore, pre-exposure to fluconazole increased the frequency at which cells acquired resistance to the unrelated selective agent 5-fluoroorotic acid, supporting the idea that fluconazole can induce genetic diversification. We observed similar results with the evolutionarily distant human pathogen *Candida albicans*. Our findings establish a means through which environmental stress might influence the fundamental mechanisms of adaptation in eukaryotes and further point to an unappreciated benefit of modulating Hsp90 activity as a strategy to overcome drug resistance in fungi.

Y3135C Evolutionary and functional analysis of dubious open reading frames suggest a functional role in yeast genomes. *G. Arora*[1], A. G. Rosenwald[2]. 1) Gallaudet University, Washington, DC-20002; 2) Georgetown University, Washington DC-20057.

The *Saccharomyces cerevisiae* genome has been studied extensively since it was first sequenced 20 years ago. A number of open-reading frames in this genome, are still classified as "dubious" including those that overlap open-reading frames known to encode functional proteins. We reexamined whether this status is still warranted given the large increase in data from numerous sources. These data include genomic information from other members of the *Saccharomyces* clade and transcriptome information from *S. cerevisiae* grown under a number of different conditions. Using genomic data we first examined the conservation of these ORFs compared to other members of the clade. Second, we examined existing data from tiling microarray and RNA-seq experiments. Our data show that these ORFs are in fact conserved and many of them are differentially expressed, suggesting a functional role.

Y3136A Genomic approaches in *Saccharomyces cerevisiae* reveal that response to the toxic spill chemical 4-methylcyclohexanemethanol is mediated by genes involved in pleiotropic drug response, in reactive oxygen species protection, and in UAS *INO* inositol biosynthetic regulation. *M. Ayers*, X. Rong-Mullins, C. Nassif, J. Gallagher. West Virginia University, Morgantown, WV.

In the aftermath of the January 2014 MCHM spill into the Elk River of Kanawha county, West Virginia, the lack of knowledge concerning the mode of action of this chemical within the cell became clear. The budding yeast is a key model organism with conserved eukaryotic biochemical and genetic pathways, as well as convenient functional genomics tools with which to identify how the cell responds to chemicals. Investigations into MCHM cellular response included growth assays of wild type strains, viability assays, growth assays of knockout strains, RNA-seq analysis, quantitative trait loci, and GFP-labeled protein localization microscopy. Viability and growth assays of wild type yeast revealed sensitivity to MCHM in the concentration range of approximately 0.04-0.06%, though defect in growth appears to be from inhibition of growth instead of lethality. RNA-seq analysis for differential expression of genes with MCHM treatment indicated that genes for transcription factors and ABC transporters in the pleiotropic drug response are affected. Knockout strains for drug response and oxidative stress genes were also grown in MCHM, revealing several mutants in these pathways were sensitive, and therefore required for MCHM response. The RNA-seq data also pointed to the inositol biosynthetic pathway, which is normally tightly regulated by a certain set of proteins. One gene in particular, the major negative regulator of UAS *INO* genes, *opi1*, showed sensitivity to MCHM. Many of the genes regulated by the Opi1 protein are downregulated by MCHM. Microscopy of the GFP-labeled Opi1p revealed relocalization of the negative inhibitor protein into the nucleus due to MCHM treatment in media lacking inositol, the normal signal for relocalization. Future investigation into the effects of MCHM must involve investigation of pleiotropic drug response, oxidative stress, and the inositol biosynthetic pathway to reveal mechanisms of toxicity and targets of treatment for exposure.

Y3137B Comparisons of the genomes of *Holleya sinecauda* and *Ashbya gossypii* – closing in on the minimal gene set for a free-living fungus. *Fred S. Dietrich*. Duke Univ, Durham, NC.

The filamentous fungus *A. gossypii* and the related dimorphic fungus *H. sinecauda* have compact genomes of less than 10Mb. They have small intergenic regions frequently less than 100 bases in length, no transposable elements, and short telomeric and centromeric regions. We have recently completed the genomic sequence of *H. sinecauda* and deposited it in Genbank. A comparison of the genomes identifies approximately 30 genes whose presence or absence differs between these two species. While *A. gossypii* has over 5000 protein coding genes and genes producing an RNA product, comparison with *H. sinecauda* and other hemiascomycete species suggests the minimal gene set for a hemiascomycete to grow robustly on salts, ammonia, carbon source, water is around 4800 genes.

Combining this analysis with the recent analysis of 100 *S. cerevisiae* genes (Strope et al, 2015) suggests a set of core genes in *S. cerevisiae*, a set of additional genes beyond the core set, and a set of genes found in only a subset of *S. cerevisiae* genomes. In addition to variation in the gene set adding functionality to the genome, gene duplication, horizontal gene transfer, and introgression are all contributing to the genomic plasticity of *S. cerevisiae*..

Y3138C Chromosome-specific and global effects of aneuploidy revealed by Synthetic Genetic Array analysis. *Stacie Dodgson*[1], Stefano Santaguida[1], Sharon Kim[1], Michael Costanzo[2], Anastasia Baryshnikova[3], Charles Boone[2], Angelika Amon[1]. 1) Koch Institute for Integrative Cancer Research, HHMI, Dept. of Biology, MIT, Cambridge, MA; 2) The Donnelly Centre and Dept. of Molecular Genetics, Univ. of Toronto, Toronto, Canada; 3) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

Aneuploidy, an unbalanced karyotype in which one or more chromosomes are present in excess or reduced copy number, causes an array of known phenotypes including proteotoxicity, genomic instability and slowed proliferation. However, the molecular consequences of aneuploidy are poorly understood and an unbiased investigation into aneuploid cell biology was lacking. We performed high-throughput screens for genes whose deletion has a synthetic fitness cost in aneuploid *Saccharomyces cerevisiae* cells containing single extra chromosomes. This analysis identified genes that when deleted decrease the fitness of specific disomic strains as well as those that impair the proliferation of a broad range of aneuploidies. In one case, a chromosome-specific synthetic growth defect could be explained fully by the specific duplication of a single gene

on the aneuploid chromosome, highlighting the ability of individual dosage imbalances to cause chromosome-specific phenotypes in aneuploid cells. Deletion of other genes, particularly those involved in protein transport, however, confers synthetic sickness on a broad array of aneuploid strains. Indeed, aneuploid cells, regardless of karyotype, exhibit protein secretion and cell wall integrity defects. Additionally, we identified a deubiquitinase, Ubp3, whose deletion broadly impairs aneuploid yeast cell fitness, and this synthetic effect is conserved in human cells. Thus, we were able to use this screen to identify novel cellular consequences of aneuploidy across organisms, which are dependent on both specific chromosome imbalances as well as caused by many different aneuploid karyotypes. Interestingly, the vast majority of cancer cells are highly aneuploid, so this approach could be of further use in identifying both karyotype-specific and nonspecific stresses exhibited by cancer cells as potential targets for the development of novel cancer therapeutics.

Y3139A Toxicogenomic approaches for profiling resistance to P450-activated food carcinogens and phenotyping human P450 polymorphisms in budding yeast. *Michael Fasullo*, Nicholas St. John, Julian Freedland, Cinzia Cera. State Univ New York Polytechnic Institute, Albany, NY.

The human response to environmental carcinogens is highly variable, depending upon environmental, lifestyle, and genetic factors. Genetic factors include polymorphic P450 and DNA repair genes; however, epidemiological studies may lack significance due to inadequate patient numbers. We used budding yeast (*Saccahromyces cerevisiae*) as a model organism to determine genetic susceptibility to food-associated carcinogens, including benzo[a]pyrene dihydrodiol (BaP-DHD), aflatoxin (AFB_1) and heterocyclic aromatic amines (HAAs). Budding yeast does not contain P450s that convert these compounds into genotoxic metabolites, so we introduced expression vectors that contain specific human P450 and NAT2 genes into yeast deletion library collection.. In yeast, either CYP1A2 or CYP1A1 activates AFB_1, while both CYP1A2 and NAT2 are required for activation of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ). To measure genotoxic effects, we measured recombination and mutation frequencies, Rad51 foci, growth inhibition and DNA adducts. To determine resistance genes, we used a high throughput approach for screening the yeast deletion library expressing specific P450 genes. Screens for aflatoxin resistance identified checkpoint, RNA metabolism, and mitochondrial maintenance genes, several of which are risk factors in cancers. We are now performing screens to identify genes involved in resistance to IQ. Preliminary data identified both recombinational repair and DNA damage tolerance genes. Further high throughput analysis will be performed using other food carcinogens, including 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx). These studies thus represent a novel approach for phenotyping P450 polymorphisms and open new avenues for exploring resistance to P450-activatable compounds. Grant Support: National Institutes of Health, 1R15ES023685-01.

Y3140B Polygenic Analysis of Ethanol Tolerance and Maximal Ethanol Accumulation capacity in *Saccharomyces cerevisiae*. *Annelies Goovaerts*[1,2], Steve Swinnen[1,2], Thiago Pais[1,2], Maria Foulquié-Moreno[1,2], Johan M. Thevelein[1,2]. 1) KU Leuven, Leuven, Belgium; 2) VIB, Leuven, Belgium.

Introduction

Most traits of industrial importance in yeast are polygenic, therefore complex and difficult to study. In our laboratory, we have developed a technology called 'pooled-segregant whole-genome sequence analysis', which has been successfully applied to complex traits such as high ethanol tolerance and maximal ethanol accumulation capacity. The aim of this study was to identify new genes underlying high ethanol tolerance and maximal ethanol accumulation capacity by combining both pooled-segregant whole-genome sequence analysis and pooled-segregant RNA expression analysis.

Methods

A haploid strain displaying the superior trait of interest was crossed with a haploid inferior lab strain. Segregants of this cross showing the phenotype of the superior parent were selected and pooled together. The genomic DNA of the pool and the parents was extracted and sequenced by Illumina HiSeq2000. The SNP variant frequency of the pooled DNA was plotted against the SNP chromosomal position, to map the quantitative trait loci (QTLs). Reciprocal hemizygosity analysis (RHA) was applied to identify the causative genes in the QTLs. Genome-wide gene expression analysis at a fermentation time-point where the ethanol concentration was 13.8% (v/v) was performed for the superior pool and parent strains via RNA-Seq. The new identified genes are introduced in a second-generation bio-ethanol producing strain with the Crispr/Cas9 method in order to improve the ethanol tolerance and maximal ethanol accumulation.

Results and discussion

Several QTLs were identified for the traits ethanol tolerance and maximal ethanol accumulation. Fine-mapping and RHA identified several specific causative genes for these traits of interest. RNA sequence analysis revealed 37 genes that were overexpressed in the pool of segregants and superior parent in comparison with the inferior parent. Most of these genes have a biological function related to stress tolerance. Our results reveal that a combination of pooled-segregant whole-genome sequence analysis and gene expression analysis is a promising approach to understand the genetic basis of complex traits.

Y3141C The evolutionary constraints of gene expression levels in *S. cerevisiae*. *M. J. Hickman*, A. Jackson, A. Tursi, J. Thornton, A. Smith. Rowan University, Glassboro, NJ.

It has been widely reported that some genes are expressed at a higher level than others. However, it has not been shown whether each gene is expressed consistently between studies and at the same level compared to all other genes. Here, we examined nine RNA-seq datasets and found that the mRNA level of each gene is indeed consistent relative to all other genes. The mRNA levels were also highly correlated to protein levels measured by different methods, though translational and protein turnover regulation likely prevent a perfect correlation. The consistency of gene expression level implies that there are evolutionary pressures that drive genes to maintain either low or high expression. In

order to identify these pressures, we compared expression level to the features of each gene (or associated protein), such as protein function, localization, essentiality and number of interactions. We found many possible pressures; for example, genes involved in translation or the ribosome were highly expressed while genes involved in chromosomal activities, such as replication and transcription, were lowly expressed. Furthermore, we optimized an artificial neural network, using several of these features as inputs, and could predict gene expression level with ~80% accuracy. In conclusion, these results show that gene expression level is likely constrained by several evolutionary pressures, including the biological process and cellular localization of the associated protein.

Y3142A An Integrated platform to characterize neurodegenerative disease associated proteins in yeast. *S. Ju*, S. Chen, A. Koesters, E. Hayden, Q. Zhong. Wright State University, Dayton, OH.

The molecular underpinnings of neurodegenerative disease are gradually emerging following biochemical characterization of protein aggregates in disease tissues, and the identifications of causal genes in certain familial forms of neurodegenerative disorders. With the vast majority of disease cases being sporadic and causes unknown, however, our understanding of neurodegeneration remains vastly incomplete. Becoming increasingly appreciated is also the difficulty to evaluate the extent to which a given risk factor identified in human genomes may alter susceptibility of any individual to a specific type of neurodegenerative disorder. This is largely due to a critically missing aspect of our understanding of neurodegenerative disease from the perspective of complex networks of gene-gene interactions. Such networks may consist of genes with causal or modifier effects and interactions that can be specifically targeted by gene mutations. Here we present three proof-of-principle studies using yeast as a model system to map and characterize biochemical and genetic interactions of neurodegenerative disease-associated proteins. Our studies demonstrate the feasibility to i) systematically profile functional alterations of human gene alleles associated with neurodegenerative disease; ii) to rapidly identify, at the genome scale, genes that exert effects the disease-associated protein aggregation and toxicity at the cellular level; and iii) to measure changes in protein-protein interactions by both disease-causing and putative mutations. Our long-term goal is to interpret phenotypic consequences of genetic variations associated with neurodegenerative disease from the global integrated network perspective.

Y3143B Investigating the effects of gene overexpression on genome stability in Saccharomyces cerevisiae. *Krystal Laframboise*, Grant W. Brown. Department of Biochemistry, Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada.

Accurate replication of the genome is essential for survival. The combination of both exogenous and endogenous sources of replication stress and DNA damage serve as a constant threat to the fidelity of this process. Fortunately, eukaryotic cells have a highly conserved DNA damage and replication checkpoint, which acts to recognize and repair damage to prevent genome instability. Though the mechanisms of genome maintenance have been extensively studied in *Saccharomyces cerevisiae*, almost all previous screens used to identify novel genes involved in these processes have employed loss-of-function alleles, subsequently ignoring the consequences of gene overexpression. These consequences are of particular interest as several disease states are associated with gene overexpression or gain-of-function mutations, including many human cancers. Here, we assay expression of the DNA damage-inducible gene, *RNR3*, using reporter synthetic genetic array methodology to identify genes causing genome instability when overexpressed. We find 41 of ~5100 genes screened result in increased *RNR3* expression, including known DNA repair genes and transcriptional regulators of *RNR3*, confirming the capability of our screen to robustly identify genes of functional relevance. 58.5% of genes inducing *RNR3* in our screen had no reported connection to genome stability when compared to previous overexpression studies, leaving 24 novel, putative genome maintenance genes for follow-up. We are currently using fluorescence microscopy to visualize the formation of various DNA damage-specific foci to ensure the mechanism of *RNR3* induction caused by these novel candidates is related to genome instability and is not the result of transcriptional regulation. Furthermore, since 17 of our candidates have human homologues, our data will not only provide insight into novel pathways involved in genome maintenance in *S. cerevisiae*, but may also reveal novel pathways promoting oncogenesis in humans.

Y3144C Analyzing Terminal Phenotypes in *Saccharomyces cerevisiae* Using Synthetic Genetic Array and High-Content Screening. *D. Lo*[1], O. Kraus[2], A. Verster[3], C. Boone[1,3], B. Andrews[1,3]. 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, CA; 2) Department of Electrical and Computer Engineering, University of Toronto, Toronto, Ontario, CA; 3) The Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, CA.

What happens as a cell dies? How does the breakdown of one organelle impact other subcellular compartments and structures, and how does this lead to the death of the cell? In order to further our mechanistic understanding of how cells die, we aim to study this subcellular chain of events that occur as a cell is dying, also termed a terminal phenotype. To examine terminal phenotypes, we developed methods that integrate the Synthetic Genetic Array (SGA) method with high-content screening (HCS) to enable the high-throughput, quantitative assessment of changes in subcellular morphology that occur as a cell is dying. We introduce fluorescent markers of key subcellular compartments into strains carrying temperature-sensitive (ts) alleles of essential genes, and then image cells using high-throughput confocal microscopy. We introduced a panel of 23 diagnostic GFP markers, which monitor major compartments and functions in the cell, into a representative array of 384 different ts mutants using SGA, and imaged the resulting mutants over 24 hours at the non-permissive temperature. Markers tailored towards observing cell death pathways include the metacaspase Mca1-GFP, the endonuclease Nuc1-GFP, and the putative necrosis factor Nhp6a-GFP. This analysis generates rich phenotypic profiles of essential gene mutants that capture the changes in subcellular morphology that span the time from first inactivation of the gene product to the death of the cell. Preliminary results indicate that terminal phenotypes are temporally dynamic, reflecting multiple early changes in subcellular morphology that tend to be specific to the mutated gene, and later defects that are more generally associated with cell death. Furthermore, mutants that display defects in multiple subcellular compartments point

towards possible unexplored functional connections between subcellular compartments and structures. These assays will provide us with new information that will allow us to determine the mechanisms behind terminal phenotypes, and to gain further insight into the functional wiring diagram of the cell.

Y3145A Independent origins of yeast associated with coffee and cacao fermentation. *Catherine L. Ludlow*[1], Gareth A. Cromie[1], Cecilia Garmendia-Torres[2], Amy Sirr[1], Michelle Hays[3,4], Colburn Field[5], Justin C. Fay[6], Aimée M. Dudley[1]. 1) Pacific Northwest Diabetes Research Institute, Seattle, WA; 2) Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France; 3) Fred Hutchinson Cancer Research Center, Seattle, WA; 4) Molecular and Cellular Biology Program, University of Washington, Seattle, WA; 5) Montana State University, Bozeman, MT; 6) Washington University School of Medicine, St. Louis, MO.

Because of its profound importance in the fermentation of foods and beverages, the link between humans and the yeast *Saccharomyces cerevisiae* is tight and ancient. Yeast strains associated with modern winemaking reflect a history of dispersal by human activity, which has produced a population of limited genetic diversity that resides in vineyards around the globe. Here, we examine whether the same might be true of the microorganisms associated with other crops that have been transported by humans and whose products rely on fermentation, specifically coffee and cacao. We focus on one of these microbes and show that live strains of *S. cerevisiae* can be isolated from unroasted coffee and cacao beans long after they have been processed by fermentation in their countries of origin. Unlike the wine strains, coffee and cacao yeasts are genetically diverse and form discrete populations, demonstrating independent origins of distinct populations of yeast from the same human-associated activity. These populations show evidence of admixture events between three previously characterized populations, one of which is the wine population itself. Thus, our study suggests that human-associated fermentations and migration have affected the distribution and abundance of yeast used for the production of coffee and chocolate. While human transport of cultivated plants is well documented, our impact on associated microbes is only now becoming apparent.

Y3146B Design and assembly of synthetic chromosomes *VIII* and *I*. *Jingchuan Luo*[1,2], Leslie A. Mitchell[1], Kun Yang[3,4], Karen I. Zeller[4], Joel S. Bader[3,4], Jef D. Boeke[1]. 1) NYU Langone Medical Center, new york, NY; 2) Johns Hopkins School of Medicine, Baltimore, MD; 3) Department of Biomedical Engineering and Institute of Genetic Medicine, Whiting School of Engineering, JHU, Baltimore, MD 21218, USA; 4) High Throughput Biology Center, JHU School of Medicine, Baltimore, MD 21205, USA.

Synthetic biology, by definition, is to design and construct new biological parts, circuits, and pathways, or introduce biological parts, or pathways into a new system, which do not exist in nature. In 2011, Dymond *et al.* synthesized the first synthetic eukaryotic chromosome arms *synIXR* and semi-*synVIL*. In 2014, Annaluru et al synthesized the first synthetic eukaryotic chromosome *synIII. SynIII* is functional in *Saccharomyces cerevisiae*. The synthetic yeast grows as well as wild type. As part of *Saccharomyces cerevisiae* (Sc) 2.0 project, I am working on assembly of synthetic chromosomes *VIII* and *I*. So far, 84% of *synVIII* was completed. *SynI* is one of the "coolest" chromosomes in Sc2.0, because we plan to attach it to the end of another synthetic chromosome. I have developed a CRISPR based method to fuse one chromosome to another chromosome. I have fused *chrI* to the longest chromosome arm (*chrIV* right arm, ~1MB) or shortest chromosome arm (*chrIX* right arm, 90KB) in budding yeast. Interestingly, the relocation of *chrI* did not affect yeast growth. Currently I am studying those fusion chromosomes.

Y3147C Genome sequence of W303 provides insight into diverse evolutionary past. *Kinnari Matheson*, Lance Parsons, Alison Gammie. Princeton University, Princeton, NJ.

W303 is a laboratory strain of *Saccharomyces cerevisiae* that has been employed to study a multitude of biological processes. Many of these studies require a reference quality genome sequence for single nucleotide polymorphism identification and other high-throughput or hybridization-based molecular analyses. In this work, we present a high quality genome sequence that can be utilized for genome-wide studies. Comparative analysis with an ancestor of W303, S288c, provides insights into the evolutionary past of this laboratory strain. About 91 % of the genome sequence of W303 is identical to S288c; however, several divergent gene families have been analyzed. Selective pressures acting on flocculation genes were characterized, these represent selection against invasive strains. Remnants of ancestor wine strains have been identified on several chromosomes.

Y3148A Synthesis, debugging and consolidation of synthetic chromosomes in yeast: *synVI* and beyond. *Leslie A. Mitchell*[1,2], Ann Wang[3,4], Giovanni Stracquadanio[3,5], Zheng Kuang[1,2], Xuya Wang[1,2], Sarah Richardson[3,5], J. Andrew Martin[1,2], Roy Walker[6], Yisha Luo[6], Hongjiu Dai[7], Kang Dong[7], Zuojian Tang[8], Yizhi Cai[6], David Fenyo[8], Junbiao Dai[4], Joel S. Bader[3,4], Jef D. Boeke[1,2]. 1) Department of Biochemistry and Molecular Pharmacology, New York University Langone School of Medicine, New York City, NY 10016, USA; 2) Institute for Systems Genetics, New York University Langone School of Medicine, New York City, NY 10016, USA; 3) High Throughput Biology Center, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; 4) Key laboratory for Industrial Biocatalysis (Ministry of Education), Key laboratory of Bioinformatics (Ministry of Education), Center for Synthetic and Systems Biology, School of Life Sciences, Tsinghua University, Beijing 100084, China; 5) Department of Biomedical Engineering and Institute of Genetic Medicine, Whiting School of Engineering, Johns Hopkins University, Baltimore, MD 21218, USA; 6) Center for Synthetic and Systems Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JL; 7) GenScript, Piscataway, NJ 08854, USA; 8) Center for Health Informatics and Bioinformatics, New York University Langone School of Medicine, New York City, NY 10016, USA.

The Synthetic Yeast Genome project, Sc2.0, aims to build a designer genome to power growth of the yeast *Saccharomyces cerevisiae*. Here we describe the design, rapid assembly, and characterization of Sc2.0 chromosome *VI*, dubbed *synVI*. Growth defects observed in the *synVI* strain

were mapped to the synthetic chromosome, including one that affected mitochondrial behavior. We mapped the mitochondrial defect to a series of synonymous coding sequence changes in the essential gene *PRE4*, encoding a proteasomal subunit; coding changes reduced Pre4 protein accumulation by about half. Completing Sc2.0 necessitates a method to backcross synthetic chromosome strains to wild type and to combine multiple synthetic chromosomes into a single strain. We developed a method to rapidly cross synthetic chromosomes to any strain of interest without sequence alteration and built a poly-synthetic strain encoding *synIII*, *synVI*, and *synIXR*. This work sets the stage for completion of the world's first synthetic, designer eukaryotic genome.

Y3149B Bar-seq analyses to determine the mode of action of compounds derived from feijoa fruit. *M. Mokhtari*[1], D. Miller[2], D. Gresham[2], A. Munkacsi[1]. 1) Centre for Biodiscovery, School of Biological Science, Victoria University of Wellington, Wellington, New Zealand; 2) The Center for Genomics and Systems Biology, Department of Biology, New York University, New York.

The feijoa fruit (*Feijoa sellowiana*) is a rich source of bioactive compounds such as tannins, terpenoids, alkaloids, and flavonoids. Of the 95 compounds reported thus far, these compounds exhibit a diverse suite of bioactivity including antimicrobial, antifungal, anticancer, and antiviral activity. To characterize the genetic and cellular mechanisms of action of two potentially novel compounds derived from feijoa fruit, we used Bar-seq analyses to identify the genes and pathways mediating the bioactivity of these compounds. Next-generation sequencing was used to sequence the barcodes in the heterozygous and homozygous diploid libraries. The barcode sequences were mapped to the reference barcodes and differential abundance analyses were used to compare barcode abundance in treated and untreated genomic libraries. The least represented genes in the heterozygous diploid library will be identified as candidate primary targets of the compounds, while the least represented genes in the homozygous diploid library will be identified as candidate targets downstream of the primary target. The results of our Bar-seq analyses will be presented at the meeting via experiments in yeast and mammalian cells. .

Y3150C A possible role for eIF5A post-translational modification in yeast Ty1 retrotransposition. *Alexis Morrissey*, Jill Keeney. Juniata College, Huntingdon, PA.

Retrotransposons are genetic elements capable of forming a virus-like particle after translation and inserting their sequence into other parts of the genome. This insertion can result in mutations, remove mutations, or even alter the overall length of the genome. Retrotransposons are found in all eukaryotic organisms, including humans. It has been hypothesized that transposons help to create genetic variation and may have a role in aging. Ty1, the most frequently found retrotransposon in S*accharomyces cerevisiae,* provides a model for studying retrotransposition mechanisms in yeast. We observed an increase in the retrotransposition of Ty1 when the gene *FMS1* was overexpressed. *FMS1* is an enzyme that catalyzes the formation of spermidine, a molecule used for the post-translational modification of the elongation factor eIF5A. This elongation factor has been shown to play an important role in the efficient translation of polyproline motifs, specifically those containing three or more prolines in a row. We hypothesize that the connection between *FMS1* and Ty1 transposition is due to the presence of polyproline motifs in Ty1. Using Ty sequences from UniProt and a constructed Java program, we were able to analyze all classes of Ty retrotransposons (1-5). We found that Ty1 retrotransposons have the highest number of polyproline motifs with an average of 2.3±0.7, the second highest being the Ty2 class with an average of 1±0. Our study is specifically looking at Ty1-H3 due to its extensive use in genetic screenings. Ty1-H3 has a total of three polyproline motifs in its protein sequence. Using another constructed Java program, we were able to analyze a fasta file from the Saccharomyces Genome Database containing the yeast proteome for the number of polyproline motifs in each individual protein. We found that <1% of proteins contained three or more polyproline motifs, showing that Ty1-H3 was unique when compared to the whole proteome. Additionally, we created a gene ontology map of the genes with three or more polyproline motifs using the BiNGO extension in Cytoscape. Viral gene ontology terms had notably low p-values (10^{-5}), showing the overrepresentation of this group of proteins. Current and future experiments are aimed at determining whether specific polyproline motifs within the Ty1-H3 sequence impact transposition rates.

Y3151A High-copy number gene expression in different *Saccharomyces cerevisiae* strains reveals the impact of natural variation in wild yeast. *D. Robinson*, A. Gasch. University of Wisconsin-Madison, Madison, WI.

Organisms need to have the ability to respond to external environmental changes and stresses in order to thrive in nature. *Saccharomyces cerevisiae* has the ability to respond to stresses through the environmental stress response (ESR), where the expression of stress defense genes is induced and the expression of genes involved in growth is reduced. Most of the current work examining stress-activated gene expression is done using laboratory strains of yeast. However, gene functions can vary in different genetic backgrounds, which is why it is important to explore the effects of natural variation. In this project, we transformed several different natural isolates of yeast have with a molecular barcoded yeast open reading frame (MoBY-ORF 2.0) high copy number plasmid library. These transformed wild strains were grown in the presence of various stresses (ethanol, salt, or DTT) to identify which high-copy number genes increase or decrease fitness during periods of stress. Of particular interest are high-copy number genes that have different effects on fitness in different wild isolates versus genes that produce common effects across many wild strains. Examination of the effects of gene amplification in natural yeast isolates could provide insight to the genetic diversity of the stress response and how natural variation relates to gene function.

Y3152B Investigation of the genetic basis of hybrid vigor in yeast. *N. Sampaio*, J. L. Argueso. Colorado State University, Fort Collins, CO.

Highly heterozygous organisms often display enhanced phenotypic manifestations, a phenomenon known as hybrid vigor. Despite the economic importance of hybrid vigor, the genetic mechanisms that promote these superior phenotypes are still unknown. The genomic complexity typically observed in crop plants and livestock often hinders the investigation of the specific role of heterozygosity in the superior phenotype of the hybrid progeny. Thus, this type of analysis would be greatly facilitated by the use of a tractable model organism, such as the

yeast *Saccharomyces cerevisiae*. The natural hybrid diploid yeast strain JAY270 is widely adopted in sugarcane-based bioethanol production. Our group has determined that heterozygous alleles are present in about 60% of JAY270's genome, suggesting that its robust growth and high fermentative performance may be associated with hybrid vigor. In this study, we are establishing JAY270 as a model for investigating the mechanisms through which heterozygosity may promote fitness. We are developing an approach to identify individual heteroalleles in the JAY270 genome that contribute to hybrid vigor in this strain. The genomes of 56 haploid derivatives of JAY270 (14 full tetrads) were sequenced. The haploids were then intercrossed to create a systematic collection of inbred diploids, containing varying known fractions of the original heterozygosity present in JAY270. The whole-genome information from all the parental haploids provided precise genome-wide maps of where each inbred diploid is still heterozygous and where they are homozygous for either parental allele. The relative fitness of 78 of such inbred diploids was assessed using a high throughput flow cytometry-based growth competition assay against the fully heterozygous JAY270 parent strain. In addition, the inbred diploid collection was challenged with various growth stress conditions (high temperature, salt, DNA damage, etc.). Alignment of the genomic of heterozygosity maps and correlation to high or low fitness in the inbred diploids will allow us to identify genomic regions where allele-dependent contributors to hybrid vigor reside. We will present the results of the phenotypic characterization of the inbred diploid collection, and will discuss progress in the mapping of specific loci in the genome associated with hybrid vigor. The characterization of these heteroalleles will shed new light on the genetic basis of this complex process, contributing discoveries that could have important practical implications for human health, agriculture, biotechnology, as well as for genome sciences.

Y3153C Deciphering mutational signatures of DNA repair deficiencies and cisplatin in yeast. *R. Segovia Ugarte*[1,2], Y. Shen[3], S. Jones[1,3], P. Stirling[1,2]. 1) Depart. of Medical Genetics, Univ. of British Columbia, Vancouver, Canada; 2) Terry Fox Laboratory, BCCA, Vancouver, Canada; 3) Canada's Michael Smith Genome Sciences Centre, BCCA, Vancouver, Canada.

Introduction: Maintenance of genome integrity is critical to the cell, however exogenous and endogenous stresses menace the integrity of the genome. Overwhelming a cell's DNA repair capacity can expose the cell to the accumulation of mutations and, in humans, predispose to cancer development. Understanding the consequences of defective DNA repair pathways and their association with specific mutational processes in tumor genome sequences is difficult to achieve. A powerful method to study the effect of defects in DNA repair mechanisms is the extraction of mutational signatures from whole-genome sequencing of model organisms carrying defined genetic alterations or exposed to mutagens. We hypothesized that mutation accumulation in *S. cerevisiae* caused by deletions of *RAD1, MUS81,* or *SGS1* genes, coupled with exposure to cisplatin will result in specific mutational profiles that can be used to inform repair mechanism and potentially mutation signatures in cancer.

Methods: We sequenced whole genomes of 104 populations of diploid *S. cerevisiae* that were WT or carried homozygous deletions of *RAD1, MUS81,* or *SGS1*. For each genetic background 12 parallel lines were propagated for 1000 generations through single-cell bottlenecks or exposed for 3 hours to 100 uM cisplatin. Genomic DNA was isolated from single clones for whole-genome sequencing.

Results and conclusions: We identified 985 SNVs, 123 indels, 107 CNVs, and 36 aneuploidies. Rad1-deficient genomes exposed to cisplatin were characterized by an increase in single nucleotide substitutions at C/G base pairs. C to N mutations were defined by a GpCpN sequence context, which indicates that the surrounding sequence influences the base to be inserted on the opposite strand. Moreover, these genomes featured dinucleotide substitutions, particularly CpT to TpA. This data reflects the chemistry of cisplatin and indicates Rad1/XPF critically protects the cell against platinum hypermutation. Sgs1-deficient genomes propagated for 1000 generations revealed CNV events with the majority of breakpoints mapped to transposable element loci and other genetic features with high transcriptional frequency. Overall the data indicates the role of Sgs1/BLM in maintaining genomic regions difficult to replicate like retroelements. Together these data define new mutation signatures for DNA repair deficient cells whose mechanism are now under investigation.

Y3154A The yeast mating pathway as a model for complex trait genetics. *Stephanie Zimmerman*[1], Michael Dorrity[1], Josh Cuperus[1], Christine Queitsch[1], Stanley Fields[1,2]. 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Howard Hughes Medical Institute, University of Washington, Seattle, WA.

Genome-wide association studies have identified many variants associated with complex traits, but in general these variants explain only a small fraction of the estimated heritability of the trait. Several hypotheses have been proposed to explain this "missing heritability." Complex traits could be caused by rare undiscovered variants with relatively large effect sizes, by the additive action of many individually small-effect variants, or by significant gene-by-gene or gene-by-environment interactions that modify otherwise small-effect variants. We are using the yeast mating pathway as a model complex trait to experimentally assess the relative impact of additive and epistatic genetic variation. Yeast mating is controlled by a well-characterized pheromone-responsive MAP kinase pathway. We used a technique called deep mutational scanning to profile the effect on mating of thousands of variants in three mating pathway genes: *STE5* (scaffold protein), *STE7* (MAP kinase kinase) and *STE12* (transcription factor). We transformed yeast null for one of these genes with a library of ~100,000 plasmids, each carrying a variant of the gene. We mated the transformants, selected for diploids, and sequenced the population of variants before and after mating by high-throughput sequencing. Mutations that impair gene function are depleted in the population after mating, while mutations that allow a wild-type level of mating are enriched. From these experiments, we identified variants in all three genes with the full spectrum of effect sizes, from null to wild-type. In addition, we tested the mating ability of the same population of variants in the presence of an Hsp90 inhibitor and at high temperature. Hsp90 is a chaperone protein that buffers the effects of mutations in many genes. From these data, we will identify variants that are sensitive to a known genetic modifier and the environment (high temperature), and determine the prevalence of buffered genetic variation in these genes. Finally, we identified ~600 small-effect variants in each gene, and in future experiments we will combine these small-effect variants in two mating pathway genes at a time. This experiment will allow us to assess simultaneously the degree of epistasis between tens of thousands of variants in both physically interacting and non-interacting proteins.

Y3155B Global analysis of genes and metabolites influencing chronological lifespan. *Haley Albright*[1], Daniel Smith[1], John Rodgers[1], Rick White[2], John Hartman[1]. 1) University of Alabama at Birmingham, Birmingham, Alabama; 2) University of British Columbia, Vancouver, Canada.

We are using phenomic analysis of the *S. cerevisiae* yeast gene deletion strain (YGDS) library to systematically investigate genes, pathways, and environmental factors that influence aging, using the chronological lifespan (CLS) model of aging for post-mitotic cells. Phenomic analysis consists of quantitative high-throughput cell array phenotyping (Q-HTCP) of the YGDS, which yields growth curve parameters associated with every gene that are used to estimate their contribution to CLS. The *met17-Δ0* or *MET17* allele status can influence CLS, and methionine restriction is known to extend lifespan in higher eukaryotes. The genomic collection of deletion strains libraries exists for both genetic backgrounds and we recently completed genome-wide CLS analysis using Q-HTCP for both libraries. Growth curve parameters, from Q-HTCP analysis, were plotted for each gene deletion strain against the parameters for the reference (background) strain. CLS phenotypes were then assigned based on the shift in growth curve parameters for the deletion strains with respect to the reference strain. We identified 1,379 long-lived strains and 277 short-lived strains out of 5,664 total strains in the *met17-Δ0* deletion library. In the *MET17* deletion library, we identified 1,285 long-lived and 367 short-lived strains out of 5,464 total. In depth analysis of these results is ongoing to confirm CLS phenotypes assigned from preliminary data and to gain insight about effects of methionine pathway interactions on CLS.

To further investigate the *met17-Δ0* effect on CLS, metabolite profiling is being used to gain additional insight about possible physiological correlates. Preliminary analysis reveled 234 metabolites with significant 3-term (time, media, genetic background) interactions. When only two-terms are considered, time and media or genetic background, we identified 335 and 157 differentially expressed metabolites, respectively. We are developing a method using isotopic labeling to predict chemical formulas for the observed metabolite masses, which will aid the effort to integrate data from the genome-wide CLS screens and metabolite profiling experiments. The ultimate goal is to understand how genetic and metabolic pathways are connected with respect to influencing cellular aging, to better explain how mitochondrial function, nutrient sensing, calorie restriction and other phenomena constitute what appear to be aging mechanisms conserved broadly across eukaryotic evolution.

Y3157A Homology curation at SGD: budding yeast as a model for eukaryotic biology. *Stacia R. Engel*, Robert S. Nash, Edith D. Wong, J. Michael Cherry, The SGD Project. Stanford University, Palo Alto, CA.

The foundation for much of our understanding of basic cellular biology has been learned from the budding yeast *Saccharomyces cerevisiae*. Studies with yeast have also provided powerful insights into human genetic diseases and the cellular pathways in which they are involved. Here we present an update on new developments at the *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org/), the premier community resource for budding yeast. We are expanding the scope of SGD to include high quality manually curated information regarding functional complementation between yeast and human homologs. This new information is provided in meaningful ways allowing data mining and discovery by integrating these data into this encyclopedic online resource. In addition to introducing our presentation of these newly curated data we will highlight other new developments, such as written summaries about yeast genes and their mutant phenotypes, their human homolog disease associations, and presentation of the yeast/human ortholog set. We also associate sequence changes with variations in cellular phenotypes and protein function. SGD maintains these different datatypes, and distributes them to the scientific community via the web and file transfer. These expanded efforts are part of our continuing mission to educate students, enable bench researchers and facilitate scientific discovery. This work is supported by a grant from the NHGRI (U41 HG001315).

Y3158B Systematic identification of human/yeast complementation pairs to create a platform for testing tumor-specific variants. *A. Hamza*, E. Tammpere, M. Kofoed, C. Keong, J. Chiang, G. Giaever, C. Nislow, P. Hieter. University of British Columbia, Vancouver, BC, CA.

While the pace of discovery of somatic mutations in tumor genomes has rapidly accelerated, deciphering the functional impact of these variants has become rate-limiting. Furthermore, linking somatic variants to specific cancer hallmarks, such as chromosome instability (CIN), is a major challenge in the cancer field. The budding yeast, *Saccharomyces cerevisiae*, has been utilized to define cellular pathways and catalog a comprehensive list of yeast genes required for the maintenance of chromosome stability (yeast CIN genes). Human orthologs of these yeast genes are candidate CIN genes whose tumor-specific variants may contribute to chromosome instability and tumorigenesis. By 'humanizing' a yeast strain using cross-species complementation, yeast can facilitate direct screening of these somatic mutations with the dual benefit that the variants are characterized in the context of the human gene and screened rapidly in a model eukaryote. Here, we outline an experimental approach to identify a list of human genes that can replace their yeast orthologs and complement a loss-of-function phenotype. We report the screening of essential and non-essential yeast CIN deletion mutants for complementation by their human counterparts. Replaceability was scored by examining rescue of growth defects caused by deletion of the essential yeast gene or by assaying for rescue of drug sensitivities for non-essential yeast genes. The resultant list of human gene/ yeast mutant complementation pairs has been used to test the functional consequences of cancer somatic mutations directly in yeast, while also providing a list of complementing genes as a resource to the field.

Y3159C Anticancer ruthenium complex KP1019 induces metabolic retooling in *Saccharomyces cerevisiae*. *Pamela Hanson*[1], Laura Stultz[1], Sarah Sharman[1], Avery Newcomb[1], James Mobley[2]. 1) Birmingham-Southern College, Birmingham, AL; 2) University of Alabama at Birmingham, Birmingham, AL.

In early clinical trials the anticancer ruthenium complex KP1019 (*trans*-[tetrachlorobis(1*H*-indazole)ruthenate(III)]) stabilized disease in five of six evaluable patients and displayed no dose-limiting toxicity, suggesting that this drug may be a viable alternative to platinum-based chemotherapeutics. Although KP1019 has been shown to damage DNA in both cancer cell lines and the budding yeast *Saccharomyces cerevisiae*, this ruthenium complex's mechanism of action remains unclear. Alternative explanations for KP1019's toxicity stem from the drug's ability to induce oxidative stress and to bind to diverse small molecules and proteins within cancer cells. Here we use an unbiased proteomic

approach to characterize *S. cerevisiae*'s response to KP1019. Bioinformatic analysis of induced proteins confirmed that KP1019 activates DNA damage and oxidative stress response pathways. Clustering and analysis of both induced and repressed proteins revealed complementary metabolic retooling. Observed changes in protein expression suggest increased synthesis of methionine and the antioxidant glutathione. Moreover, yeast treated with KP1019 increased expression of evolutionarily conserved enzymes of the pentose phosphate pathway (PPP), including the glucose-6-phosphate dehydrogenase Zwf1, the 6-phosphogluconolactonase Sol4, and the transketolase Tkl2. KP1019-dependent induction of the rate-limiting PPP enzyme Zwf1 was verified by flow cytometry of Zwf1-GFP yeast treated with varying concentrations of the drug. Culturing yeast in media containing the PPP substrate xylose both induced Zwf1-GFP and increased resistance to KP1019, suggesting that upregulation of the PPP contributes to drug tolerance. This conclusion was further supported by KP1019-dependent repression of the PPP regulator Pho13. Deletion of the phosphatase-encoding gene *PHO13* has previously been shown to upregulate the PPP, and here we show that *pho13Δ* yeast are more resistant to KP1019 than an isogenic wild-type control. Since glutathione and the PPP have well-established roles in cellular adaptation to oxidative stress, these data suggest that oxidative stress contributes to KP1019 toxicity and that metabolic re-tooling is an important contributor to cellular tolerance of this drug. Ultimately, metabolic biomarkers may prove suitable for use in predicting patient responses to this promising chemotherapeutic.

Y3160A Yeast RNA-binding protein Ssd1 and human FUS, implicated in ALS, share localization and features in yeast. *C. Kurischko*[1,2], G. Petsko[1], J. Broach[2]. 1) Weill Cornell Medicine, New York, NY; 2) College of Medicine, Penn State Univ., Hershey, PA.

Ssd1 is a nucleo-cytoplasmic shuttling protein with multiple roles in the life of its target mRNAs, from transcription and maturation to polarized localization, translation and decay. Interaction between Ssd1 and its target mRNAs initiates during transcription in the nucleus and, depending on the extent of its phosphorylation by the RAM kinase Cbk1, results in subsequent localization of the mRNAs either to the bud for translation or to stress granules (SG) and P-bodies (PB) for mRNA storage or decay. Ssd1 lacking a nuclear localization signal ($Ssd1^{(417\text{-}427)11A}$), and thus excluded from nuclear entry, forms large cytoplasmic aggregates that colocalize with Hsp104 and Sis1, markers of "Insoluble Protein Deposition" sites (IPOD). Overexpression of $Ssd1^{(417\text{-}427)11A}$ enhances the mild toxicity of wild type Ssd1. Activation of a separate cryptic NLS within Ssd1 or deletion of its prion-like domain suppresses IPOD formation of $Ssd1^{(417\text{-}427)11A}$. Thus, nuclear shuttling of Ssd1 is required to prevent prion-like, non-productive aggregate formation.

The human RNA-binding protein Fused in Sarcoma (FUS) is implicated in ALS, a fatal neurodegenerative disease. While not a homolog of Ssd1, FUS shares domain structures with Ssd1 and, when expressed in yeast, localizes in the same pattern as Ssd1, including formation of IPODs upon overexpression. Accordingly, our studies on Ssd1 provide a framework for understanding the molecular basis of FUS structure. We use these observations to obtain insight into processes in which FUS may be involved in mammalian cells, particularly neurons. This could help to understand the underlying causes of neurodegenerative diseases, in which mutated RNA-binding proteins play a central role.

Y3161B Unbiased functional annotation of compound libraries using yeast chemical genomics. *Sheena C. Li*[1], Scott W. Simpkins[2], Justin Nelson[2], Jeff S. Piotrowski[3], Hamid Safizadeh[2], Karen Kubo[4], Nikko Torres[6], Grant Brown[6], Yoshikazu Ohya[4], Ming-Wei Wang[5], Minoru Yoshida[1], Chad L. Myers[2], Charles M. Boone[1,5]. 1) RIKEN Center for Sustainable Resource Science, Wako, Saitama, JP; 2) Computer Science and Engineering, University of Minnesota, MN, USA; 3) Yumanity Therapeutics, Cambridge, MA, USA; 4) University of Tokyo, Kashiwa, Chiba, Japan; 5) The National Center for Drug Screening, Shanghai, China; 6) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, ON, Canada.

To elucidate the molecular mechanism of bioactive compounds in an unbiased manner, we used chemical genomics in budding yeast to systematically screen very large and diverse compound collections, with the goal of linking unknown compounds to their cellular targets.

A high-throughput pipeline using various barcoded yeast mutant collections in a drug hypersensitive genetic background was developed. We designed a diagnostic pool of 310 non-essential deletion mutants to minimize the amount of compound required for screening and to maximize dynamic range. In addition, we constructed temperature-sensitive and heterozygous diploid mutant collections for all essential genes to obtain higher resolution chemical genetic information. Chemical genetic signatures for thousands of compounds were efficiently and rapidly generated via highly multiplexed next generation sequencing of DNA barcodes in the mutant strains. Targets were predicted using two strategies: For the diagnostic non-essential pool and the essential temperature-sensitive collection, we compared chemical genetic signatures with genome-wide synthetic lethal data to predict compound functionality at the biological process level. We complemented this analysis with a drug-induced haploinsufficiency approach to determine precise gene targets using the heterozygous diploid collection.

We applied this system to screen eight diverse compound libraries. Using the diagnostic pool approach, we assessed over 18,000 compounds for target specificity, and identified high confidence target process predictions for ~2000 unique compounds. Functional diversity of different compound libraries was characterized by mapping predicted targets onto the global yeast genetic interaction network. We then used the set of compounds with high confidence predictions for further chemical genetic screens with temperature-sensitive and heterozygous diploid collections for essential genes. Validation studies have confirmed predictions at the biological process level and also at the specific gene target level, including compounds with multiple predicted modes of action.

Y3162C Identifying novel small molecules for improved antifungal drug treatment. *Kevin F. Murphy*[1], Dina Wassaf[2], Angela N. Koehler[3], Martha L. Bulyk[4,5]. 1) Department of Biology and Health Sciences, McNeese State University, Lake Charles, LA; 2) Broad Institute, Cambridge, MA; 3) Koch Institute for Integrated Cancer Research at MIT, Cambridge, MA; 4) Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 5) Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

Invasive fungal infections (IFIs) are associated with high rates of morbidity and mortality and pose a serious health concern for severely immunocompromised patients. Fungal resistance to current drug therapies is largely due to the transcriptional upregulation of membrane associated efflux pumps, which expel from the cell a myriad of structurally diverse molecules, including antifungal drugs, thus severely reducing their effectiveness against these pathogens. Additionally, resistance can arise by overexpression of drug target genes within the ergosterol biosynthesis pathway. The fungal-specific zinc cluster (Zn_2Cys_6) family of transcription factors (TFs) is primarily responsible for the upregulation of these efflux pumps and ergosterol biosynthesis enzymes and thereby mediating pleiotropic drug resistance (PDR) in yeast. Thus, these TFs offer an attractive and rational target for the development of new antifungal drugs.

In pursuit of this goal, we aimed to identify small molecules capable of inhibiting the DNA-binding ability of Zn_2Cys_6 TFs regulating PDR in yeast. An initial screen utilizing small molecule microarrays (SMM) was employed to identify compounds capable of binding the DNA-binding domain of Zn_2Cys_6 TF Pdr1p from *Candida glabrata*. In our initial SMM-based screen, a library of 15,000 different compounds was examined and yielded 76 unique compounds that specifically bound to Pdr1p. Small-molecule "hit" compounds consisted of several different structural classes, including 44 azetidine and sulfonamide-based compounds. Future studies will examine the potential of these lead compounds for development of improved antifungal drugs in the treatment of IFIs.

Y3163A Discovering Novel Inhibitors of Deubiquitinases *in vivo*: Strategies using Budding Yeast. *N. Pascoe*[1], Michael Costanzo[2], Sachdev Sidhu[1,2,3], Charles Boone[1,2,3]. 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Terrance Donnelly Center for Cellular and Biomolecular Research, University of Toronto, Toronto Ontario, Canada; 3) Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada.

Deubiquitinating enzymes (DUBs) are key regulators of the ubiquitin pathway that catalyze the removal of ubiquitin from target substrates. Hence, these enzymes affect a wide variety of cellular processes by influencing the localization, stability and function of their targets. Importantly, inhibition of DUB activity has emerged as a promising therapeutic strategy to treat several diseases including various cancers & Alzheimer's. Despite this, a paucity of specific and potent inhibitors aimed at DUB pathways has hindered attempts to exploit them for therapeutic benefit.

To address this issue, we developed a strategy using ubiquitin (Ub) as a scaffold to engineer specific & potent DUB inhibitors, called Ub variants (UbV). We have generated massively diverse and combinatorial libraries, comprising >$7x10^{10}$ unique UbVs. Using phage-display we have screened our library to identify inhibitors of ~8/95 human DUBs. Although effective, an inability to purify many disease-relevant DUBs has limited the applicability of our *in vitro* screening system. Thus, we have developed a method based on the yeast two-hybrid (Y2H) system to detect UbV-DUB interactions *in vivo*. In a pilot study using a smaller library consisting of 10^5 unique UbVs, we identified UbVs that bind the DUB USP2 specifically, thus validating our approach. We are currently adapting this system to allow for the high-throughput screening of massive UbV libraries *in vivo*. In conjunction with established *in vitro* screening methods, we will use our *in vivo* screening platform to discover novel inhibitors against the full panel of human DUBs. Together, this work will make seminal contributions towards furthering our understanding of DUB function and alleviate a formidable bottleneck that obstructs the development of more powerful therapeutics aimed at the ubiquitin system.

Y3164B Modulation of yeast chronological lifespan by TOR signaling in the context of replication stress. *Sean M. Santos*[1], Chandler Stisher[1], Darryl Outlaw[1], Haley Albright[1], John Rodgers[1], Rick White[2], John L. Hartman, IV[1]. 1) University of Alabama at Birmingham, Birmingham, AL; 2) University of British Columbia, Vancouver, Canada.

The target of rapamycin (TOR) pathway is an evolutionarily conserved nutrient sensing pathway that is repressed during caloric restriction, which is associated with extension of lifespan in yeast and higher eukaryotes. Replication stress, induced by inhibition of RNR and depletion of dNTP pools, shortens yeast chronological lifespan (CLS), which can be alleviated by glucose restriction or deletion of *SCH9* (a downstream mediator of TOR signaling). To further investigate possible interaction between TOR signaling and replication stress, we used quantitative high throughput cell array phenotyping (Q-HTCP) of the complete collection of the yeast knockout and knockdown (YKO/KD) strains to identify genes that influence the TOR and RNR pathways. Based on this analysis and evidence from the literature, we hypothesize vacuolar acidification is a process where the TOR and RNR pathways functionally intersect, and suspect this gives rise to genetic interaction that could be important in determination of lifespan. There are several observations we are trying to relate with one another: Vacuolar acidification in glucose depleted media is optimal at pH = 7, and neutral pH is also associated with increased CLS, but whether increased vacuolar function affects, or is affected by, CLS is unclear. Interestingly, HU treatment can be associated with higher pH over time. We developed a high throughput pH assay to better associate pH and CLS across the deletion collection and CLS. Although hydroxyurea-treated yeast exhibit decreased viability initially, we found CLS to be paradoxically greater with increasing age, depending on MET17/met17-Δ0 status. Deletion of *MET17* extends CLS, yet it shortens late CLS after HU treatment whereas having a functional *MET17* increases lifespan after HU treatment. Based on these findings, we are investigating a model whereby perturbation of RNR activity damages/kills some cells in S-phase, but may also induce inhibition of TOR signaling that facilitates G1/G0 arrest of surviving cells, which ultimately promotes extension of CLS.

Y3165C Using Yeast to Screen for Drugs for the Treatment of Inherited Parkinson's Disease. *K. A. Strynatka*[1], P. P. Poon[1], C. R. McMaster[1,2]. 1) Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia, CA; 2) Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, CA.

Current therapies for Parkinson's disease (PD) are limited to managing signs and symptoms; there is no treatment available that prevents or significantly delays progression of the disease. Approximately 10% of PD cases are inherited forms of the disease. Specifically, mutations in the

kinase LRRK2 have been shown to cause PD. Previous studies showed that LRRK2 kinase activity is regulated by ArfGAP1 and that decreasing ArfGAP1 expression results in a decrease in toxicity of mutant LRRK2. Therefore, small molecules that inhibit ArfGAP1 may be a potential therapy for PD. To that end, we screened for compounds that inhibit human ArfGAP1. We expressed human ArfGAP1 under the control of a titratable promoter in the yeast *Saccharomyces cerevisiae*. Expression of human ArfGAP1 in yeast proved toxic to the cell. This toxic phenotype was exploited to perform a high-throughput small-molecule screen for compounds that inhibit ArfGAP1, which would restore viability to the cell. A panel of small molecules was screened, including 5000 pharmacologically active compounds and off-patent FDA-approved drugs, and 100 000 novel small molecules. Six compounds were identified as potential inhibitors of ArfGAP1, all with a similar core structure suggestive of inhibition of the same target. These are being tested for their capacity to inhibit ArfGAP1 directly, and reduce LRRK2 toxicity *in vitro* and *in vivo*.

Y3166A Discovery of plant extracts that greatly delay yeast aging by targeting certain signaling pathways and modulating lipid metabolism. *V. Titorenko*, V. Lutchman, V. Svistkova, P. Dakik, A. Arlia-Ciommo, Y. Medkour. Concordia Univ., Montreal, Canada.

The yeast *Saccharomyces cerevisiae* has been successfully used as a model organism for discovering signaling pathways and chemical compounds that modulate cellular aging, define organismal lifespan, and influence organismal fitness not only in yeast but also in various multicellular eukaryotes. Using a robust quantitative assay for measuring yeast chronological lifespan, we screened a library of plant extracts (PEs) for extracts that can extend longevity of chronologically aging yeast. We identified six PEs (i.e. PE4, PE5, PE6, PE8, PE12 and PE21) whose longevity-extending efficiencies greatly exceed those of currently known anti-aging compounds, such as resveratrol, rapamycin, spermidine, caffeine, and metformin. Our analysis of the Gompertz mortality function revealed that each of these six PEs is a geroprotector which delays the onset and reduces the rate of yeast chronological aging by eliciting a hormetic stress response. Our genetic, biochemical, and mass spectrometry-based lipidomic analyses uncovered the following mechanisms by which these PEs delay yeast chronological aging by targeting certain signaling pathways and modulating lipid metabolism: 1) PE4 attenuates the inhibiting effect of the pro-aging TOR (target of rapamycin) signaling pathway on the anti-aging AMP-activated protein kinase (AMPK/Snf1), increases the intracellular concentration of phosphatidic acid (PA), and decreases the concentration of triacylglycerols (TAG; the major form of energy storage in yeast and other eukaryotes); 2) PE5 mitigates the pro-aging cAMP/PKA (cAMP/protein kinase A) signaling pathway, elevates the concentration of PA, reduces the concentration of TAG, and rises the concentration of cardiolipin (CL; a phospholipid synthesized only within mitochondria in yeast and other eukaryotes); 3) PE6 targets currently unknown pro- and/or anti-aging pathways of longevity regulation, increases the concentration of PA and phospholipids other than CL, and decreases the concentration of TAG; 4) PE8 weakens the inhibiting effect of cAMP/PKA signaling pathway on the anti-aging AMPK/Snf1, enlarges the concentrations of PA, CL and all other phospholipids, and lessens the concentration of TAG; 5) PE12 activates the anti-aging protein kinase Rim15 (on which the pro-aging TORC1 and cAMP/PKA signaling pathways converge), and decreases the concentrations of CL and TAG; and 6) PE21 attenuates the pro-aging protein kinase Sch9 (which is activated by the pro-aging TOR and Pkh1/2 signaling pathways), elevates the concentrations of PA and phospholipids other than CL, and reduces the concentration of TAG.

Y3167B Identification of a Natural Product that Disrupts the Fungal Cell Wall Integrity Pathway by Targeting Hsp90. *Siddharth Tripathi*, Qin Feng, Melissa Jacob, Xing-Cong Li, Alice Clark, Ameeta Agarwal. University of Mississippi, University, MS.

Due to the narrow activity spectrum of echinocandin antifungal drugs and the emergence of drug resistance, new therapies are needed to potentiate echinocandins such as caspofungin (CAS). We have identified a sesquiterpene quinone compound named puupehenone (PUUP) that enhances CAS activity in CAS-resistant strains of *Candida albicans* and also in the inherently CAS-insensitive pathogen *Cryptococcus neoformans*. To investigate PUUP's CAS-potentiating mechanism, we conducted a transcript profiling study in the model yeast *Saccharomyces cerevisiae*. Cells were exposed to solvent, PUUP, CAS, and PUUP + CAS, and the RNA extracted was subjected to RNA-Seq analysis. To further investigate PUUP's mechanism of action, a fitness profiling study was performed to monitor the sensitivity of whole-genome yeast deletion mutants to PUUP. To confirm PUUP's effect on Hsp90, a well-established promoter-reporter assay system was used that monitors the rat glucocorticoid receptor (GR), an Hsp90 client protein. An additional client protein, Mpk1 was monitored by Western analysis. Transcript profiling with CAS + PUUP revealed that genes in the cell wall integrity pathway (CWIP) that were strongly induced by CAS alone were not induced by CAS + PUUP; thus, PUUP synergizes with CAS by preventing cell wall repair through the CWIP. Further studies revealed that PUUP targets Hsp90. The transcript profile of PUUP was similar to that of the Hsp90 inhibitor celastrol. Genes encoding chaperones and co-chaperones involved in heat shock response were strongly induced by PUUP. To confirm that PUUP targets Hsp90, we monitored the rat GR, an Hsp90 client protein, and observed that PUUP inhibited GR induction in a concentration-dependent manner. Our fitness profiling experiment identified 28 mutants with increased hypersensitivity to PUUP. Of these, 14 mutants have been previously reported to show hypersensitivity to other Hsp90 inhibitors. We also showed that the overexpression of four different Hsp90-related proteins resulted in significant resistance to PUUP. We also observed that PUUP inhibited the activation of the yeast protein Mpk1, another Hsp90 client protein. Because Mpk1 is a critical kinase in the CWIP pathway, this result demonstrates that PUUP inhibits the CWIP by disrupting Mpk1 activity. In summary, we have identified a natural product that disrupts the fungal cell wall integrity pathway by targeting Hsp90.

Y3168C *Saccharomyces* Genome Database: How to find what you are looking for. *Gail Binkley*, Travis K. Sheppard, Kalpana Karra, Pedro H. R. de Assis, Shuai Weng, Edith D. Wong, J. Michael Cherry, The SGD Project. Stanford University, Department of Genetics, Palo Alto, CA.

Everyone has experienced the frustration of being unable to find the information you want on a website because you have only a vague idea what you are looking for and don't know what search term(s) to use. Here we present several ways to find and explore data at SGD using new search methods. "Exact match" searches are effective and fast when it is already known that specific information exists. However, "exact match" searches do not allow browsing of all available data, and can sometimes obscure the context of the returned results, making it difficult

to discover new connections and uncover novel relationships. To address these issues, the *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org) has recently implemented several new ways to find and explore yeast data. SGD's "exact match" search has been replaced with a new faceted search based on Elasticsearch, which allows filtering in multiple categories and speeds query performance. Faceted navigation provides immediate feedback and easy navigation so users can quickly refine and refocus their search based on the returned results. Other recent website additions include SGD's Variant Viewer, which employs a combination of data filters and visualizations to display novel sequence variants for open reading frames within a reference panel of 12 widely used *S. cerevisiae* genomes. Datasets are also now easier to find in SGD. Over 225 expression and other high-throughput datasets can now be filtered by curated metadata and displayed using JBrowse, a JavaScript- and HTLM5-based genome browser. This work is supported by a grant from the NHGRI (U41 HG001315).

Y3169A Classifying Microscopy Images with Deep Learning. *Oren Kraus*[1,3], Jimmy Ba[1], Charles Boone[2,3,4], Brenda Andrews[2,3,4], Brendan Frey[1,3,4]. 1) Electrical and Computer Engineering, University of Toronto, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, Ontario, Canada; 3) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada; 4) Banting and Best Department of Medical Research, University of Toronto, Canada.

High content screening (HCS) technologies have enabled large scale imaging experiments for studying cell biology and for drug screening. These systems produce hundreds of thousands of microscopy images per day and their utility depends on automated image analysis. Recently, deep learning approaches that learn feature representations directly from pixel intensity values have dominated object recognition challenges. The recognition tasks in these challenges typically consist of a single centered object and existing models are not directly applicable to microscopy datasets. Here we develop an approach that combines deep convolutional neural networks (CNNs) with multiple instance learning (MIL) in order to classify and segment microscopy images using only whole image level annotations. MIL is a framework that enables supervised learning models to train on datasets that only have labels for sets of data points.

We introduce a new neural network architecture that uses MIL to simultaneously classify and segment microscopy images with populations of cells. Building supervised classifiers based on segmented single cells remains time consuming and difficult for researchers. Combining CNNs with MIL enables training classifiers with whole microscopy images, even images containing mixed populations, using whole image level labels. We base our approach on the similarity between the aggregation function used in MIL and pooling layers used in CNNs. We show that training end-to-end MIL CNNs outperforms several previous methods on both mammalian and yeast datasets without requiring any segmentation steps. On a publically available drug screen of MFC-7 breast cancer cells (Broad Bioimage Benchmark Collection, image set BBBC021v1) we achieve 97% accuracy at predicting the mechanism of action of different treatments. On a yeast protein localization dataset, we achieve 96% accuracy at predicting localization for proteins that localize to a single subcellular compartment.

Y3170B Saccharomyces Genome Database: Outreach and online training services. *Kevin A. MacPherson*, Kyla S. Dalusag, Olivia Lang, Sage T. Hellerstedt, Stacia R. Engel, J. Michael Cherry, The SGD Project. Stanford University, Stanford, CA.

The Saccharomyces Genome Database (SGD; http://www.yeastgenome.org) is the leading community resource for the budding yeast S. cerevisiae. SGD provides high-quality, manually curated information on the yeast genome and offers a wide variety of tools and features that have made it an indispensable resource for many researchers. However, lack of familiarity with the SGD interface and tools can present a significant barrier to new users and prevent current users from making full use of these resources. To inform our user community about new developments at SGD, improve familiarity with SGD features and tools, and increase public awareness of the importance of yeast to biological and biomedical research, SGD has engaged in a variety of online training services and outreach efforts. Here we present the SGD Webinar Series, a series of interactive webcasts aimed at demonstrating the SGD website and the value of yeast as a model organism, and the SGD YouTube channel, which currently provides over 30 useful help videos and quick tutorials (http://www.youtube.com/SaccharomycesGenomeDatabase). We will continue to develop these services to provide outreach to students and scientists on the significance and beauty of biology, and facilitate greater use and understanding of the resources made available by SGD. This work is supported by a grant from the NHGRI (U41 HG001315).

Y3171C A Morphology Profile Pipeline for Genome-wide Screens in *Saccharomyces cerevisiae*. *N. Sahin*[1,2], E. Styles[1,2], A. Verster[3], Q. Morris[1,2], B. Andrews[1,2]. 1) University of Toronto, Toronto, Canada; 2) The Donnelly Centre, Toronto, Canada; 3) University of Washington, Seattle, Washington.

Synthetic genetic array (SGA) analysis coupled with high-content screening (HCS) in *Saccharomyces cerevisiae* has provided a wealth of information on functional genomics. Until recently, genetic interactions in SGA analysis have used colony size as a proxy for cellular fitness. Although this metric has proven to be robust, higher resolution phenotypes such as subcellular morphology cannot be assessed. Since there are various perturbations in *S. cerevisiae* in which mutant growth is normal despite morphological abnormalities within subcellular compartments, it would be of great benefit to the yeast community to complement existing colony size data with cell morphology data. Using the SGA-HCS approach, the Boone and Andrews Labs at the University of Toronto, have produced an image-based dataset of subcellular mutant phenotypes in the context of genome-wide perturbations. To analyze these massive datasets of 900,000 images, our labs have developed a machine learning strategy that has been able to successfully detect and classify about half of all the observed and published phenotypes. However, it is challenging to computationally analyze and model a total number of 100 classifiers for all the expected phenotypes with the existing pipeline. Thus, to complete the analysis, I expanded on optimizing the existing pipeline by constructing classifiers for missing phenotypes, and score the genes generating aberrant morphologies. So far, the optimized pipeline can classify a substantial amount of the mutant phenotypes as

preliminary. In order to validate genes resulting with similar mutant phenotypes, I generated the morphology profiles for each gene and performed a preliminary trend analysis on morphology profiles by comparing them to the genetic interaction network to identify genes with high fitness but aberrant morphology. By obtaining complete profiles, we can construct a new informative network for our collections to use alongside the fitness scores from genetic interaction networks. Comparing the biological interpretations of the genetic interaction network and the morphology profiles can reveal further information on biological enrichment and functional analysis that might have been overlooked by the multiplicative model of the fitness measurements alone. This analysis will allow for the identification of connections between discrete biological processes, the prediction of novel gene function, and the generation of a clearer understanding of basic eukaryotic cell biology.

Y3172A HSF-type transcription factors regulate morphogenesis in the human fungal pathogen *Candida albicans*. *V. Basso*, S. Znaidi, V. Lagage, C. d'Enfert, S. Bachellier-Bassi. Institut Pasteur, Paris, FR.

Candida albicans is a diploid fungus, commensal of most healthy individuals, but also one of the most prevalent human fungal pathogens. *C. albicans* has the ability to switch between a unicellular yeast form and filamentous forms (pseudohyphae or hyphae). This switch is important for virulence: yeast cells contribute to the colonization of mucosa and dissemination into the bloodstream, hyphal forms are important for crossing barriers, invasion of tissues and formation of biofilms. The transition is triggered by environmental cues (temperature, pH, CO_2 …) and leads to the activation of hypha specific genes (HSGs). The transcriptional network behind the morphogenetic switch is very complex. Here, we report the role of the HSF-type transcription factors (TF) Sfl1, Sfl2 and Skn7.

Sfl1 and Sfl2 antagonistically regulate the morphogenetic switch: Sfl1 has a negative role, whereas Sfl2 enhances filamentation in response to temperature. On the other hand, deletion of *SKN7* is impairing hyphal growth on solid inducing media, while its overexpression (OE) triggers filamentation in the absence of hypha-inducing cues. We performed genome-wide ChIP analyses to uncover chromosomal regions bound by these TFs, and transcript profiling to identify genes regulated differentially upon their OE. Sfl1 and Sfl2, through divergent motifs, positively and negatively regulate a common set of targets, including repressors of hyphal growth (*SSN6*, *NRG1*, *RFG1*), activators of hyphal development (*UME6*, *BRG1*, *TEC1*) and yeast specific genes (*RME1*, *RHD1*, *YWP1*). Additionally, Sfl2 binds to and turns on the expression of many HSGs. We have shown that Sfl1 and Sfl2 interact with other regulators of morphogenesis (*EFG1*, *UME6*, *TEC1*, *BRG1*). Strikingly, Efg1 binds to targets of Sfl1 and Sfl2, and immunoprecipitates with either proteins. Furthermore, Skn7 binds to regions on the *C. albicans* genome, that also exhibit binding sites for other morphogenesis regulators, including Efg1, Ndt80, Sfl1 and Sfl2. Epistatic studies led us to a model where Skn7 modulate genes involved in hyphal growth, including several key factors of filamentation (*DEF1*, *UME6*, *CPH1* and *CZF1*). Moreover, because Skn7 is also necessary for adaptation of *C. albicans* to oxidative stress, we measured intracellular Reactive Oxygen Species (ROS) levels upon induction of filamentous growth, and showed that Skn7 limits the increase of ROS associated with filamentation on solid media. In conclusion, we propose a regulatory network where *C. albicans* HSF-type regulators Sfl1, Sfl2 and Skn7 coordinate morphogenesis. This model fits in the wider and more complex morphogenetic transcriptional circuitry, implicating intimate functional interactions with other regulators.

Y3173B Dissecting *CTF4*'s role in DNA replication through a synthetic dosage lethality genetic interaction network. *E. Bryant*[1], R. J. D. Reid[2], R. Rothstein[2]. 1) Columbia University, New York, NY; 2) Columbia University Medical Center, New York, NY.

Chromosome Transmission Fidelity 4 (*CTF4*) plays an important role in maintaining replication fork stability by forming a homo-trimer that tethers the lagging strand DNA polymerase α (Polα) to the CMG helicase (Cdc45/Mcm2-7/GINS). *CTF4* genetically interacts with more than 70 different genes annotated to DNA replication, recombination or repair. *CTF4* deletion is especially sensitive to defects in DNA replication fork progression, and joint molecule resolution. Interestingly, *CTF4* overexpression ($CTF4^{OE}$) and *ctf4Δ* share many genetic interactions, and both of these genetic perturbations result in increased Rad52-YFP foci formation (an indicator of DNA repair centers), and increased a-faker frequency (an indicator of genomic instability). However, $CTF4^{OE}$ is uniquely sensitive to loss of 9-1-1 DNA damage checkpoint signaling (Rad17/Mec3/Ddc1), whereas *ctf4Δ* is uniquely sensitive to loss of the fork protection complex (Mrc1/Tof1/Csm3). Interestingly, mutation of the conserved Ctf4 binding site on either the CMG helicase, or Polα, fails to recapitulate *CTF4*'s genetic interactions, which suggests that *CTF4*'s role at the replication fork may be more complex than the current CMG-Ctf4-Polα tethering model. To elucidate *CTF4*'s other possible roles in DNA replication and repair we have overexpressed an array of *CTF4* mutations in combination with a small collection of 200 *CTF4* genetic interaction network mutants. To improve the resolution of these experiments we have developed a system to measure arrayed colony growth over time in various sensitizing conditions. By comparing the resulting genetic interaction profiles of *CTF4* mutations we have begun to separate the function of *CTF4* domains. Preliminary results suggest that Ctf4 may physically interact with several other replication factors, and may also have a role in signaling the DNA replication checkpoint. These experiments highlight the utility of overexpression as a genetic perturbation that can complement a synthetic lethal network analysis to reveal separation of function and biological insight.

Y3174C Using genetic interactions to dissect the cellular response to cisplatin. *E. Bryant*[1], R. J. D. Reid[2], I. Sunjevaric[2], A. Canat[2], E. Rizk[1], L. Crowley[2], R. Rothstein[2]. 1) Columbia University, New York, NY; 2) Columbia University Medical Center, New York, NY.

Cisplatin, a frontline chemotherapy agent for ovarian, breast and lung cancers, is in a class of DNA damaging agents that lead to replication fork stalling and collapse, apoptosis and cell death. Cisplatin sensitivity is affected by a number of DNA repair pathways including nucleotide excision repair, the multiple pathways that collectively make up post replication repair, and processes that specifically incise DNA interstrand crosslinks. Although much is known about the genetic control of these DNA repair mechanisms, the interactions and overlapping functions of these pathways are not completely understood. Furthermore, efforts to catalog the genes affecting cellular sensitivity to cisplatin has either defined a few, well studied genes, or has defined a large number of genes that may only be peripherally involved in sensitivity. We therefore reexamined datasets of cisplatin sensitivity in *Saccharomyces cerevisiae* to define a gene set for systematic double mutant analysis. The CLIK

(Cutoff Linked to Interaction Knowledge) algorithm expanded the number of likely cisplatin sensitive genes in individual high throughput studies, and additional genes were identified via growth curve analysis of gene disruption libraries using multiple cisplatin concentrations. Pilot experiments have highlighted the importance of tailoring the drug concentration for each query mutation to optimally identify epistatic versus synergistic genetic interactions. Analysis of a translesion polymerase, Polζ (Rev3-Rev7) and the homologous recombination related SHU complex (Shu1-Shu2-Psy3-Csm2) demonstrates that optimal detection of synergy between these complexes requires a low concentration of cisplatin. On the other hand, detection of epistasis among Polζ complex members requires a much lower concentration of cisplatin than the SHU complex. Interestingly, synergy was detected between SHU complex members Shu1 and Shu2, which suggests a possible redundancy between these two factors. These experiments lay the foundation for a high-fidelity cisplatin dependent epistasis map that will be used to disambiguate relationships between the many gene and pathways involved in managing DNA crosslinks.

Y3175A Functional interaction network of the conserved NDR kinase Orb6. *C. Chen*, I. Nuñez, D. Wiley, G. D'Urso, F. Verde. University of Miami, Miami, FL.

NDR (nuclear dbf2-related) kinases represent an evolutionarily conserved subclass of AGC kinases. NDR kinases control cell morphogenesis in different organisms, ranging from yeast to mammals. Our lab previously found that Orb6 kinase regulates cell polarity by spatial control of Cdc42 GTPase (*Das et al., Curr Biol. 2009* and *Das et al., Science. 2012*).

Our lab used an ordered fission yeast gene deletion library to perform a genome-wide screen for gene functions that display synthetic lethality or suppression interactions with an analog-sensitive Orb6 kinase mutant (orb6-as2). The potential modulators of the NDR kinase Orb6 were further refined by using computational, microscopy, mass spectrometry, and 2-hybrid analysis approaches. By this genome-wide screen, our lab has identified two genetically separable functions of Orb6, cell morphogenesis and P-body formation, mediated by two direct substrates, Gef1 (*Das et al., MBoC 2015*) and Sts5 (*Nuñez et al., under review*), respectively. Also, two other novel functions of Orb6 kinase identified from the same screens are (1) stress response and (2) autophagy regulation. Current work is focusing on the intermediate role of Orb6 kinase in connecting diverse extracellular stress signals and autophagy modulation.

Y3176B Measuring protein-protein assemblies with a molecular ruler in living cells. *A. Chrétien*, I. Gagnon-Arsenault, C. Lamothe, A. K. Dubé, A. Dion-Côté, C. R. Landry. Laval University, Quebec, Quebec, CA.

Protein quaternary assembly is central to many cellular processes. Several methods have been developed to detect and measure protein-protein interactions in model organisms. Some methods allow the identification of members of complexes but do not provide information on their spatial relationships. Other methods allow the detection of binary and direct interactions between physically associated proteins but do not provide direct insights into the larger quaternary architecture. In order to dissect the organisation of protein complexes both in terms of composition and spatial relationship among the subunits, it would be optimal to have a method that combines the two approaches described above. Here, we show that Protein-fragment Complementation Assay (PCA) can meet these needs. PCA is based on the association of complementary reporter fragments that reveals protein-protein interactions upon complementation. The reporter fragments are fused to the proteins of interest by a short peptide linker that determines the maximal physical distance at which fragments can complement. We found that we can modulate the length of the linkers to detect novel protein-protein interactions on a proteome-wide scale and more distant protein-protein interactions within large protein complexes. Overall, we show that this modified PCA can act as a molecular ruler for measuring protein-protein interactions and proximity in living cells.

Y3177C A network of correlated phenotypes contributes to pleiotropy in yeast single-cell morphology. *Kerry Geiler-Samerotte*[1], Austin Taylor[2], Chelsea Ramjeawan[2], Harris Lazaris[2], Annalise Paaby[3], Mark Siegal[2]. 1) Stanford University, Palo Alto, CA; 2) New York University, NY, NY; 3) Georgia Institute of Technology, Atlanta, Georgia.

Introduction: Pleiotropy (i.e. when one gene influences multiple phenotypes) is central to major problems in biology. It contributes to difficulty predicting the fitness effect of mutations and also can lead to development of drugs with undesired side effects. Despite the importance of pleiotropy, there are fundamental disagreements about its prevalence, mechanism, and definition. One particularly intractable issue when studying pleiotropy has been the definition of independent phenotypes, especially in high-dimensional studies in which many related and potentially correlated phenotypes are surveyed. To achieve a fuller understanding of pleiotropy's extent and molecular underpinnings, we develop a novel approach to describe the network of correlated phenotypes that contribute to pleiotropy in yeast single-cell morphology.

Methods: We used high-throughput microscopy and image analysis to quantify hundreds of single-cell morphological features in 374 lines from a cross between two genetically diverged yeast strains. We identified 12 pleiotropic loci (QTL) that each contribute to variation in 2 or more traits. But does each QTL influence multiple unrelated traits or does some fraction of pleiotropy result because genetic effects cascade through a network of phenotypes that exert influence on one another? To determine which phenotypes are correlated in the absence of genetic effects, we leveraged hundreds of single cell measurements from within each of the 374 clonal yeast populations. We used advanced statistical methods that tease apart phenotypic correlations present within strains from those that arise between strains.

Results: *Within* clonal yeast populations, over 80% of phenotype pairs are more correlated than after random permutation, suggesting that a connected network of phenotypes underlies cell morphology. Correlations increase *between* genetically distinct yeast strains for ~35% of phenotype pairs, suggesting that genetic effects contribute additional pleiotropy beyond what would occur due to the underlying structure of the phenotype-phenotype network. As for QTL effects, pleiotropy is achieved through indirect effects cascading through the phenotype-phenotype network roughly 2/3 of the time. A QTL on chromosome 13 is the exception. It affects the most phenotypes in this study (43);

experiments confirm these effects are due to a single gene, CYK2. Our analysis suggests that CYK2 influences 22 phenotypes directly and 21 indirectly through the phenotype-phenotype network. In sum, our novel approach can enhance knowledge of (1) phenotype-phenotype networks, and (2) the extent and nature of pleiotropy. Both types of knowledge further our understanding of the relationship between genotype and phenotype.

Y3178A Expanding the yeast genetic toolkit: developing a pooled assay for genetic interactions. *M. Jaffe*[1], J. D. Smith[1], R. P. St. Onge[1], G. Sherlock[1], S. F. Levy[2]. 1) Stanford Univ., Stanford, CA; 2) Stony Brook Univ., Stony Brook, NY.

Two genes are said to interact when the fitness of a double mutant deviates from the multiplicative fitness of the two corresponding single mutants. *Saccharomyces cerevisiae* is an ideal system to measure these interactions quantitatively and in high-throughput because of its compact genome and its wealth of genetic tools. In order to improve current screening methods, we developed, and present here, two iterations of a pooled assay of genetic interactions named iSeq. These pool-based methods increase the scalability of measurements across growth conditions, because, in contrast to prior methods, a pool of mutants must only be generated once before assaying multiple conditions. In the first iteration of iSeq, we generated ~400 mutant strains representing 45 possible single or double gene deletions, with multiple strains per genotype. Starting with deletions from the yeast deletion collection, we introduced a novel DNA double-barcoding technology to each double deletion strain to use as its molecular identifier during pooled growth. iSeq fitness and interaction score measurements for each strain in the pool were reproducible across three independent cultures for three growth conditions tested (ρ = 0.91-0.99). These measurements also correlated with an independent OD-based growth assay (ρ = 0.68-0.69). However, we observed high variability in estimates across strains carrying the same gene deletion(s), but unique barcodes (N = 4—16 per genotype). Whole-genome sequencing of 73 double mutant and parental strains revealed segregating and *de novo* mutations were common and accumulated over the two rounds of mating and selection used to generate strains. This genetic variability likely led to the observed fitness differences, and observations of aneuploidy were often associated with lower fitness estimates. To reduce this genetic variability for future screens, we are developing a second iteration of iSeq that relies on an inducible CRISPR/Cas9 gene repression system to generate single and double mutants without any mating or selection steps. As validation, we are using this system to perform a preliminary screen for genetic interactions of 9x459 genes, also in a pooled format. Next, we aim to apply this technology to discover novel gene functions and further understand the topology of the genetic interaction network and its dynamics across growth conditions.

Y3179B A Gene Network Model of Cellular Aging and its Applications. *H. Qin*. Spelman College, Atlanta, GA.

Why would a genotypically homogeneous population of cells live to different ages? We propose a mathematical model of cellular aging based on gene interaction network. This model network is made of only non-aging components, and interactions among genes are inherently stochastic. Death of a cell occurs in the model when an essential gene loses all of its interactions. The key characteristic of aging, the exponential increase of mortality rate over time, can arise from this model network with non-aging components. Hence, cellular aging is an emergent property of this model network. The model predicts that the rate of aging, defined by the Gompertz coefficient, is proportional to the number of active interactions per gene and that stochastic heterogeneity is an important factor in shaping the dynamics of the aging process. Hence, the Gompertz parameter is a proxy of network robustness. Preliminary studies on how aging is influenced by power-law configuration, synthetic lethal interaction, and allelic interactions can be modeled. A general framework to study network aging as a quantitative trait has also been found, and the results has implication on missing heritability.

Y3180C Identifying novel factors underlying stress resistance in the pathogenic yeast *Candida glabrata*. *L. C. Ames*[1], A. Cook[1], G. Cromie[2], E. Jeffery[2], A. Dudley[2], K. Haynes[1]. 1) University of Exeter, Exeter, Devon, GB; 2) Pacific Northwest Diabetes Research Institute, Seattle, W.A., USA.

The ability of pathogens, such as *Candida* species, to adapt to stresses encountered in the host environment is vital for survival and the establishment of infection. In particular, they must mount a robust response to reactive oxygen species in the phagosome. Despite highly homologous stress response pathways, *C. glabrata* is intrinsically more stress resistant than its close relative, the non-pathogenic yeast *Saccharomyces cerevisiae*. To identify novel factors contributing to such stress resistance in *C. glabrata*, mutants were generated for characterisation using two methods. Firstly, 96 *C. glabrata* mutants resistant to tBOOH, an oxidative stress-inducing chemical, were produced by EMS mutagenesis. Secondly, *in vitro* passaging experiments were used to force evolution of tBOOH- and H_2O_2-resistant phenotypes in *C. glabrata*.

Characterisation of EMS mutants revealed that the acquisition tBOOH resistance was accompanied by a fitness trade-off where mutants either became less fit under non-stress conditions or became more susceptible to another type of stress. Most notably, 70 % of tBOOH-resistant EMS mutants became more susceptible to the antifungal drug fluconazole. Surprisingly, *C. glabrata* strains passaged in H_2O_2 showed no fitness defect under stress-free conditions. The virulence of stress-resistant mutants is currently being investigated in a newly-optimised *Galleria mellonella* model of *C. glabrata* infection.

Whole genome sequencing was performed on all stress-resistant EMS and passaged mutants, revealing over 4000 polymorphism calls and 14 aneuploidy events. Recreation of selected polymorphisms into the parental strain background, by site directed mutagenesis, will identify mutations contributing to stress resistance in *C. glabrata*.

Y3181A Perseverance and hetero-resistance, the epigenetic ability of a subpopulation of pathogenic yeasts to survive and grow in drug, contributes to the appearance of drug resistance via different genomic mechanisms. *Judith Berman*[1], Alex Rosenberg[1], Noa Wirthheimer[1],

Esther Weindling[1], Noam Shahar[1], Alon Dahan[1], Aleeza Gerstein[1,2], Inbal Hecht[1], Ronen BenAmi[1]. 1) Tel Aviv University, Ramat Aviv, IL; 2) University of Minnesota, Mpls, MN.

The terms resistance, tolerance, persistence and heteroresistance have been used very generally in the current literature to describe the ability of an organism to survive and grow in the presence of an antimicrobial drug. Furthermore, clinical failures are often due to non-resistant (susceptible) strains that persist *in vivo* and likely seed recurrent infections. Resistance is heritable, with growth of ~ all cells in the population up to a given drug concentration. Studies in bacteria have started to define tolerance and persistence much more precisely. By contrast, the use of these terms remains very vague in studies of fungal pathogens such as Candida species. Within different *Candida albicans* isolates we detect different degrees of subpopulations of cells that grow slowly but consistently in drug. We developed and adapted assays to measure this subpopulation and, based on its growth dynamics, we call it "Perseverance". Perseverence is slow, steady growth in drug that is seen for >10% of the population, is concentration-independent, but time-dependent. Furthermore, and in contrast to tolerance in bacteria, these subpopulations exhibit shorter lag times in drug relative to sensitive strains. The degree to which high perseverence is connected to the rate of appearance of frank resistance will be presented.

By contrast, in *Candida glabrata,* we find that clinical strains exhibit hetero-resistance, in which very small subpopulations (<0.1%) survive in drug. Hetero-resistance is also heritable and it allows subpopulations of a clinical strain to persist in the host. Furthermore, genomic analysis indicates that different subpopulations exhibit different gene expression profiles.

Importantly, clinical assays currently measure neither persistance or hetero-resistance and thus these properties of strains are not considered in clinical decisions. We suggest that perseverance and hetero-resistance are important parameters contributing to the evolution of resistance and thus the persistence and recurrence of and infection, which must be considered in the clinical setting. .

Y3182B Structure/Function Analysis of the Hif1 Histone Chaperone in *Saccharomyces cerevisiae*. *Nora S. Dannah*, Jeffrey Fillingham. Ryerson University , Toronto, ON, CA.

Understanding how eukaryotic cells assemble their chromatin is a significant research subject in part because several human pathologies including cancer are associated with defects in chromatin assembly. Transporting of newly synthesized histones H3/H4 occurs in a stepwise fashion and is regulated by a variety of protein factors including histone chaperons. In the budding yeast *Saccharomyces cerevisiae*, the Hif1 protein is an evolutionarily conserved H3/H4-specific histone chaperone and a member of the nuclear Hat1 complex that catalyzes the deposition-related acetylation of newly synthesized histones H4. Hif1 as well as its human homolog NASP have been implicated in an array of chromatin-related processes including histone H3/H4 transport, chromatin assembly, DNA repair and telomeric silencing. In this study, we elucidate structural and functional aspects of Hif1. Through targeted mutational analysis, we demonstrate that the acidic region of yeast Hif1 which interrupts the TPR2 is essential for physical interaction with the Hat1-complex. We also demonstrate that Hif1 requires its C-terminal basic patch for nuclear localization. Furthermore, we provide evidence for the involvement of Hif1 in regulation of histone metabolism by showing that cells lacking *HIF1* are both hypersensitive to histone H3 overexpression. Finally, we describe a functional link with a transcriptional regulatory protein Spt2 possibly linking Hif1 to transcription-associated chromatin reassembly. Taken together, our results provide novel mechanistic insights into Hif1 functions and establish it as a key player in various chromatin-associated processes.

Y3183C Hsp90 perturbations affect genome integrity in *Candida albicans*. *K. Dong*[1*], A. Forche[2], S. Milne[1], L. Alaalm[1], J. Berman[3], S. Diezmann[1]. 1) University of Bath, Bath, GB; 2) Bowdoin College, Brunswick, USA; 3) Tel Aviv University, Tel Aviv, Israel.

One of the leading fungal pathogen of humans, *Candida albicans*, does not appear to engage in canonical sexual recombination. This is surprising given *C. albicans*' proclivity to effectively switch between being a commensal and an opportunistic pathogen and rapidly evolve drug resistances. The molecular chaperone Hsp90, a central regulator of protein homeostasis, governs virulence and drug resistance in *C. albicans*. This then raises the question: how does an 'obligate' diploid generate genetic variations? Here, we address this question by identifying if and how Hsp90 regulates genome integrity in *C. albicans*. Forche *et al*. (2011) demonstrated that different stresses elevated Loss-of-Heterozygosity (LoH) rates across the *C. albicans* genome. We hypothesize that alterations of either Hsp90 level or function result in LoH and thus promote genome diversification. In line with this hypothesis, we conducted fluctuation assay showing that multiple modes of Hsp90 perturbation increase LoH rate, suggesting that Hsp90 is required for the maintenance of a diploid, heterozygous genome but compromised Hsp90 function promotes the genomic rearrangement. More importantly, our SNP-RFLP analyses revealed that the types of LoH events are contingent on the mechanisms used to abrogate Hsp90 function. Pharmacological inhibition increased frequency of local recombination events such as gene conversion or break-induced recombination, whereas high temperature stress caused whole-chromosome homozygosis. This very novel observation suggests that *C. albicans* may utilize distinct forms of LoH to deal with disparate stressors. This concept is supported by our *in vitro* fungal drug resistance test which showing a biological relevant pattern. Hence, our work provides evidence that Hsp90 governing LoH rates and types is one of principles underpinning *C. albicans* genome plasticity, which aids in the generation of mosaic karyotypes with novel phenotypes. This indicates that Hsp90 affects a pathogen's virulence through genome modulations, eventually imparting both theoretical and clinic novel knowledge.

Y3184A Rme1 controls chlamydospore formation in the human pathogenic yeast *Candida albicans*. *A. Hernandez Cervantes*[1,2], S. Znaidi[1,2], V. Basso[1,2], F. Vincent[3], F. Dalle[3], M. E. Bougnoux[1,2], N. Sertour[1,2], S. Bachellier-Bassi[1,2], C. d'Enfert[1,2]. 1) Pasteur Institute, Paris, France; 2) INRA, USC2019, F-75015 Paris, France; 3) UMR 1347, Agroécologie AgroSup/INRA/uB 21065 Dijon, France.

Candida albicans is the most common pathogenic fungus of humans, normally found as a harmless commensal colonizing skin and mucosal epithelium of 30-70% healthy individuals. This yeast causes systemic infections in the absence of a proper immune system, by gaining access to

the bloodstream mainly through translocation across the intestinal barrier. A characteristic of this organism is its capability to react to variations of the environment by growing either as unicellular yeasts or in filamentous forms (true hyphae or pseudohyphae). The ability to switch between yeast and filaments is a virulence trait, important for the success of *C. albicans* both as a commensal and as a pathogen. Upon specific growth conditions, *C. albicans* can produce chlamydospores, which are large refractile cells with thick cell walls, often forming on suspensor cells attached to hyphae, neither dead nor long-term survival cells, likely to be a specialized and alternative form of growth of *C. albicans.* Previous studies in our laboratory suggested that the *C. albicans* transcription factor Rme1, ortholog of *Saccharomyces cerevisiae* regulator of meiosis, was involved in the yeast-hyphae transition. In order to determine the *in vivo* function of Rme1, we performed ChIP-on-chip and transcriptomics analyses on an inducible overexpression (OE) strain, showing that Rme1 binds to 724 promoter regions, upregulating 622 genes and downregulating 357 genes. The most strongly upregulated genes are markers of chlamydospore development. To explore the role of Rme1 in this morphogenetic program, *RME1* OE and knock-out (KO) mutant strains were grown on Potato Carrot Bile Agar medium under microaerophilic conditions in the dark for 7 days. The OE strain formed masses of chlamydospores as compared to the SC5314 wild type strain, whereas the KO strain was unable to form these structures. Moreover, we observed that the expression levels of *RME1* and its targets were increased in *C. albicans* clinical isolates that overproduced chlamydospores. In addition, we observed that *RME1* OE could restore the defect in chlamydospore development displayed by the *hog1*ΔΔ and *efg1*ΔΔ KO strains, suggesting that Rme1 acts as a bottleneck regulator in the chlamydospore formation pathway. Taken together, our results confirm that the function of ScRme1 in the regulation of meiosis has been lost in *C. albicans* and has been rewired towards the control of a complex regulatory network necessary for chlamydospore formation.

Y3185B Isolation and characterization of a manganese tolerant mutant of *Saccharomyces cerevisiae*. *M. Kishida*, T. A. Do, T. Sakai, Y. Tanaka, H. Iwata, M. Furuta. Osaka Prefecture Univ, Sakai, Osaka, JP.

Manganese contamination in water is one of the most serious problems in southeastern countries, including Vietnam, because the large amount of manganese provides the toxicity in cells. Bioremediation using microorganisms is expected to be a useful technique to remove manganese from contaminated water. We employed to breed a yeast strain having the manganese accumulation ability from the brewing yeast *Saccharomyces cerevisiae*. In this study, we indicate that the growth and the manganese accumulation of the isolated yeast strains cultured in manganese ion-containing media.

Strains used were *S. cerevisiae* BY4741 as the wild-type strain and some manganese accumulation mutants bred from BY4741. The used medium was YPD (2% glucose, 1% yeast extract, and 1% peptone) with various concentrations of manganese chloride, if necessary. To test the toxicity of the manganese ion, the growth profile of yeast was determined by turbidity measurement at 660 nm. The concentration of manganese in cells was measured by atomic absorption spectrophotometry after the digestion of yeast cells by the concentrated nitric acid and then an adequate dilution.

Manganese accumulation mutants were screened from the growing strains on YPD with the high concentration of manganese ion (Mn^{2+}) in which BY4741 could not grow, as follows. Overnight cultured BY4741 was inoculated into YPD containing various concentrations of Mn^{2+} and tested for the growth. BY4741 could not grow more than 10 mM of Mn^{2+}. To select manganese tolerant mutants, BY4741 was inoculated in YPD with 10 mM of Mn^{2+} and cultured at 30°C for 24 h. Survival strains were inoculated again in the same medium and cultured at the same condition. Then, the variants that accumulated more manganese than BY4741 were isolated by repeat screening of survivors in 10mM Mn^{2+}-containing YPD media. Finally, nine mutants which accumulated manganese were isolated and named IM1 to IM9. One variant showing the highest manganese accumulation, IM3, accumulated over 4-fold more manganese than its parent strain BY4741, and could grow under 25 mM Mn^{2+}. Effects of culture temperature and pH on manganese accumulation were analyzed in IM3. The maximum accumulation was shown at 30°C while the maximum growth was shown at 37°C. The manganese accumulation was recognized from pH 4.0-9.0 and was highest at pH 6.0. Interestingly, IM3 could grow a little at pH 9.0 when manganese was added to the culture media, while IM3 could not grow without manganese addition. This reason is unclear but it may show the possibility that Mn^{2+} uptake affects the intracellular pH in IM3. We try to analyze the effect of the other stress on manganese accumulation in IM3.

Y3186C Phylogenetic profiling for the elucidation of heme-iron acquisition in pathogenic yeasts. *D. Kornitzer*[1], S. Yaish[1], L. Nasser[1], Z. Weissman[1], G. Horev[2], H. Dvir[3]. 1) Faculty of Medicine, Technion-I.I.T., Haifa, IL; 2) Bioinformatics Knowledge Unit, Technion-I.I.T., Haifa, IL; 3) Technion Center for Structural Biology, Technion-I.I.T., Haifa, IL.

Iron acquisition represents a particular challenge for pathogenic microorganisms, because of the iron withholding mechanisms deployed by the host. Many microbial pathogens, including fungi such as *Candida albicans*, have therefore evolved mechanisms for extracting iron from hemoglobin, the largest iron store in the host, by removing the heme cofactor from the globins, and transferring it to the microbe's cytoplasm. Elucidation of this pathway in *C. albicans* by genetic means was however hampered by the fact that this organism is not suited for forward genetic screening, and that the pathway is absent from the model yeast *S. cerevisiae*. Screening of a *C. albicans* library introduced in yeast enabled the initial identification of a family of extracellular heme-binding proteins involved in heme-iron utilization. These proteins all include a CFEM sequence signature, consisting of 8 cysteines with conserved spacing. We solved the structure of the hemophore Csa2, revealing that the CFEM domain adopts a novel protein fold, held together by 4 disulfide bonds generated by the 8 conserved cysteines, and that it displays a unique heme-iron coordination mechanism. CFEM proteins involved in heme utilization are either secreted, or are GPI-anchored at different locations in the cell envelope. These proteins all are able to capture heme from hemoglobin and to transfer it between themselves, consistent with a pathway in which heme is extracted from hemoglobin outside the cell and is then transferred from one CFEM protein to the next until it reaches the plasma membrane - whereupon it is endocytosed by a mechanism previously shown to involve the ESCRT pathway. A missing link was the putative transmembrane heme receptor that connects the extracellular CFEM network with the endocytic pathway. To identify candidates for such a receptor, we resorted to phylogenetic profiling: taking advantage of the hundreds of fungal genomes available, we

screened for genes jointly present with the CFEM heme transfer cascade genes across fungi. This enabled us to identify a new class of plasma membrane proteins that are essential for heme uptake. The hemoglobin-iron utilization pathway that emerges from these studies, which is distinct from bacterial systems, indicates that fungi have evolved a unique solution to the heme-iron acquisition problem. Phylogenetic profiling is shown here to represent a powerful method for unraveling cellular pathways in organisms that are recalcitrant to conventional genetic screening.

Y3187A Assembling whole eukaryotic genomes from mixed microbial communities using Hi-C. *I. Liachko*[1], J. Burton[1], L. Sycuro[2], A. Wiser[2], D. Fredricks[2], J. Shendure[1], M. Dunham[1]. 1) University of Washington, Seattle, WA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

De novo assembly of whole genomes from next-generation sequencing is inhibited by the lack of contiguity information in short-read shotgun sequencing. This limitation also impedes metagenome assembly, since one cannot tell which sequences originate from the same species within a population. We have overcome these bottlenecks by adapting a chromosome conformation capture technique (Hi-C) for the deconvolution of metagenomes and the scaffolding of *de novo* assemblies of complex genomes.

In modeling the 3D structure of a genome, chromosome conformation capture techniques such as Hi-C are used to measure long-range interactions of DNA molecules in physical space. These tools employ crosslinking of chromatin in intact cells followed by intra-molecular ligation, joining DNA fragments that were physically nearby at the time of crosslink. Subsequent deep sequencing of these DNA junctions generates a genome-wide contact probability map that allows the 3D modeling of genomic conformation within a cell. The strong enrichment in Hi-C signal between genetically neighboring loci allows the scaffolding of entire chromosomes from fragmented draft assemblies. Hi-C signal also preserves the cellular origin of each DNA fragment and its interacting partner, allowing for deconvolution and assembly of multi-chromosome genomes from a mixed population of organisms.

We have used Hi-C to scaffold high-quality genomes of animals, plants, fungi, as well as prokaryotes and archaea from very fragmented *de novo* assemblies. We have also been able to use this data to annotate functional features of microbial genomes, such as centromeres in many fungal species including over a dozen yeasts. Additionally, we have applied our technology to diverse metagenomic populations such as craft beer, bacterial vaginosis infections, soil, and tree endophyte samples to discover and assemble the genomes of novel strains of known species as well as novel prokaryotes and eukaryotes. This method's ability to reconstruct multi-chromosome genomes has led to discovery of novel yeast hybrids directly from mixed communities.

The high quality of Hi-C-based assemblies allows the simultaneous assembly and scaffolding of numerous unculturable genomes, placement of plasmids within host genomes, and microbial strain deconvolution in a way not possible with other methods.

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Y3188B Phylogenetic and phenotypic characterization of yeasts from detritivorous beetles. *D. J. Wohlbach*, K. A. Mueller, K. Muirhead. Dickinson College, Carlisle, PA.

Detritivorous beetles of the temperate deciduous forest biome thrive in low-nutrient environments with an abundance of cellulosic material, and it is speculated that yeast endosymbionts aid in the metabolism of such materials within the gut of these beetles. To investigate the biodiversity of microbes in this niche, we isolated over 200 yeast strains from different beetles living in the Pennsylvania area. Isolates contained representatives from both Ascomycete and Basidiomycete fungi, including several putatively novel species. Phylogenetic analysis characterized the evolutionary relationship of isolated yeasts; physiological and morphological growth tests helped to characterize the phenotypes of novel species. Interestingly, we found that the majority of yeast endosymbionts were able to metabolize xylose, a five carbon 'woody sugar,' faster than glucose. This research contributes to the study of yeast biodiversity in a particular niche and future characterized strains could improve methods used for lignocellulosic biofuel production.

Y3189C Convergent evolution of phosphate- and thiamine-regulated phosphatases: The *PMU* gene family in *C. glabrata* is analogous to the *PHO5* gene family in *S. cerevisiae*. *D. D. Wykoff*, C. L. Iosue, N. F. Shaik, S. G. Leone, M. T. Peel, P. Toubia, A. Brower, J. Glowala. Villanova Univ, Villanova, PA.

Acid phosphatase genes are present in most Ascomycete genomes and are related based on sequence similarity to the *PHO5* gene in *Saccharomyces cerevisiae*. In *S. cerevisiae, PHO5* is regulated by inorganic phosphate starvation, and *PHO3* (a *PHO5* homolog) is regulated by thiamine starvation. *Candida glabrata* does not have *PHO5* homologs. Previously, we determined that *PHO5* was functionally replaced in *C. glabrata* with the *PMU2* gene, which is part of a three gene family tandemly duplicated on chromosome 11. Using RNA-seq, we now determine that *PMU3* is highly induced during thiamine starvation, that induction is dependent on the thiamine starvation regulators, *THI3* and *PDC2*, and that *PMU3* encodes a thiamine pyrophosphatase (TPPase). We determined that only Pmu3p (and not Pmu2p or Pmu1p) is a TPPase using a functional assay with thiamine pyrophosphate (TPP) as a sole source of thiamine that can only be accessed by TPPase activity. The RNA-seq data demonstrate that ten genes are highly induced during thiamine starvation and are *THI3* dependent in *C. glabrata*. Unlike *S. cerevisiae*, the regulator *THI3* is not induced during starvation, and additionally, *C. glabrata* does not have a *THI2* homolog. We have characterized the highly inducible thiamine-regulated promoters using promoter-YFP fusions and flow cytometry and identified a minimal region that confers thiamine regulation. We expect that this promoter region binds *Cg*Pdc2 during thiamine starvation. The induction of both phosphate- and thiamine-regulated genes require chromatin remodeling/modification complexes for full induction of gene expression because mutants in *Cgsnf2*Δ,

*Cggcn5*Δ, and *Cgasf1*Δ are aberrant in the regulation of phosphate- and thiamine-regulated promoters. We observe that *C. glabrata* has *de novo* sub-functionalized phosphatase expression into two tandem genes and because this system has analogous behaviors and characteristics to *PHO5* & *PHO3* in *S. cerevisiae*, there might be selective reasons for this sub-functionalized phosphatase architecture.

Y3190A Strategies to produce high levels of extracellular cAMP based on cAMP-PKA and purine synthesis pathway regulation in *Saccharomyces cerevisiae*. *S. Zou*[1], P. Ma[2]. 1) Tianjin University, Tianjin, Tianjin, CN; 2) Tianjin University, Tianjin, Tianjin, CN.

Strategies to produce high levels of extracellular cAMP based on cAMP-PKA and purine synthesis pathway regulation in *Saccharomyces cerevisiae*

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cAMP, an important compound involved in various biochemical reactions and a key second messenger, is widely used in animal food additives and pharmaceutical research. In yeast, cAMP concentration is tightly regulated because of the important role of the cAMP-PKA pathway in controlling a broad array of cellular processes. Up to now, lots of research has been focused on the cAMP-mediated regulatory pathway and the regulation of intracellular cAMP levels in *Saccharomyces cerevisiae*. In this study, the extracellular cAMP level and its regulation was investigated in detail and *Saccharomyces cerevisiae* was shown to be an excellent candidate for fermentative production of cAMP after manipulating the cAMP-PKA and purine synthesis pathways and optimizing the media. First, the cAMP dependent protein kinase (PKA)-encoding genes were deleted to remove the powerful feedback inhibition of PKA on cAMP synthesis and the lack of viability of *tpk*1 *tpk*2 *tpk*3 triple mutants was further suppressed by mutations such as *yak*1 or *msn*2/*msn*4. Second, the low-affinity cAMP phosphodiesterase Pde1 was inactivated to decrease the breakdown of cAMP. Third, Bas1p and Bas2p were fused and over-expressed by co-integrating into the genome to reinforce their cooperative interaction in purine synthesis pathway and thus the production of the cAMP precursors ADP and ATP. Bas1p is a Myb-related transcription factor. It acts together with the homeodomain-related Bas2p (Pho2p) to regulate purine and histidine biosynthesis genes in response to extracellular purine limitation. Fourth, the content of yeast extract and peptone in fermentation media were increased and the ratio of carbon to nitrogen source was optimized. As a result, the extracellular cAMP level could reach 6414.3 μmol/L.

Y3191B Integrating Post-Translational Modification Data into the *Saccharomyces* Genome Database. *Sage T. Hellerstedt*, Stacia R. Engel, Shuai Weng, J. Michael Cherry, The SGD Project. Stanford University, Stanford, CA.

After translation by a ribosome, a protein can undergo modifications known as post-translational modifications (PTMs). By the covalent addition or removal of various functional groups, PTMs increase the functional diversity of the proteome. At the *Saccharomyces* Genome Database (SGD; www.yeastgenome.org), we have been actively expanding our protein data to include manually curated post-translational modification information for the model organism *Saccharomyces cerevisiae.* Examples of PTM types we are currently curating from the literature include, but are not limited to: phosphorylation, acetylation, methylation, ubiquitination, and sumoylation. These data are displayed in the amino acid sequence section of SGD Protein pages as highlighted residues, including the protein modifier when available. Post-translational modification information is often critical to understanding mature protein function and regulation, thus we include the PTM data in SGD as part of our continuing mission to educate students, enable bench research, and facilitate scientific discovery. This work is supported by a grant from the NHGRI (U41 HG001315).

Y3192C Multi-omic analysis of yeast strains evolved for xylose fermentation reveals a new connection between sugar sensing and oxygen response. *Kevin S. Myers*, Nicholas M. Riley, Trey K. Sato, Joshua J. Coon, Audrey P. Gasch. Great Lakes Bioenergy Research Center, University of Wisconsin - Madison, Madison, WI.

Biofuels produced by microbes, including *Saccharomyces cerevisiae*, have promise as a non-petroleum based energy system. Biofuel production from agricultural waste, such as corn stover, would provide an important advance because it would not compete with food sources. However, corn stover contains high concentrations of xylose, which *S. cerevisiae* cannot naturally ferment, for reasons that are not understood. This defect renders nearly a third of the potential carbon in corn stover biomass unutilized. To address this, an engineered *S. cerevisiae* strain containing bacterial xylose isomerase was subjected to directed evolution for over 200 generations. Strain Y127 was evolved from a stress-tolerant wild yeast strain (Y22-3) for aerobic xylose fermentation; strain Y128 was further evolved from Y127 to ferment xylose anaerobically. Whole-genome sequencing identified responsible mutations in the evolved strains, but the physiological effect of these mutations remains unclear. To better understand the physiology of these strains, we performed genome-scale analyses to study differences in gene expression (RNA-seq), protein levels (label-free quantitative mass-spec proteomics), and phosphorylation abundance (mass-spec phosphoproteomics) on the parental strain and the two evolved strains, in the presence of glucose or xylose and under aerobic or anaerobic growth conditions. Remarkably, comparison of the mRNA and protein changes revealed a disconnect between mRNA and protein abundance in the unevolved strain growing anaerobically on xylose, and a defect in mounting the hypoxic response when cells are grown anaerobically on xylose. Over-expression of AZF1 significantly improved anaerobic xylose fermentation in the evolved Y128 strain, but only during anaerobic growth on xylose. Comparative analysis across the multi-omic datasets implicates downstream targets of Azf1 and upstream regulatory pathways that enable anaerobic xylose fermentation. These results present important new insights into how anaerobic xylose fermentation can be engineered in yeast and reveal new connections between sugar and oxygen responses.

Y3193A Quantitative phosphoproteomics identifies regulatory feedback between inositol polyphosphate signaling and yeast pseudohyphal growth. *Kaitlyn Norman*, Christian Shively, Anuj Kumar. University of Michigan, Ann Arbor, MI.

Inositol polyphosphates are important eukaryotic second messengers with diverse roles in chromatin remodeling, telomere maintenance, mRNA export, and regulation of the phosphate starvation response. Despite strong research interest, relatively little is known regarding the regulation of inositol polyphosphate signaling in yeast or metazoans. Through studies incorporating quantitative phosphoproteomic analysis of kinase signaling pathways required for yeast pseudohyphal growth, we report here an unexpected regulatory interrelationship between inositol polyphosphate signaling and the cellular response to nitrogen stress. *S. cerevisiae* exhibits a dramatic growth response to nitrogen limitation, characterized by the transition from a vegetative yeast-like form to a filamentous form with chains of elongated pseudohyphal cells. Pseudohyphal growth is regulated through signaling networks encompassing MAPK and Snf1p pathways. We find that kinase-dead mutants of Ste11p, Ste7p, Kss1p, and Snf1p exhibit differential phosphorylation of the inositol polyphosphate kinases Arg82p, Vip1, and Kcs1p. Homozygous mutants deleted for *IPK1*, *VIP1*, and *KCS1* display distinct pseudohyphal growth phenotypes under conditions of nitrogen limitation. Profiling of inositol polyphosphates upon growth in media with limited nitrogen reveals striking increases in the abundance of numerous inositol polyphosphate species, including IP_3, IP_7, and IP_8. Further, deletion mutants of the pseudohyphal growth MAPK *KSS1* and *SNF1* exhibit altered profiles of IP_7 isomers by HPLC analysis. In sum, we present data indicating regulatory control of inositol polyphosphates by Kss1p and Snf1p pathways, as well as results identifying regulatory control of pseudohyphal growth through inositol polyphosphate signaling. These findings are consistent with a role for inositol polyphosphate second messengers during the pseudohyphal growth transition, particularly with respect to downstream inositol polyphosphates and pyrophosphates that may function in a complex feedback control system with pseudohyphal growth regulators.

Y3194B Omics approaches for discovery of aging-delaying and anti-tumor compounds and defining mechanisms of their action. *V. Titorenko*, A. Leonov, A. Arlia-Ciommo, Y. Medkour, V. Svistkova. Concordia Univ, Montreal, PQ, CA.

Caloric restriction (CR) and dietary restriction (DR) extend lifespan across species and improve health by delaying the onset of age-related diseases. All currently known aging-delaying chemical compounds 1) mimic lifespan-extending and health-improving effects of CR and DR without restricting caloric and nutrient intake; and 2) target signaling pathways that are under the stringent control of calorie and/or nutrient availability. It was believed therefore that all longevity pathways are “adaptable” by nature because they modulate longevity only in response to certain changes in the extracellular and intracellular nutrient and energy status of an organism. However, it is possible that that some longevity pathways could be “constitutive” or “housekeeping” because they control longevity irrespective of calorie and/or nutrient availability. We designed a high-throughput chemical genetic screen for compounds that increase the chronological lifespan of yeast under CR by modulating such housekeeping pathways. Our screen identified lithocholic bile acid (LCA) as a geroprotector which significantly delays the onset and reduces the rate of yeast chronological aging. Using proteomics, lipidomics, metabolomics, and cell biological approaches, we found that LCA delays yeast chronological aging by: 1) remodeling lipid metabolism in the endoplasmic reticulum, lipid droplets, and peroxisomes - thereby preventing liponecrotic programmed cell death caused by the age-related accumulation of fatty acids; 2) remodeling the repertoire of mitochondrial membrane lipids - thereby reducing the number of mitochondria, increasing their size, expanding their cristae, elevating the abundance of respiratory supercomplexes in the inner mitochondrial membrane, and altering the age-related dynamics of changes in mitochondrial respiration, membrane potential, and reactive oxygen species; 3) attenuating age-related mitochondrial fragmentation - thereby suppressing mitochondria-controlled apoptosis; and 4) promoting “mitohormesis” through the activation of several stress response-related processes in mitochondria. Our findings also imply that, in addition to its robust aging-delaying effect, LCA exhibits a potent and selective anti-tumor effect in cultured human neuroblastoma, glioma, breast cancer, and prostate cancer cells by activating both the intrinsic and extrinsic pathways of apoptotic death.

Y3195C Quantitative proteomics of the yeast Hsp70/Hsp90 interactomes during DNA damage reveals chaperone-dependent regulation of ribonucleotide reductase. *Andrew Truman*[1], Laura Knighton[1], Isaac Sluder[1], Donald Wolfgeher[2], Stephen Kron[2]. 1) University of North Carolina at Charlotte, Charlotte, NC; 2) University of Chicago, Chicago, IL 60637.

The highly conserved molecular chaperones Hsp90 and Hsp70 are indispensible for folding and maturation of a significant fraction of the proteome, including many proteins involved in signal transduction and stress response. To examine the dynamics of chaperone-client interactions after DNA damage, we applied quantitative affinity-purification mass spectrometry (AP-MS) proteomics to characterize interactomes of the yeast Hsp70 isoform Ssa1 and Hsp90 isoform Hsp82 before and after exposure to methyl methanesulfonate (MMS). Of 256 proteins identified and quantified via 16O/18O labeling and LC-MS/MS, 142 are novel Hsp70/90 interactors. Nearly all interactions remained unchanged or decreased after DNA damage, but 5 proteins increased interactions with Ssa1 and/or Hsp82, including the ribonucleotide reductase (RNR) subunit Rnr4. Inhibiting Hsp70 or 90 chaperone activity destabilized Rnr4 in yeast and its vertebrate homolog hRRM2 in breast cancer cells. In turn, pre-treatment of cancer cells with chaperone inhibitors sensitized cells to the RNR inhibitor gemcitabine, suggesting a novel chemotherapy strategy.

Y3196A Humanization of entire yeast pathways via CRISPR/Cas9. *A. Akhmetov*[1], A. Kachroo[1], E. Marcotte[1,2,3]. 1) University of Texas at Austin, Austin, TX; 2) Center for Computational Biology and Bioinformatics, University of Texas at Austin, Austin, TX; 3) Department of Molecular Biosciences, University of Texas at Austin, Austin, TX.

We have recently found that a large number of S. cerevisiae genes can be "humanized", i.e. the yeast gene is deleted and replaced with its human ortholog to generate viable strains. We have also observed several gene modules (pathways or complexes) which are comprised mostly of genes that can be humanized (Kachroo et al. (2015) Science, 348:921-925). The sterol biosynthesis pathway, which is common between yeast and humans (producing the precursors of ergosterol and cholesterol in them respectively) is one such pathway; 17 of the genes in it

(many of them essential) can be individually humanized.

We have sought to investigate the possibility of higher order humanizations of an entire gene module (simultaneous humanization of multiple genes), using the sterol biosynthesis pathway as a starting point. We have developed a rapid, high-throughput approach based on CRISPR/Cas9 technology which allows us to easily and efficiently perform genetic engineering at several yeast loci. We have also investigated the utility of methods for combining modifications at several different loci in one strain. We expect that module-scale humanization will yield valuable insights into the evolution of species. In addition to this, this system is a promising platform for systematically investigating interactions between human genes, both in their wild-type as well as mutant forms or allelic variants. Since experiments with humanized yeast are much less labor and cost intensive than alternatives such as cultured human cells, they offer the possibility of probing human allelic variation on a greater scale than has been possible before.

Y3197B Program number not assigned

Y3198C Long-term real-time imaging of budding yeast with on-demand perturbation via a microfluidic examination trap. *J. T. Helton*, P. J. Hung. EMD Millipore, Hayward, CA.

The budding yeast Saccharomyces cerevisiae has been commonly used to study the biology of aging and growth rate. Yet it is difficult to image cells long-term due to their mobility to escape the focal plane as well as the rapid growth nature to overcrowd the imaging area. To address this we have developed a microfluidic device that is capable of mechanically restraining cells in a 72 × 72 × 4 µm (L x W x H) elastic trap area for long-term, real-time observation with 6 programmable on-demand flow switches. The design provides continuous dissection of excess daughter cells outside the well-defined trap site to prevent cell congestion in the 3mm x 3mm x 10 µm chamber. In addition, cells can be held for several days in a focused monolayer and continue to replicate throughout their entire lifespan during imaging. Specifically in the 72 hour experiment, we monitored growth of single budding yeast cells at a fixed xyz position under high magnification to form a monolayer culture without overcrowding the chamber. Image analysis tool was used to track and quantify the number of daughter cells a yeast mother cell produced. Software-driven perturbation provided precise media flow to the thriving colony which was verified by introducing two-color fluorescent to measure viability. Together, the resulting dynamic cell dissection in tightly controlled focused area with long-term real-time imaging offers considerable potential for cell analysis.

Y3199A A tandem-integration interaction sequencing platform. *Xianan Liu*[1,2], Sasha Levy[1,2]. 1) Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, NY; 2) Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY.

Combinatorial biological screens, such as those that assay genetic interactions between mutations or gene deletions, or that assay physical interactions between proteins, have historically been limited in throughput by the requirement to test for interactions one-at-a-time. A potentially higher-throughput alternative would be to provide each combination with a unique barcode and measure the interaction score of each barcoded genotype *en masse* in cell pools via barcode sequencing (bar-seq). However, construction and storage of a uniquely barcoded strain for each combination is impractical for genome-scale interaction screens. Here, we introduce a pair of novel double barcoding systems that allow us to easily construct and assay extremely large combinatorial libraries. The key innovation is a tandem “landing pad” in the yeast genome that allows two barcoded plasmid libraries to be brought to the same genetic location. Pooled growth of double barcode libraries and paired-end amplicon sequencing can be used infer the relative fitness of each pairwise interaction in a population. We show that this system can easily scale up to yeast pools containing 10^7 or 10^9 double barcodes, depending on whether barcode pairs are brought together by sequential integration or mating. As a proof-of-principle, we generated a library of ~3000 double barcoded haploid and diploid yeast strains, each with a different pair of auxotrophic rescue plasmids. We are currently assaying these pools across several environments to determine the fitness error rates of the assay. Additionally, we are porting our tandem integration platform to mammalian cell systems. This platform will be useful for a variety of applications including assays of interactome dynamics, as well as mammalian protein-protein interaction and CRISPR/Cas9-based genetic interaction assays.

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Zebrafish Development and Genetics

Poster Session Abstracts

Z6001A Investigating the role of tetraploid intermediates in melanoma progression. *R. Darp*[1,2], S. Gujja[1], Y. Edwards[1], D. Pellman[3], L. Zon[4], N. Ganem[5], C. Ceol[1,2]. 1) University of Massachusetts Medical School, Program in Molecular Medicine, Worcester, MA, USA; 2) University of Massachusetts Medical School, Department of Molecular, Cellular and Cancer Biology, Worcester, MA, USA; 3) Howard Hughes Medical Institute, Department of Pediatric Oncology, Dana-Farber Cancer Institute, Children's Hospital and Department of Cell Biology, Harvard Medical School, Boston, MA, USA; 4) Howard Hughes Medical Institute, Stem Cell Program and Division of Hematology/Oncology of Boston Children's Hospital and Dana Farber Cancer Institute, Harvard Stem Cell Institute, Harvard Medical School, Boston, MA, USA; 5) Departments of Pharmacology and Experimental Therapeutics and Medicine, Division of Hematology and Oncology, Boston University School of Medicine, Boston, MA, USA.

Tetraploid cells and their aneuploidy progeny have been proposed as intermediates in tumor development. Support for this hypothesis comes from deep sequencing studies that suggest at least one third of solid tumors have undergone a genome doubling event during their progression and additional studies that have implicated genome doubling as an important step early in tumor progression. These studies propose that nascent tetraploid tumor cells can more easily sample genetic configurations that are advantageous for growth because they, as compared to diploid cells, are buffered against deleterious effects of mutations and changes in gene dosage. Studies using a melanoma-prone *Tg(mitfa:BRAFV600E);p53(lf)* strain suggest a role for tetraploidy and genome doubling in the genesis of tumors in these fish. We have found that, unlike wild-type melanocytes, melanocytes in *Tg(mitfa:BRAFV600E)* animals are binucleate and tetraploid. In tissue culture, newly-formed tetraploid cells undergo a *p53*-dependent G1 cell cycle arrest. A similar arrest likely occurs in zebrafish melanocytes, as melanocytes in *Tg(mitfa:BRAFV600E); p53(lf)* animals exhibit 8N and greater DNA content, suggesting bypass of an arrest that halts progression of tetraploid *Tg(mitfa:BRAFV600E)* melanocytes. These data implicate tetraploids generated by increased BRAF pathway activity as a contributor to melanoma initiation. To gain insight into the mechanism by which *BRAFV600E* generates binucleate cells, we have established a lentiviral-based doxycycline inducible *in vitro* model system to induce *BRAFV600E* expression. We are currently using live-cell imaging approaches to determine the mechanism by which these binucleates arise in culture. We are also conducting retrospective bioinformatic analyses to determine whether melanomas in *Tg(mitfa:BRAFV600E);p53(lf)* zebrafish progress through tetraploid intermediates, supporting the notion that the combination of oncogenic *BRAFV600E* and mutated *p53* causes the generation and progression of nascent tetraploid melanoma cells.

Z6002B Optical Control of Cancer Initiation in Zebrafish. *Zhiping Feng*[1], Vriz Sophie[2], Michel Volovitch[2,3], Ludovic Jullien[4], Shuo Lin[5], Shimon Weiss[1,6], David Bensimon[6,7]. 1) Department of Molecular, Cellular and Integrative Physiology, UCLA, USA; 2) Collège de France, Center for Interdisciplinary Research in Biology, France; 3) Department of Biology, ENS, France; 4) Department of Chemistry, ENS, France; 5) Department of Molecular, Cell and Developmental Biology, UCLA, USA; 6) Department of Chemistry and Biochemistry, UCLA, USA; 7) Laboratoire de Physique Statistique, ENS, France.

Although cancer initiation and evolution have been extensively studied, they are not, as of yet, fully understood. In particular, two outstanding questions, the effectiveness of oncogenic transformation and the role of the local microenvironment on cancer initiation, require the investigation into early events of cancer initiation and the study of the fates of individual cells and their progenies. Several models have attempted to answer how cancer arises from individual transformed cells. However, current probes of cancer development are restricted to the collective properties of many thousands of cells. Recently, we developed a technology that allows for the control of protein activity and gene expression in single cells through light activation. In this work, we utilize this method to activate in a small number of cells in a live zebrafish a typical oncogene, K-RasG12V, and investigate effects of these changes on tumorigenesis under varied genetic backgrounds. We successfully demonstrated the spatiotemporal control of oncogene expression in live zebrafish. Furthermore, we investigated different tumorigenic phenotypes by transiently or permanently activating K-Ras at varied developmental stages. We believe that our studies could shed new light on cancer initiation and growth and provide new tools for target validation and testing of novel anti-cancer drugs.

Z6003C Synergy between Loss of *NF1* and Overexpression of *MYCN* in Neuroblastoma Is Mediated by the GAP-related Domain. *Shuning He*[1], Marc R. Mansour[1,2], Mark W. Zimmerman[1], Dong Hyuk Ki[1], Hillary M. Layden[1], Koshi Akahane[1], Eric D. de Groh[3], Antonio R. Perez-Atayde[4], Shizhen Zhu[5], Jonathan A. Epstein[3], A. Thomas Look[1]. 1) Dana-Farber Cancer Institute, Boston, MA; 2) University College London, London, UK; 3) Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; 4) Children's Hospital Boston, Boston, MA; 5) Mayo Clinic, Rochester MN.

Earlier reports have shown that hyperplasia of sympathoadrenal cell precursors during embryogenesis in *Nf1*-deficient mice is independent of Nf1's role in down-modulating RAS-MAPK signaling. By contrast, loss of *nf1* leads to aberrant activation of RAS signaling in *MYCN*-induced neuroblastomas that arise in these precursors, and the activity of the GTPase-activating protein (GAP)-related domain (GRD) is sufficient to suppress the accelerated onset of neuroblastoma in *nf1*-deficient zebrafish. Thus, even though neuroblastoma is a classical "developmental tumor" of childhood, NF1 uses a very different mechanism to suppress malignant transformation than it does to modulate normal neural crest cell growth. We also show marked synergy in tumor cell killing between the MEK inhibitor trametinib and the retinoid isotretinoin *in vivo* in primary neuroblastomas. Thus, our model system has considerable translational potential for investigating new strategies to improve the treatment of very high risk neuroblastomas with aberrant RAS-MAPK activation.

Z6004A Somatic deficiency of DNA polymerase α causes tissue-specific nuclear atypia and apoptosis in Zebrafish. *A. Y. lin*, K. C. Cheng. Penn State Hershey College of Medicine, Hershey, PA.

Nuclear atypia is a morphological abnormality noted by histology that is common and diagnostically important in cancer diagnosis, but its mechanisms are not understood. A zebrafish genetic screen for mutations that cause nuclear atypia yielded a mutant, *huli hutu* (*hht*) with a

pleiotropic phenotype that included nuclear atypia in gastrointestinal cells, apoptotic nuclear fragmentation in neurons of the brain and eye, reduced head and eye size, and body curvature. The causative mutation was a frameshift in *pola2*, which encodes subunit B of DNA polymerase α (Pol α), that results in a premature stop codon at the 38th amino acid position of the 600 amino acid protein. Loss of function mutations of *pola2* in other model organisms such as *S. cerevisiae* and *Arabidopsis* are known to cause rapid growth arrest, delay in S phase progression, and cell division defects. In contrast, *hht* can live up to 7dpf as a result of wild-type maternal *pola2* mRNA detected in mutant eggs by allele-specific PCR. Subsequent somatic loss of wild-type mRNA results in atypical cellular morphology, abnormal mitoses, and tissue deformation in homozygous mutant larvae. This *hht* phenotype is strikingly similar to that observed in zebrafish with CRISPR/Cas9-mediated loss of function mutations in *pola2* and other DNA polymerase α subunits, *pola1, prim1, and prim2,* indicating an effect that is not subunit-specific, but rather due to deficiencies in DNA polymerase activity. In support of this idea, inhibition of DNA synthesis associated with aphidicolin or hydroxyurea treatment also yielded tissue-specific *hht*-like phenotypes. Prolonged survival and development allows *hht* to demonstrate tissue-specific changes in cellular and nuclear morphology, suggesting that different tissues may have different requirements for DNA polymerase α activity during development.

Z6005B PHF6 keeps hematopoietic lineage development in check. *S. Loontiens*[1], K. Durinck[1], I. van de Walle[2], E. Janssens[1], S. Vanhauwaert[1], F. Moore[3], P. Rondou[1], G. Dewyn[1], C. De Bock[4], J. Cools[4], D. Langenau[3], P. Van Vlierberghe[1], T. Taghon *[2], F. Speleman *[1], * shared last authorship. 1) Center for Medical Genetics, Ghent Univeristy, Gent, BE; 2) Departement of Clinical Chemistry, Microbiology and Immunology, Ghent University, Ghent, BE; 3) Harvard Stem Cell Institute, Boston, Massachusetts, USA; 4) Laboratory for the Molecular Biology of Leukemia, Center for Human Genetics, University of Leuven and Center for the Biology of Disease, Vlaams Instituut voor Biotechnologie, Leuven, BE.

T-cell acute lymphoblastic leukemia (T-ALL) is a genetically heterogeneous disease. The *PHF6* gene is frequently targeted by loss-of-function mutations or deletions, with the highest prevalence in *TLX1* or *TLX3* rearranged T-ALLs. We aim to gain insights into the role of PHF6 during normal thymocyte development and malignant transformation. To this end, transcriptome wide perturbation effects were measured following PHF6 knock down in the *PHF6* wild-type T-ALL cell lines Jurkat and ALL-SIL. In parallel, gene expression profiles were established from cord blood CD34^{+} progenitor T-cells, cultured for short-term on an OP9-DL1 feeder layer, with stable knockdown of PHF6. Modulation of *PHF6* expression induced robust and broad transcriptional regulatory effects of PHF6 including on *IL7R* expression. *IL7R* encodes a cytokine receptor critically involved in normal T-cell development and acts as a *bona fide* oncogene in subsets of T-ALLs. Using the *in vitro* differentiation assay for all lineages starting from CD34+ cells we also identified a broader role for PHF6 beyond thymocyte differentiation acting as a master regulator of early hematopoietic differentiation along the T-cell, B-cell, myeloid and NK-cell lineages (Durinck et al., in preparation). To further test this *in vivo*, we performed *phf6* gene inactivation by injection of gRNAs and Cas9 protein into the one-cell stage zebrafish embryos yielding varying out-of-frame in/dels. In addition, we used a TALEN based PHF6 knock out zebrafish line (Moore et al., Plos One, 2012).
Upon *phf6* knockdown accelerated T-cell maturation was seen in support of our *in vitro* data in human cells. Interestingly, transcriptional changes in the *phf6* knock out lines were noted for *lmo2* and *notch1*, both key players in zebrafish hematopoiesis. Using optimized of RT-qPCR and RNA sequencing on sorted lymphocytes we are currently evaluating the transcriptional effects of *phf6* down regulation during lymphocyte development. We will present findings on whole kidney marrow tissue for changes in hematopoietic population distribution in wild type versus *phf6* knock out zebrafish. In conclusion, we were able to show for the first time a conclusive role for PHF6 in hematopoiesis using *in vitro* and in vivo modeling in human and zebrafish respectively.

Z6006C Investigating Colorectal Cancer Metastasis to Liver in Zebrafish. *S. Mukhopadhyay*[1], M. Mesmar[1], J. Gustafsson[1,2], C. Williams[2], M. Bondesson[1]. 1) University of Houston, Houston, TX; 2) Karolinska Institutet, Solna, Sweden.

Chemokines are known to play a role in cancer. C-C chemokine Receptor type 6 (CCR6) and its sole ligand, CCL20 have been reported to be up-regulated in colorectal cancer (CRC) cells. We are studying the chemotaxis of CCR6-expressing CRC cells to CCL20, whose expression is highest in the liver. It has been suggested that it is due to this chemoattraction that 75% of the CRCs metastasize to the liver. Hence, disruption of the CCR6-CCL20 interaction is one strategy for inhibiting CRC liver metastasis. This hypothesis is being tested using a variety of *in vitro* assays and *in vivo* in zebrafish. We investigated CCR6 RNA and protein expression in different CRC cell lines, and selected the highest expressing cell lines for microinjection into zebrafish embryos, following tracking of the fluorescence-labeled migrating cells. When injected to the perivitelline space, SW480 and HT29 cells micro-metastasize to the vasculature. We are now injecting CRC cells into the zebrafish intestine following imaging of cancer cell migration to evaluate whether zebrafish xenografting is a viable model for studies of liver metastasis.

Z6007A aMOTIV microscopy: mechanical characterization of the *in vivo* tissue microenvironment, a step towards living mechanical histology. *K. Tanner*. National Cancer Institute, Bethesda, MD.

Microscale heterogeneities in tissue rheological properties such as stiffness and viscosity strongly influence cell fate and malignancy. However, outstanding questions about the timescales of interactions (measured as a range of frequencies), length scales and type of interactions sensed by cells within tissues that are physiologically relevant remain unanswered. What is needed is the ability to resolve and quantitate minute forces that cells sense in the local environment (on the order of microns) within thick tissue (~mm) and 3D culture models, that approximate clinically relevant *in vivo* architecture and signaling cues, allowing for real time characterization of cell-ECM dynamics. We performed active Microrheology using an Optical Trap In Vivo (aMOTIV) microscopy using an *in situ* calibration method to obtain exact trap stiffness at each probe to quantify local applied forces with high spatial and temporal resolutions. This allowed us to determine tissue mechanics at length scales (nm-mm) and frequencies (1-10,000's Hz) unobtainable by bulk rheology, which misses the cell-scale

heterogeneities, or with passive microrheology, which misses the interesting non-linear stress/strain curves seen with active probing then apply defined strains, Applicable to thick tissue, this technique allowed us to distinguish mechanical heterogeneities with micrometer spatial resolution at penetration depths up to 500 μm. After initial characterization of 3D cell culture gels, we applied our technique in zebrafish, Danio rerio; the first time in situ calibration and microrheology has been applied to a living model vertebrate organism. Our initial data indicates a broad range of elastic moduli, with measurements in the tail ranging ~ 10s to 1000s of Pascals, while the brain is significantly softer (10's to 100's Pa). We show here an advanced technique for micro-rheological characterization *in vitro* and *in vivo*, to accurately quantify physical determinants of the local microenvironment. This allows for accurate measurements mechanical properties in living organisms and in tissue models to definitively account for microenvironmental impact on individual cells and organogenesis.

Z6008B Studying the functionality of the homologous repair pathway in zebrafish embryos: heading for an in vivo functional test to evaluate the pathogenicity of BRCA2 variants identified in breast/ovarian cancer patients. *Jeroen Vierstraete*[1,2], Andy Willaert[1], Kris Vleminckx[1], Petra Vermassen[1], Paul Coucke[1], Leen Pieters[2], Anne Vral[2], Kathleen Claes[1]. 1) Center for Medical genetics Ghent, Ghent University Hospital, Belgium; 2) Department of Basic Medical Sciences, Ghent University, Belgium.

Aims:

Since the introduction of next generation sequencing, the challenge for genetic testing moved from developping mutation detection methodologies towards adequate variant interpretation. We propose a novel in vivo approach to study the functionality of BRCA2 missense variants in zebrafish. We aim to develop an in vivo functional assay to measure in zebrafish embryos the capacity of homologous recombination (HR) for human BRCA2 mRNA containing variants of unknown clinical significance (VUS).

Methods:

To evaluate the efficiency of HR repair we induce DNA double strand breaks (DSB) in zebrafish embryos by irradiation. We use yH2AX and RAD51 foci assays as markers for DSB and HR repair respectively. We generated zebrafish brca2 knockdown models by morpholino injection and Crispr-Cas9 mutagenesis, and obtained a mutant line from the European Zebrafish Resource Center (EZRC). Rescue experiments will be performed with wild type human BRCA2 mRNA and mRNA containing the VUS of interest.

Results:

We developed a protocol for visualising and quantifying RAD51 foci in zebrafish embryonic tissue. Brca2 knockdown by morpholino results in an almost complete absence of RAD51 foci. Similar results have been generated in the EZRC line, and are currently being generated in the Crispr-Cas9 knockout model. In a next step we will rescue the phenotype by microinjection of wild type human BRCA2 mRNA and mRNA containing VUS to study the effect of these VUS on HR capacity.

Conclusions:

The zebrafish genome contains nearly all genes involved in different DNA repair pathways in eukaryotes, including HR, in which BRCA2 plays a major role. Therefore, zebrafish provides an ideal in vivo model for studying variants in genes involved in DNA damage and repair.

Z6009C Probing cancer genomes using tissue-specific genome editing. *R. Vyas*, A. M. Venkatesan, M. Kasheta, C. J. Ceol. University of Massachusetts Medical School, Worcester, MA.

Site-directed nucleases have recently emerged as potent tools for gene disruptions in various model organisms, including disruptions of tumor suppressor genes. However, whole organism tumor suppressor gene disruption is time-consuming and often causes deleterious effects such as lethality. To mitigate these problems, we have developed somatic cell gene-targeting approaches. Our strategy is to target candidate tumor suppressor genes specifically in melanocytes. Using the miniCoopR system, in which transgene-expressing melanocytes are reconstituted in a *mitfa(lf)* background, we have expressed TALENS in melanocytes and validated targeted knockdown of *p53* in melanomas. In order to achieve greater targeting efficiency, we are using CRISPR-Cas system. With our improved genome-editing system we aim to target candidate tumor suppressors that are recurrently deleted in human and zebrafish melanomas. Additionally, we are assessing the function of these candidate tumor suppressors in human melanoma cell lines using down-regulation and over-expression studies and will present our progress. Combining the throughput afforded by genetics in zebrafish with functional in vitro studies has enabled our identification of novel melanoma oncogenes. We now extend that approach to tumor suppressor genes.

Z6010A Understanding the mechanistic roles of Integrin Alpha 6 in tumor development using humanized zebrafish model system. *Ashley B. Williams*, Shauntell N. Luke, Andy Diamanduros, Vinoth Sittaramane. Georgia Southern, Statesboro , GA.

Present day cancer incidence and mortality rates indicate the need for effective cancer diagnostic tools and targeted cancer therapeutic strategies. Recent studies have focused on the biological pathways of cells and tumor microenvironments to identify putative biomarkers and potential drug targets as diagnostic and therapeutic tools. Human integrins, transmembrane adhesion receptors, have become the focal points in these studies, specifically Integrin Alpha 6 (*ITGA6*) which has been implicated in major tumor progression roles. Several human tumors like pancreatic, prostate, breast and ovarian cancers exhibit increased levels of ITGA6 expression (Marthick JR et al., 2012; Lathia JD et al., 2010; Hirozumi S et al., 2006; Johnatty S et al., 2010). *ITGA6* has been implicated in tumor angiogenesis, metastasis and cancer stem cell maintenance (Primo L et al., 2010; Wang J et al., 2013; Carrion B et al., 2013). These characteristics make *ITGA6* an excellent candidate for potential drug or diagnostic target, however the mechanism by which *ITGA6* imparts tumor progression is unclear. Cell culture studies have indicated ITGA6 could be cleaved extracellularly to increase metastasis (King TE et al., 2008; Cress AE et al., 2014). But, zebrafish with organismal structures and vascular network, presents a complete *in vivo* model to track metastasis and angiogenesis. In the present study we aim to identify the specific role of *ITGA6* in tumor development by using a humanized zebrafish model, where Di-I labeled human prostate cancer cells (PC3) are

transplanted into *Tg*(*Fli1a:gfp*) zebrafish embryos that is overexpressed with various forms of human ITGA6. To test the domain specific role of *ITGA6*, Human full length or Truncated extracellular or Mutated non-cleavable ITGA6 RNA is injected to the zebrafish PCs tumor xenograft. Our studies indicate that truncated *ITGA6* overexpression significantly upregulate tumor metastasis compared to full-length *ITGA6* overexpression. Similarly, mutated *ITGA6* significantly decreased the tumor metastasis. The results suggest that cleaved *ITGA6* increases tumor metastasis potentially aiding in extracellular matrix remodeling. The cellular role of *ITGA6* will be evaluated by transplanting *ITGA6* siRNA transfected PC3 cells for zebrafish tumor xenografts. We anticipate these experiments will help establish the cell and non-cell autonomous roles of *ITGA6* during tumor development. Further, we expect to use high resolution imaging techniques to track the migration of single cancer cells an *in vivo* system to understand the dynamics of tumor metastasis.

Z6011B Identification of melanoma progenitor cells remaining after regression in zebrafish models. *S. Wojciechowska*[1], A. Capper[1], Z. Zeng[1], J. Lister[2], E. E. Patton[1]. 1) MRC Institute of Genetics and Molecular Medicine, MRC Human Genetics Unit & Edinburgh Cancer Research Centre, University of Edinburgh, Western General Hospital, Crewe Road South, EH4 2XR, UK; 2) Department of Human and Molecular Genetics and Massey Cancer Center, Virginia Commonwealth University, School of Medicine, Sanger Hall 11-014 1101 E. Marshall Street Richmond, VA 23298-0033, USA.

Melanoma is the most deadly form of skin cancer that kills over 20,000 Europeans each year and incidence continues to rise rapidly. $BRAF^{V600E}$ inhibitors have led to clinically significant improvements in outcomes for melanoma patients, yet many patients with metastatic melanoma rapidly succumb to the disease due to eventual chemoresistance, or insensitivity to the drug. Thus, it is critical to identify new therapies that can act alone, or be combined with available treatments for enhanced efficacy and/or to overcome drug resistance.

Evidence from human melanoma indicates that the melanocyte lineage is critical for melanoma survival and contributes to therapeutic resistance. MITF is a highly conserved "master melanocyte regulator" with a complex role in melanoma. Our lab has developed two temperature sensitive zebrafish melanoma models (earlier published $BRAF^{V600E}$ *mitf* and a new BRAF-independent model *p53 mitf*), both carrying the $mitf^{v7}$ splice site mutation that enables us to conditionally control its endogenous activity by adjusting the water temperature. We showed that the MITF activity is crucial for melanocyte survival and that both mutated BRAF and p53 deficiency are oncogenic with low levels of MITF, and result in fish nevi and melanoma resembling the pathology of human disease. Complete inhibition of MITF activity leads to rapid tumor regression, but once its activity is restored the melanomas recur at the same site as the original tumor. This suggests that a subpopulation of cancer initiating cells remains following melanoma regression and is capable of repopulating the tumor. We have been able to show that some cells remaining at the sites of regression express $BRAF^{V600E}$ and we are currently searching for other markers. Our goal is to identify the molecular signatures of these proposed melanoma stem cells and to develop approaches to visualize and target these subpopulations. We are using histopathology studies, molecular and lineage tracing imaging methods and melanocyte lineage transgenes in our genetic zebrafish models, as well as zebrafish melanoma cell culture to study the nature of the cell of origin for the tumor recurrence.

Z6012C Assessing the Lineage Fate of First Vs. Second Heart Field Derived Cells in Cardiac Development and Regeneration. *J. Choubey*[1,2], I. Scott[1,2,3]. 1) University of Toronto, Toronto, Ontario, CA; 2) The Hospital for Sick Children, Toronto, Ontario, CA; 3) Heart & Stroke/ Richard Lewar Center of Excellence, Toronto, Ontario, CA.

Surgical intervention for patients with some forms of congenital heart diseases (CHD) has increased patient survival to adulthood; however post surgical stress contributes to a high incidence of heart failure, with the right ventricle being more susceptible to ventricular failure. Developing novel therapies for CHDs requires an understanding of the underlying biological mechanisms driving cardiac progenitor cell specification and differentiation. Two distinct progenitor populations, termed the first heart field (FHF) and the second heart field (SHF), have been shown to drive cardiac development . Whereas the FHF contributes to the left ventricle (LV) , the SHF derived cells contribute to the right ventricle (RV), inflow and outflow tracts in the mammalian heart. Unsurprisingly, SHF defects are a major contributor of CHDs. Recently, second heart field progenitors in zebrafish have been shown to give rise to three cardiovascular lineages in the OFT and myocardium in the distal ventricle; thus displaying a multipotent characteristic.

My project focuses on studying the underlying biological mechanisms and the intrinsic competence of cardiac progenitor cells to differentiate into multiple lineages within the heart. There are currently no studies that have assessed the lineage specification of FHF and SHF progenitors in the zebrafish adult heart. This will help us to understand how the two distinct progenitor populations give rise to the adult heart and its structures; thus allowing us to model CHDs in zebrafish. I'm employing a Cre recombinase-mediated lineage-tracing of cells expressing *cmlc2* in order to assess the lineage specification of FHF and SHF progenitors in the zebrafish heart. The *cmlc2* gene product is found in the sarcomere of cardiomyocytes undergoing differentiation. I am using a tamoxifen-inducible Cre driven by *cmlc2* regulatory sequences, Tg(*cmlc2*:ERCreER).This line, when crossed with Tg(*cmlc2*:loxP-GFP-STOP-loxP-mCherry) will allow visualization of cardiomyocytes with mCherry fluorescence after tamoxifen induced excision of loxp-flanked STOP sequences . The SHF population begins differentiation later, and adds onto the linear heart tube gradually; expressing the differentiation marker later. Thus, administering tamoxifen to the double transgenic embryos prior to SHF differentiation will lead to the labelling of FHF specific cells with mCherry fluorescence. The late differentiating progenitor population will not undergo excision of the loxp-flanked STOP sequences, and will be marked with GFP fluorescence.

Z6013A Sensory cilia functions in zebrafish. *Judith Bergboer*[1], Cameron Wyatt[2], Christina Austin-Tse[1], Jonathan Raper[3], Emre Yaksi[4], Iain Drummond[1]. 1) MGH/HMS, Charlestown, MA; 2) Neuroelectronics Research Flanders, Leuven, Belgium; 3) University of Pennsylvania, Philadelphia, PA; 4) Kavli Institute for Systems Neuroscience, Trodheim, Norway.

Primary cilia mediate signal transduction by acting as an organizing scaffold for cell membrane receptors, signaling proteins, and ion channels.

Ciliated olfactory sensory neurons (OSNs) organize olfactory G-protein coupled receptors and ion channels on dendritic cilia and generate a cAMP-coupled influx of calcium ions as a primary signal in odorant detection. In the zebrafish olfactory placode, ciliated OSNs and microvillus OSNs constitute the major OSN cell types with reported distinct odorant sensitivity. Here we report single cell analysis of transgenic zebrafish expressing the calcium indicator GCaMP5 in OSNs to directly image cilia-dependent OSN responses to odorants in live zebrafish larvae. *Oval/ift88* mutant (*ift88 -/-*) and *ift172* knock-down zebrafish showed fewer and severely shortened OSN cilia without a reduction in total OSN number. OSN response amplitude to bile acids, and food odor were significantly reduced in *ift88* mutants and *ift172*-deficient embryos while the responses to amino acids were not significantly changed. We have also generated CRISPR/Cas9 mutants of the syndromic ciliopathy genes Cep290 and BBS4 which show delayed defects specifically in sensory cilia structures. Combining ciliopathy gene mutations with biosensor transgenes provides a quantitative model for studying sensory ciliogenesis in zebrafish and a platform for therapeutic screening. Our results also implicate *ift172*-deficiency as a novel cause of anosmia.

Z6014B Positional cues within the nucleus underlie the dynamic chromosome events of meiosis in zebrafish. *S. M. Burgess*, Y. P. Blokhina, A. Nguyen, D. B. Chu, H. Roberts, T. A. Newman, B. W. Draper. Univ California, Davis, Davis, CA.

Chromosome aneuploidy is a leading cause of birth defects and miscarriages in humans. The majority of aneuploidies are generated by errors in segregating homologous chromosomes at the first meiotic division. The incidence of chromosome segregation errors is higher in female mammals than males. Nonetheless, meiosis is largely understudied in females since the events of meiotic prophase I occur in the fetal ovary. The zebrafish model overcomes many of the limitations of using mice because gametogenesis occurs throughout adulthood in both sexes, the progeny number in the hundreds and embryos develop outside of the body. We will describe a comprehensive toolkit we have developed to study the chromosome events of meiotic prophase in zebrafish at the molecular, genetic and cellular levels. We found that both male and female zebrafish follows the canonical pathway of meiotic progression seen for fungi, plants, and mammals; that is, SPO11-induced double-strand breaks (DSB) are required for pairing and synapsis of homologous chromosomes. As seen for human males, synapsis (SYCP1 loading) initiates near telomeres and zippers inward until chromosomes are fully synapsed at pachytene. In zebrafish, the extension of the chromosome axis (SYCP3) also initiates near telomeres and extends just ahead synapsis. There is a dramatic localization of DSBs (RAD51 foci) to the sites of clustered telomeres in the bouquet stage. Synapsed telomere regions are not always associated with RAD51 foci and many RAD51 foci are not associated with telomeres. These data suggest that multiple chromosomal loci undergoing DSB repair may coalesce to a single region of the nucleus during the earliest stages of pairing and synapsis and bring into question the mechanism of pairing. Further analysis of the *spo11* mutant shows that females are fertile, however, embryos are largely deformed due to high levels of aneuploidy; by contrast, *spo11* males fail to make sperm. While the meiotic program occurs until chromosome axes have formed, synapsis is absent. Together these findings point to zebrafish as an excellent model to study the dynamic events of meiotic prophase. Similarities and differences between multiple model organisms point to a conserved relationship between telomere clustering, DSB repair and synapsis initiation as contributing to the successful execution of the meiotic program.

Z6015C *marsyas:* a zebrafish mutant in GBF1 showing defects in epithelial integrity. *Thomas A. Hawkins*[1], Liana Goodings[1], Patrick Toolan-Kerr[1], Richard J. Poole[1], Rodrigo M. Young[1], Heather L. Stickney[1], Florencia Cavodeassi[2], Quenten P. Schwartz[3], Stephen W. Wilson[1]. 1) Div. Biosciences, UCL, London, UK; 2) Centro de Biologia Molecular Severo Ochoa, Universidad Autonoma de Madrid, Madrid, Spain; 3) Center for Cancer Biology, University of South Australia, Adelaide, Australia.

The zebrafish mutant *marsyas* (*mrys)* was identified through a screen for skin defects using the vital dye R18 at 3dpf. *mrys* mutants display skin defects from 36hpf with the outer layer of skin cells sloughing off from the basal layer and ineffective formation of all fins. *mrys* mutants also have defective blood vessel formation, displaying bleeds across their circulatory system concurrent with defects in the skin. Apico-basal polarity of epidermal cells appears unaffected.

Initial bulked-segregant mapping indicated a lesion on chromosome 13 and subsequent whole genome sequencing identified a premature stop codon in GBF1 (golgi brefeldin-A resistance factor 1). This lesion was confirmed by high-resolution mapping. We are currently attempting phenocopy and rescue of the phenotype to confirm the mutation as causative.

GBF1 is an ArfGEF implicated in the entry of vesicles to the *cis*-golgi, feeding primarily the exocytosis pathway of vesicle trafficking although it is also implicated in endocytosis by some studies. The drosophila GBF1 mutant *gartenzwerg* has defects in tracheal and other tubular structure formation, suggesting a comparable phenotype.

We are currently carrying out further phenotypic analysis, including cell-autonomy tests, electron microscopy and GFP tagging of golgi and other organelles to closely examine the phenotype. Our primary question concerns whether the phenotypes we observe originate from a failure of the vesicular delivery of protein(s) important for epithelial integrity or whether the phenotype is caused by defective balance between membrane compartments of epithelial cells.

Z6016A Differential Lectin Binding and Coronary Angiography in Zebrafish and Giant danio. *O. Shifatu*, A. May, J. Quinn, D. Lafontant, W. He, P. Lafontant. DePauw University, Greencastle, IN.

Lectins are carbohydrate-binding proteins commonly used in the study of glycoprotein expression in mammalian hearts. However lectins have received little use in fish models and non-models species. We hypothesize that lectin binding may reveal specific and differential patterns of glycoprotein expression in fish hearts. Here, we determine the binding patterns of commonly used lectins, including wheat germ agglutinin (WGA), Ulex europaeus agglutinin (UEA lectin), Bandeiraea simplicifolia lectin (BS lectin), Concanavalin A (Con A), Ricinus communis (RCA), and Lycopersicon esculentum lectin (tomato lectin) in zebrafish and giant danio hearts. Our results show that WGA stained fish cardiac myocyte

borders in patterns similar to that seen in mammalian hearts, with staining markedly stronger in the fish compact hearts. Con A showed broad and strong staining in fish ventricles. Interestingly, BS lectin reacted poorly to a number of fish species including zebrafish, pearl danio, common koi, goldfish, and Buenos Aires tetra. By contrast BS lectin strongly stained coronary vessels of giant danio, and several pleco and gourami species. Importantly, enhanced BS lectin biding to vascular endothelium compared to endocardium allowed for 3D coronary angiography in the giant danio, and for quantitation of vascular reconstruction during regeneration. Differential lectin reactivity was further observed in developing zebrafish hearts. In conclusion, our study demonstrates that lectins are simple but important tools for studies in model fish species, and in adult and regenerating giant danio heart.

Z6017B Type-Specific Cells Differentiate into Neurons in Spinal Cord of Zebrafish Embryos after Hypoxic Stress or Injury. *Chih-Wei Zeng*[1], Yasuhiro Kamei[2], Yun Yu[1], Chih-Tien Wang[1], Huai-Jen Tsai[3]. 1) Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan; 2) National Institute for Basic Biology, Okazaki, Japan; 3) Institute of Biomedical Science, Mackay Medical College, New Taipei City, Taiwan.

Neuron stem/progenitor cells (NSPCs) of zebrafish central nervous system (CNS) are known to thrive during recovery after hypoxia, but not all cells have been fully characterized. To address this, zebrafish transgenic *huORFZ* embryos were exposed to hypoxia, followed by oxygen recovery (OR), and GFP was exclusively expressed in some CNS cells. Among these GFP-expressing cells, most were NSPCs and radial glia cells (RGs), along with a few oligodendrocyte progenitor cells (OLPs) and oligodendrocytes (OLs), all termed as hypoxia-responsive recovering cells (HrRCs). After hypoxia, HrRCs did not undergo apoptosis, but GFP(-) neurons did. Among HrRCs, only GFP(+)-NSPCs and GFP(+)-RGs proliferated, migrated, and differentiated into functional neurons during OR. When some HrRCs were ablated in the spinal cord of hypoxia-exposed *huORFZ* embryos, swimming performance was impaired, suggesting that HrRCs are involved in neuronal regeneration. In *huORFZ* embryos treated with spinal cord injury, GFP-expressing cells displayed characteristics similar to HrRCs. These results imply that type-specific cell populations in zebrafish spinal cord can respond sensitively to hypoxia and play a role in neural regeneration.

Z6018C The neurosteroids alfaxalone and allopregnanolone protect larval zebrafish against PTZ-induced deficits. *P. R. Lundegaard*[1], T. Dyhring[2]. 1) University of Copenhagen, Copenhagen, DK; 2) Saniona A/S, Ballerup, DK.

The zebrafish has emerged as a vertebrate model system amenable to study human disease states. Zebrafish models of human neurobiological disorders offer the opportunity to identify novel therapeutic treatments through phenotype-based chemical modifier screens and larval zebrafish have previously been suggested as an experimental model of epilepsy-related pathogenic states.
Using alternating light and dark conditions, combined with a sub-convulsive concentration of pentylenetetrazole (PTZ) we show that the most pronounced effects were obtained with the neurosteroids alfaxalone and allopregnanolone. In addition, neurosteroid and benzodiazepine drug combinations were found to significantly improve phenotypic rescue in larval zebrafish, as reflected by a reduction in the minimal effective drug concentrations needed to reverse locomotion in the alternating light/dark assay.

Z6019A Optogenetic Control of Cell Ablation for Regeneration Studies of Spinal Cord Injuries. *K. Mruk*, J. Chen. Stanford University, Stanford, CA.

Spinal cord injuries (SCIs) affect multiple cell types. After the initial injury, additional tissue loss occurs by a self-propagating cascade of cellular and biochemical mechanisms that exacerbate the SCI. Animal models capable of regeneration are likely our best resource for understanding and ultimately treating SCIs. The zebrafish central nervous system (CNS) shares many organizational, cellular and molecular pathways with humans, yet it is capable of functional regeneration even after complete spinal cord transection. Zebrafish models of SCI are unique, valuable tools for studying spinal cord regeneration, particularly because the optical transparency of larvae permits real time observation of the CNS and optogenetic manipulations. To better understand how the CNS responds to SCI, we are developing optogenetic approaches to selectively ablate cells in the zebrafish spinal cord and monitor regeneration in real time. Applications of our optogenetic technologies and characterization of the SCIs they cause in zebrafish larvae will be discussed.

Z6020B Discovery of novel psychotrophic agents using zebrafish larval behavioral assays. *Ashley B. Williams*, Danielle Lott, Abid Shaikh, Vinoth Sittaramane. Georgia Southern, Statesboro, GA.

Psychotropic agents are a loosely defined group of drugs which affect consciousness through pharmacological action on the nervous system. Current psychotropic agents act as anxiolytics, antidepressants, hallucinogens and anesthetics. These compounds may reduce anxiety, relieve pain, and compensate for mental irregularities, but are also responsible for serious complications, including paralysis and death. The discovery of novel psychotropic agents which exhibit high potency and low toxicity remain in high demand. The present study aims to identify the psychotropic potential of novel organic compounds such as quinolone derived trifluoromethyl alcohols and 4-Methylcyclohexanemethanol (MCHM). The behavioral screening of MCHM and quinolone compounds revealed specific defects in motor activity of zebrafish larvae (5 dpf). Our data show that zebrafish larvae treated with MCHM and quinolone derivatives exhibit a reduction in motor activity in lower concentration (1-10uM) exposure. However, at higher concentrations (25uM) it results in loss of balance and failure to respond to touch. The reduction in motor activity is reversible and specific without any toxicity. Simple pharmacokinetic studies reveal that the effects were very rapid, sustained for the entire duration of exposure and reversible up to 72 hours without any toxicity. Further studies with ionic indicators such as CoroNa Green (labels Na^+) and DiH-MEQ (labels Cl^-) on larvae indicate that these compounds specifically block sodium channels on the mitochondria rich cells at 72 hpf. Therefore, our studies have identified some specific and novel organic compounds with profound and specific

pharmacological activity. We are currently characterizing other neuroactivity mechanisms of these compounds such as their ability to modulate other ionic transport channels.

Z6021C Vanderbilt Phenotypic Discovery Resource:Screening, Discovery, Crowdsourcing. *C. H. Williams*[1], C. C. Hong[1,2]. 1) Vanderbilt University, Nashville, TN; 2) Vanderbilt Univ. Med. Ctr., Nashville, TN.

Phenotypic changes are the ultimate cause of disease, whether the molecular pathology is multigenic, congenital, acquired or multifactorial. The result is that there are likely multiple modes to any given "treatment". Viability is the first phenotype observed in any organism, and arises from a complex interplay of signaling molecules across multiple cell and tissue types. Defects in these functional units form the basis of the phenotypic changes that result in disease. Phenotypic screening in zebrafish presents numerous advantages, including low cost, genetic tractability, aqueous drug delivery, and the biological in vivo features that preselect for favorable properties such as selectivity, target engagement and bioavailability. Here we describe a resource of compounds being screened that modulate critical nodes during embryonic development. These compounds will be associated into a chemotype-phenotype database that is publically available in an effort to crowd source discovery through the various stages of early drug development.

Z6022A Melanophore-iridophore interactions during adult pigment pattern maintenance in zebrafish. *E. J. Bain*, L. M. Saunders, D. M. Parichy. University of Washington, Seattle, WA.

Boundary formation and maintenance are critical aspects of multicellular development. These processes have been well studied in the embryo; however, boundaries must be maintained beyond embryonic stages into adulthood. The pigment pattern of adult zebrafish offers a tractable system in which to study post-embryonic boundaries. It consists of four to five dark stripes of black melanophores separated by lighter interstripes of yellow xanthophores and iridescent iridophores. Positive and negative interactions between pigment cell types are required to initiate and reiterate this pattern, but homeostatic roles of these interactions are not well understood. While iridophores help position and terminate melanophore stripes, melanophores prevent iridophore expansion into the stripe region. Using a temperature-sensitive allele, we experimentally manipulated melanophore numbers during boundary maintenance to query effects on iridophore patterning. We find that iridophores accumulate ectopically in stripe regions when melanophores die, and disperse when melanophores differentiate. These observations suggest that melanophores prevent iridophores from trespassing into stripe regions and signal their presence via some molecular cue. We have identified candidate genes for such signaling by RNA-Seq, and we are using conditional CRISPR/Cas9 mutagenesis to test roles for these candidates during stripe maintenance. These data will further our understanding of the molecular bases for post-embryonic boundary maintenance which can then be used to explore how defects in these mechanisms lead to boundary failure in the context of human disease.

Z6023B Using zebrafish to probe how Cdx transcription factors specify the posterior spinal cord. *Alana V. Beadell*, Robert K. Ho. University of Chicago, Chicago, IL.

Vertebrate *cdx* genes are members of the parahox gene cluster and are homologs of *Drosophila* Caudal. They are expressed in the posterior tissues of every major group of bilaterians, where they control growth and development of the posterior embryo. In zebrafish, and likely all vertebrates, Cdx homologs have acquired an additional role, namely, specification of the spinal cord: *cdx* genes are among the earliest expressed in the spinal cord territory during development and are necessary and sufficient for spinal cord fate within the zebrafish posterior neural plate. Additionally, unlike the brain and anterior spinal cord, cells of the posterior spinal cord are not specified by the end of gastrulation and instead must be continually generated and patterned from precursor cells within the tailbud as the embryo elongates. Work from our lab and others indicates that Cdx plays a key role in specifying/committing posterior cells to the posterior spinal cord fate, as well as maintaining the posterior growth zone itself from which the caudal tissues of the embryo emerge.

In this work, we ask: how does zebrafish Cdx4 specify/commit posterior cells to the spinal cord fate? Currently, we do not know at what step or steps, from precursor specification to neural differentiation, Cdx acts in this process. To understand the role of Cdx in posterior neural cell fate, we developed a scalable loss-of-function assay that takes advantage of the CRISPR/Cas9 system to generate F0, genetically mosaic loss-of-function zebrafish embryos. We use this assay, a powerful and efficient alternative to morpholino knockdown and akin to RNAi-based screens in other systems, to query a candidate gene list of Cdx4 direct and indirect effectors compiled from multiple high-throughput experiments. We have confirmed several candidates as expressed in the spinal cord, responsive to Cdx4 levels, and whose loss-of-function causes ectopic expression of hindbrain marker genes within the spinal cord territory as loss of Cdx4 does. Some of these candidates, like the helix-loop-helix protein Id1, are known regulators of neural differentiation; others, like the metalloproteinase Adamts18, are poorly characterized. In particular, we have found that mosaic knockdown of multiple components of the BMP signaling pathway also produce ectopic marker gene expression in our assay.

Creating mutant lines for some of these Cdx4 effector loci, we are further characterizing their role in posterior neural development. This work should shed light on the generation of posterior neural tissue from the multipotent stem cell-like niche within the vertebrate tailbud.

Z6024C A transcriptomics analysis of *tbx5a* and *tbx5b* during early fin and heart development. *Erin Boyle Anderson*, Robert Ho. University of Chicago, Chicago, IL.

Holt-Oram syndrome is a human condition that occurs in 1 in 100,000 births resulting in developmental defects in both heart and hand/arm. It is a result of haploinsufficiency due to a mutated copy of *TBX5*. Unlike humans, zebrafish have two copies of *tbx5*: *tbx5a* and *tbx5b*. Both Tbx5a and Tbx5b-deficient embryos display defects in heart and pectoral fin development. Although the role of Tbx5a in zebrafish fin development and the role of Tbx5 in heart and limb development in other animals has been well studied, much less is known about the roles

Tbx5b plays during zebrafish development.

From expression data and morpholino data, it appears that Tbx5a and Tbx5b have overlapping but distinct roles in the development of the fin and heart. The role of Tbx5b in the lateral plate mesoderm was investigated and compared to the previously characterized functions of Tbx5a. RNA-sequencing identified targets of Tbx5a and Tbx5b and allowed for an unbiased comparison and approach towards identifying differences between Tbx5a and Tbx5b function at multiple time points during lateral plate mesoderm migration. These transcriptomics methods reveal similarities and differences between Tbx5a and Tbx5b function that may be reflected in their transcriptional networks.

Z6025A Vegf signaling promotes vasculogenesis and arterial specification by upregulating Etv2 / Etsrp expression. *D. Satish Casie Chetty*[1,2], Megan S. Rost[1,2], Jacob Enriquez[1], Jennifer A. Schumacher[1], Kristina Baltrunaite[1], Andrea Rossi[3], Didier Y. R. Stainier[3], Saulius Sumanas[1]. 1) Cincinnati Children's Hospital, Cincinnati, OH; 2) University of Cincinnati College of Medicine, Cincinnati, OH; 3) Max Planck Institute for Heart and Lung Research, Bad Nauheim Germany.

Vasculogenesis involves the differentiation of vascular endothelial progenitors de novo from undifferentiated mesoderm, their migration and coalescence to form the major embryonic vessels and the acquisition of arterial or venous identity. Vascular Endothelial Growth Factor (Vegf) signaling has been implicated in regulating arteriovenous specification and overall vascular endothelial differentiation during embryonic vasculogenesis. However, it is not known how Vegf signaling results in different functional outcomes. Here we examined the role and mechanisms of Vegf signaling during vascular endothelial differentiation and arteriovenous specification in zebrafish embryos. Chemical inhibition of Vegf receptor (VegfR) signaling resulted in a loss of arterial specification and downregulation of overall vascular endothelial marker expression. Conversely, Vegfaa overexpression resulted in the expansion of arterial markers and increase in the overall vascular endothelial differentiation. Our results further suggest that Vegf signaling affects arterial differentiation by modulating the expression of the ETS transcription factor Etv2/Etsrp. *Etv2* expression was downregulated in VegfR inhibited embryos, while endothelial specific Etv2 overexpression was sufficient to rescue arterial marker expression in Vegf inhibited embryos. *vegfaa* genetic mutants, similar to VegfR inhibited embryos, displayed absent arterial differentiation and reduced numbers of vascular endothelial cells. These results argue that Vegf signaling plays two distinct roles during vasculogenesis, by promoting arterial specification and proliferation of vascular endothelial cells, and Vegf promotes arterial specification by upregulating Etv2 expression.

Z6026B Roles of RyR-mediated intracellular calcium mobilization in muscle development and function. *A. A. Chagovetz*, E. Ritchie, D. Gunther, M. Jurynec, D. Grunwald. University of Utah, Salt Lake City, UT.

The Ryanodine Receptor (RyR) intracellular calcium release channels (CRC) regulate release of calcium from intracellular stores in the ER/SR. Though expressed in many cell types, they are best known for their roles in muscle contraction; indeed loss-of-function mutations are associated with myopathy in humans. We propose this narrow view of the RyR function fails to account for the full range of phenotypes observed in humans carrying RYR1 mutations, which include slow muscle cell defects. Here we elucidate unexpected functions of RyR in the zebrafish embryo. Our lab has previously demonstrated multiple Hedgehog-dependent cell specification events, including generation of somite muscle cells and dorsal root ganglia, require RyR-mediated calcium mobilization. We report the results of our efforts to identify the specific combinations of *ryr* genes that support these developmental functions. We have i) reinvestigated the expression of *ryr* genes, ii) generated null *ryr* alleles, and iii) characterized phenotypes for single and compound mutants using a combination of histology, live imaging and behavioral assessments.

Z6027C Zebrafish *GCaMP6s* transgenic lines for imaging calcium activities *in vivo*. *J. Chen*, J. Li-Villarreal, L. Solnica-Krezel. Washington University School of Medicine, St. Louis, MO.

Intracellular calcium signaling plays important roles to regulate cell activity during embryogenesis and in adult organisms. In this study, we generate stable transgenic lines, *Tg[ßactin2:GCaMP6s]*^{stl351}to leverage the ultra-sensitive calcium indicator GCaMP6s together with the transparent characteristics of zebrafish embryos**,** for improved *in vivo* calcium imaging during early embryogenesis. During the cleavage stages, calcium signaling is associated with the cleavage furrow progression in zebrafish. We recently discovered that embryos lacking maternal and zygotic function of atypical cadherin *dachsous1b* (MZ*dchs1b)* display abnormal cleavages (Li-Villarreal et al., Development, 2015). Using the *Tg[bactin:GCaMP6s]*, we observed that MZ*dchs1b* embryos displayed various furrow positioning, propagation, and deepening defects at cleavage stages.

During the blastula stages, we detected higher frequency of calcium transients in the enveloping layer (EVL) cells, and a longer dorsal-biased calcium signaling window (3-5hpf) in the WT blastomeres compared with earlier studies. We further report the direct visualization of calcium signaling in the gastrula dorsal forerunner cells (DFCs), which are formed by ingression of dorsal surface epithelium cells at the margin in a Nodal signaling dependent manner. Interestingly, we found that excess Nodal signaling not only leads to the increased number of calcium transients, but also an increase of calcium transient duration specifically in the DFCs. Current studies are addressing whether embryos dorsalized by inhibition of BMP activity show altered pattern of calcium signaling in the EVL. We anticipate that the *GCaMP6s* transgenic lines described here offer a valuable addition to the zebrafish toolbox that will enable to visualize many more dynamic calcium events at embryonic and larval stages as well as in adult animals.

Z6028A Zebrafish *dyrk1aa*, an orthologue of human Down syndrome gene *DYRK1A*, plays a role in cerebrovascular development. *Hyun-Ju Cho*[1,2*], Se-yeol Yang[2], Kweon Yu[1,2,3], Jeong-Soo Lee[1,2,3]. 1) Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, South Korea; 2) Korea University of Science and Technology (UST), Daejeon, South Korea; 3) Korea Institute of Science and Technology (KIST), Seoul,

South Korea.

Human *DYRK1A* is one of the crucial genes within the Down's syndrome critical region of human chromosome 21. DYRK1A has been implicated in neuronal development and neurodegenerative disorders, but its roles in the brain vascular development is not clear. Here we generated a zebrafish knockout mutant for *dyrk1aa* gene (*dyrk1aa*^{kbr1}), one of the two zebrafish orthologues of mammalian *DYRK1A,* using the TAL endonuclease technique. Approximately 30% of the length and branching points of the central arteries (CtAs) situated in the hindbrain were reduced in *dyrk1aa*^{kbr1} at 52hpf compared to the wild type (wt) control. Consistently, similar vascular defects were observed in a dose-dependent manner upon the treatment of harmine, a well-characterized DYRK1A inhibitor, while forced expression of *dyrk1aa* mRNA rescued CtA defects of *dyrk1aa*^{kbr1}. Overexpression of *dyrk1aa* mRNA in the wt background led to the increased length and branching points of CtAs, corroborating the role of *dyrk1aa* in promoting vascular formation in the developing brain. Currently we investigate the involvement of signaling pathways important for the *dyrk1aa*-regulated cerebrovascular development by examining expression patterns of candidate target genes and exploiting reporter transgenic lines. This study will reveal underappreciated roles of *dyrk1aa* in the vascular formation during cerebral development, and shed light on the vascular pathology in DYRK1A-related diseases including Down syndrome and Alzheimer's disease. .

Z6029B A morphogenetic role for FGF signaling in zebrafish cardiac looping and ballooning. *B. Christophers*, M. Grant, R. Burdine. Princeton University, Princeton, NJ.

Three of the most common forms of human congenital heart defects result from aberrant asymmetric cardiac morphogenesis. Using zebrafish as a model, we aim to better understand the links between cellular aberrations during heart morphogenesis and the resulting cardiac defects. To that end, we have investigated the role of Fibroblast Growth Factor (FGF) signaling, which is known to couple morphogenesis to cell migration events in zebrafish, in asymmetric heart development. We have uncovered a role for FGF signaling in dextral looping that is independent of its early role in establishing left-right asymmetry. We find that treatment of embryos with the FGFR inhibitor SU5402 during different time windows results in defective cardiac looping and ballooning. Treatment from 24-30hpf affects both the position of the heart chambers as well as their morphology; however, treatment from 30-36hpf affects chamber morphology without significantly impacting chamber positioning. We thus propose that FGF signaling plays two different roles during late cardiac development: the promotion of dextral looping and of chamber expansion. We will present data that define the role of the pathway during event. .

Z6030C Maturation of Photoreceptor Cells during Zebrafish Retinal Development. *Cátia Crespo*[1], Satu Kujawski[1], Daniele Soroldoni[2], Elisabeth Knust[1]. 1) Max Planck Institute-CBG, Dresden, Dresden, DE; 2) Department of Cell and Developmental Biology, UCL, London, UK.

Photoreceptor cells (PRCs) are a highly specialized type of neurons present in the retina, which are responsible for phototransduction. Vertebrate PRCs can be divided into rods, specialized for night vision, and cones, responsible for daylight and colour vision. In zebrafish, there are 4 different types of cones: green, red, blue and UV. Mature PRCs are polarized cells, with the apical surface subdivided into the outer and the inner segment and they arise from columnar epithelial progenitor cells.

PRCs have a highly specialized structure, which is essential for their function. Several studies have focused on the role of different proteins in PRC development and phototransduction. However, a detailed characterization of the morphological changes from the precursor stage to its functional form is lacking.

In this study, we aim to characterize the maturation of the apical surface of PRCs in zebrafish. We are performing a time course of the formation of the inner and outer segments and the connecting cilium using protein markers expressed in specific regions. We are also measuring cell volumes and cell lengths every four hours from the appearance of the PRC precursors to the matured stages using reporter lines specific for the *red* and UV sensitive *PRCs*.

We used Crb2a and Crb2b for labeling the inner segment. We found that in PRC precursors Crb2a localizes in the apical membrane of the cells, changing it's location to the sub-apical region of the incipient inner segment during the maturation process. Crb2b is also present in the sub-apical region of PRCs but it only starts being expressed when the inner segment is forming.

With this detailed characterization we aim to have a better understanding of the maturation process of the apical surface of PRCs. As a next step, we intend to use these methods to investigate the role of candidate proteins in PRC maturation.

Z6031A Regulation of canonical Wnt signaling activity by zebrafish Nup62l. *Zongbin Cui*, Xiaojie Yang. Institute of Hydrobiology, CAS, Wuhan, Hubei, CN.

Canonical Wnt signaling is one of intracellular signaling pathways that play crucial roles in vertebrate embryonic development, cell fate determination and maintenance of adult tissue functions. Activation of this pathway is closely associated with cytoplasmic degradation and nuclear accumulation of beta-catenin. Once transported into the nucleus, beta-catenin forms a complex with transcription factor LEF/TCF to drive the transcription of target genes. Currently, many proteins within the membrane, the cytoplasm and the nucleus are known to affect the intracellular localization of beta-catenin and thus control the activity of canonical Wnt signaling.

Nucleoporin p62 (Nup62)localizes in the central channel of nuclear pore complexes (NPCs) and regulates nuclear pore permeability and nucleocytoplasmic transport. Zebrafish Nup62-like protein (Nup62l) is a homolog of mammalian Nup62. The *nup62l* gene is maternally expressed, but its transcripts are ubiquitously distributed during early embryogenesis and enriched in the head, pharynx, and intestine of developing embryos. Activation of the Wnt/beta-catenin pathway positively modulates *nup62l* transcription, while Bmp signaling acts downstream of Wnt/beta-catenin signaling to negatively regulate *nup62l* expression. Overexpression of *nup62l* dorsalized embryos and enhanced gastrula convergence and extension (CE) movements. In contrast, knockdown of Nup62l led to ventralized embryos, an impediment

to CE movements, and defects in specification of midline organ progenitors. Mechanistically, Nup62l acts as an activator of Wnt/beta-catenin signaling through interaction with and facilitation of nuclear import of beta-catenin-1/2 in zebrafish. Thus, Nup62l regulates dorsoventral patterning, gastrula CE movements, and proper specification of midline organ precursors through mediating the nuclear import of beta-catenins in zebrafish.

Obviously, in-depth studies of mechanisms underlying the regulation of Wnt/beta-catenin signaling activity would reveal the asymmetric and tissue-specific activation of canonical Wnt signaling during early development in zebrafish and the activity of intracellular beta-catenin in pathological contexts.

Z6032B Opto-CRISPR : a new tool for genome editing at the single cell level. *B. Ducos*, W. Zhang, D. Bensimon. LPS-ENS, CNRS, Paris, FR.

The CRISPR-Cas9 system has recently emerged as an essential tool for genome editing, acting like genetic scissors to precisely abrogate or restore gene function, or transcribe specific targets. An important issue in deciphering gene regulation in time and space is to develop strategies for conditional gene expression. Usually this approach is based on transgenic lines where the expression of the genes of interest is under the control of tissue-specific promoters that can be switched on or off using drugs or heat. While much qualitative information on genetic networks have been obtained by that method, the lack of precise control of the concentration, location and timing of the gene products impair a quantitative analysis of the physiological response to such perturbations.

To overcome this gaps our lab has developed an optical method combining the use of conditional protein release via a protein-ERT fusion and a caged ligand, namely caged-cyclofen. Upon illumination with UV light (at 370nm) or with a two-photon laser beam, active cyclofen is released in the illuminated cell(s). It binds to the hormone binding domain of a truncated estrogen receptor (ERT) fused to the protein of interest which it releases from the complex it forms with cytoplasmic chaperones. If the protein of interest is a Gal4 transcription factor, it then diffuses to the nucleus, binds to its UAS promoter and turns on the gene of interest (in particular a Cas9 gene) and a fluorescent protein (CFP) reporter expressed as a bi-cistronic gene. This method allows us to specifically express Cas9 in live zebrafish in a controlled manner at given time and spatial location and to visualize the photoactivated cells.

We shall present results where we co-injected UAS:Cas9-T2A-CFP-Ubi:EosFP and gRNA for EosFP gene at one cell stage. Caged-cyclofen was UV activated at 256 cells and the embryos imaged at 24 hpf. CFP (blue) fluorescence was observed in the photoactivated cells and was negatively correlated with EosFP green fluorescence suggesting that in these cells Cas9 was active as indeed confirmed by T7 endonuclease analysis.

We have also developed a direct photo-switchable Cas9 by fusing its gene with ERT. In this context while Cas9 is continuously expressed, it is sequestered by cytoplasmic chaperones and quickly released at the single cell level upon cyclofen uncaging.

These new tools are currently developed as zebrafish transgenic lines offering the zebrafish community a powerful tool to investigate gene functions and networks (by crossing with relevant gRNA lines or by injections of appropriate gRNAs).

Z6033C Reverse genetics screening for uveal coloboma in zebrafish using CRISPR-Cas9 mediated genome editing. *S. Dutta*, B. P. Brooks. NEI/NIH, Bethesda, MD.

The vertebrate eye development begins with the symmetric, bilateral evagination of the diencephalon to form optic vesicle (OV). As development continues the OV invaginates to form bilayer optic cup, the invagination of OV is asymmetric and creates a gap in the ventral region of the developing eye, known as the optic fissure. To continue normal development, the two edges of this fissure must fuse. Any abnormality in the optic fissure closure results in a potentially blinding congenital ocular malformation known as uveal coloboma, account for 10% of childhood blindness. Several modes of inheritance have been documented, yet, most cases are sporadic, impeding effective genetic counseling for families affected by the disease. Although the embryology has long been understood, the genetic and developmental mechanisms behind optic fissure closure defects are still largely unknown.

CRISPR/Cas9 mediated genome engineering technology made it possible to perform targeted mutagenesis in a fast and economical way in zebrafish that facilitate large-scale phenotyping efforts in mutant embryos. We have used CRISPRs to generate knock out (KO) lines for previously described *nlz1* and *nlz2* gene that are necessary for normal optic fissure closure (1) and *nlz1* and *nlz2* CRISPRs KO embryos exhibited ocular coloboma (2). Genes that are dynamically regulated at the edges of the optic fissure during the process of closure are likely to be important for proper execution of this developmental process. Our lab identified 164 annotated genes dynamically regulated during optic fissure closure in the mouse using laser capture microdissection (LCM) and microarray (1), and the function of 90% of the genes during eye development are unknown. To understand the function of the genes expressed during optic fissure closure, we are generating CRISPRs KO zebrafish lines and screen for ocular coloboma, and other eye abnormalities in the KO embryos. In future our research will shed light into genes and genetic network that are crucial for optic fissure closure and eye abnormalities as well as to move forward in the genetic research of uveal coloboma.

1. Brown JD et al. (2009) Expression profiling during ocular development identifies 2 *Nlz* genes with a critical role in optic fissure closure. *PNAS* 106(5):1462-7.

2. Dutta S et al. (2015) *nlz1* is required for cilia formation in zebrafish embryogenesis.*Dev Biol*. 2015 Oct 15; 406(2):203-11.

Z6034A Tbx5a functions in migration of cardiac and forelimb precursors of the anterior lateral plate mesoderm in zebrafish. *L. Fong*, E. Boyle Anderson, H. K. Stinnett, R. K. Ho. University of Chicago, Chicago, IL.

The *tbx5a* mutant, *heartstrings*, has a lethal phenotype where the primary heart tube fails to loop during development and a pectoral fin is not formed. Our lab group has previously shown that cells within the anterior lateral plate mesoderm fail to converge in Tbx5a knockdown

conditions during limb initiation. We have hypothesized that during limb initiation, Tbx5a may play an early role in the ability of lateral plate mesoderm to migrate towards a localized signaling source of an Fgf molecule, namely Fgf24.

We have further elucidated a fate map for the Tbx5a-positive cells of the lateral plate mesoderm to include the heart tissues and have studied the dynamics of cardiac precursor migration using timelapse imaging. One interesting facet of this study is that the formation of the heart occurs asymmetrically, with looping of the heart tube towards the left side. We will report upon the specification and migration of the lateral plate mesoderm on the right versus left sides of the wild-type embryo during heart formation. Here we will also address whether cardiac precursors migrate topologically in a manner similar to limb bud precursors, or if the migration dynamics of the heart precursors are significantly different to those of the limb bud precursors.

Studying the dynamics of the migration in this process has generated several questions: Does Tbx5a have a similar early migration function in other systems such as cardiac precursors? Do similar ligand-receptor interactions found to play important roles during limb bud development also occur during the migration of heart precursors? Alternatively, could Tbx5a be affecting cell fate decisions within the anterior lateral plate mesoderm? To address these issues, we will also report upon the specification and migration of the lateral plate mesoderm precursors in Tbx5a knockdown embryos compared to wild-type embryos.

Z6035B Investigating role of breast tumor kinase/protein tyrosine kinase 6 (Brk/PTK6) during zebrafish development using TALEN-generated knockout alleles. *S. Foster*[1], S. Glover[1], A. Challa[1], K. Chatti[2]. 1) University of Alabama at Birmingham, AL; 2) University of Hyderabad Campus, Hyderabad, India.

Breast tumor kinase/protein tyrosine kinase 6 (Brk/PTK6) was initially discovered in a screen of tyrosine kinases overexpressed in metastatic breast cancer tissue. It is currently known to be expressed in several cancer types, and cell culture studies indicate a pro-oncogenic role for PTK6. PTK6-null mice are viable, but show hyperproliferation of colonic villi and hyperactivation of AKT. Contrasting evidence supporting a pro-apoptotic role of PTK6 has also been reported. In cultured non-cancer cells, PTK6 appears to sensitize the cells to inducers of apoptosis. The contrasting functions of PTK6 are likely to be a result of cell-type specific genetic interactions, biochemical regulation and environmental effects during oncogenesis. In order to gain a comprehensive understanding of the developmental role of PTK6 genes and to potentially explain their contrasting effects on cell growth and differentiation in vertebrates, we generated several indel alleles of the two zebrafish genes *ptk6a* and *6b* using TALENs. Our current findings suggest that homozygous mutant animals are viable presumably because these two genes complement each other, or because of maternal expression of the genes that can rescue the zygotic loss-of-function. Further characterization is underway to generate and analyze double mutants as well as maternal-zygotic mutant animals for each of these genes to identify any developmental roles.

Z6036C Temporal and spatial requirements for Nodal-induced anterior mesendoderm and mesoderm in anterior neurulation. *N. Gonsar*[1,2], A. Coughlin[1], J. A. Clay-Wright[1], B. R. Borg[1], L. M. Kindt[1], J. O. Liang[1]. 1) University of Minnesota, Duluth, MN; 2) Gustavus Adolphus College, St Peter, MN.

Zebrafish with defective Nodal signaling have a phenotype analogous to the fatal human birth defect anencephaly, which is caused by an open anterior neural tube. Previous work in our laboratory found that anterior open neural tube phenotypes in Nodal signaling mutants were caused by lack of mesendodermal/mesodermal tissues. Defects in these mutants are already apparent at neural plate stage, before the neuroepithelium starts to fold into a tube. Consistent with this, we found that the requirement for Nodal signaling maps to mid-late blastula stages. This timing correlates with the timing of prechordal plate mesendoderm and anterior mesoderm induction, suggesting these tissues act to promote neurulation. To further identify tissues important for neurulation, we took advantage of the variable phenotypes in Nodal signaling-deficient *sqt* mutant and Lefty1-overexpressing embryos. Statistical analysis indicated a strong, positive correlation between a closed neural tube and presence of several mesendoderm/mesoderm-derived tissues (hatching glands, cephalic paraxial mesoderm, notochord, and head muscles). However, the neural tube was closed in a subset of embryos that lacked any one of these tissues. This suggests that several types of Nodal-induced mesendodermal/mesodermal precursors are competent to promote neurulation.

Z6037A The Effect of Timing on Wnt Induced Neural Posteriorization. *D. Green*, A. Lekven. Texas A&M, College Station, TX.

During embryonic development morphogenic signaling gradients inform cells of their relative positions so that they may take on specific fates. There has been controversy in many models whether these signaling gradients are generated spatially, temporally or a combination of these. We have used the neural ectoderm of the zebrafish as a model to study the interface between spatial and temporal morphogen gradient signaling and its effect on cell fate in anterior posterior (AP) neural patterning.

In vertebrates, all neural ectoderm cells initially have an anterior fate. Through morphogenic signals, including Wnt's and Fgf's, the cells within the neural ectoderm are informed of their spatial position and take on specific fates along the (AP) axis. The primary morphogen of early neural posteriorization, *wnt8a,* is expressed in the blastoderm margin during epiboly. *wnt8a* generates a signaling gradient and posteriorizes the neural ectoderm in a dose dependent manner, with high doses inducing a more posterior fate. This signaling event occurs over roughly 4 $^{1/2}$-hour period that includes a large degree of dynamic cell movement as the cells of the blastoderm migrate towards the vegetal pole. How the timing and duration of the Wnt8a signal affects its ability to posteriorize the neural ectoderm is poorly understood. Of particular interest is how the different regions of the neural ectoderm change in response to the Wnt8a signaling gradient at different times during neural posteriorization.

To better understand the temporal aspect of Wnt8a's signaling gradient we have used heat shock inducible lines to analyze the response of the neural ectoderm to Wnt8a signaling during early neural posteriorization. Through comprehensive analysis of the temporal response to Wnt signaling we will determine how spatial and temporal factors can integrate to establish a morphogenic signaling gradient in development.

Z6038B Zebrafish Zic2a and Zic2b play redundant roles in brain, retinal and craniofacial morphogenesis. *Y. Grinblat*, L. Roberson, I. Sedykh. Univ Wisconsin, Madison, WI.

Human Zic2, a zinc-finger transcription factor, is strongly associated with holoprosencephaly (HPE), a malformation of the forebrain, and with a unique set of dysmorphic facial features. We have previously used knockdown assays to demonstrate critical roles for zebrafish Zic2 orthologs, Zic2a and Zic2b, in the developing retina, forebrain and craniofacial cartilages. To examine the mechanism of Zic2 function in more depth, we have generated compound *zic2a;zic2b* mutants and determined that embryos homozygous for mutant alleles at both *zic2a* and *zic2b* loci develop with retinal coloboma, aberrantly shaped ventricles and greatly reduced and disorganized craniofacial cartilage. In addition, mutant embryos present with periocular hemorrhages and edema suggestive of vascular malformations. To characterize the molecular consequences of Zic2 loss-of-function, we examined transcriptomes of *zic2a;zic2b* mutant embryos vs siblings using RNAseq. The resulting gene set identified several neural crest lineages, including but not limited to chondrogenic neural crest, as strongly dependent on Zic2 function. Among the candidate zic2 targets is a homeobox gene Alx1, which is expressed in neural crest and is required for both craniofacial and retinal morphogenesis in zebrafish. Moreover, human Alx1 has been linked to a severe form of frontonasal dysplasia. Based on these data, we hypothesize that Zic2 functions in neural crest lineages that contribute to craniofacial structures and regulate formation of adjacent structures, including the ventral retina. In-depth functional analysis of Alx1 and other candidate targets of Zic2 identified by RNA-seq will test this hypothesis, clarifying the mechanism of conserved Zic2 functions during vertebrate embryogenesis.

Z6039C Using diapause as a platform to dissect and understand various signaling pathways and regulatory mechanisms during early embryo development. *CHI-KUO HU*, Anne Brunet. Stanford University, Stanford, CA.

Many organisms encounter and overcome extreme condition which is not ideal for embryo development by diapause, a unique surviving approach by which embryos put all developmental processes in temporary arrest to live through an adverse environment. Although similar dormancies are widely observed among species, still little is understood about the underlying mechanical principles of diapause. It is especially intriguing that how distinct tissues and cells in different developmental programs can altogether be suspended at the same time, and if their developmental status/identity are maintained or modified during the 'suspended state' of diapause. Zebrafish is known to suspend its early embryo development when oxygen is deprived, but only for a short period of time (up to 24 hours). A similar but more significant case of diapause can be found in turquoise killifish *Nothobranchius furzeri*, which possess an ability to suspend embryonic development at the end of somitogenesis. Living in ephemeral ponds where water presents only during the brief annual rain seasons, newly laid killifish embryos prepare the coming drought by entering diapause for months, sometimes even years, until the ponds are refilled with water again in another rain season**.** This lengthy diapause in killifish is obligatory and can still occurs in lab conditions, providing a unique opportunity to study early embryo development and their crosstalk with diapause**.** Our transcriptome profiling revealed a dramatic shift of gene expression patterns at the transition between embryogenesis and diapause. Immediately after diapause entry, more than 35% of genes changed their expression levels for over 2 folds, indicating that diapause embryos were switching into a phase distinct to embryogenesis. With many antagonistic mechanisms upregulated, various embryonic developmental programs were either put on hold or erased in diapause. Key regulators of cell differentiation such as NeuroD1, and MyoD1 were heavily downregulated, and critical morphogens of embryogenesis such as Wnt and Fgf were mostly depleted. Interestingly, with developmental processes suspended in diapause, many general or tissue-specific genes related to stress tolerance, protection and homeostasis were highly expressed for already developed tissues, especially neural tube/crest and muscles. Overall, diapause provides a unique pausing point to further dissect and understand the developmental programs and their signaling pathways in early embryo development. The research in embryonic diapause not only provides insights of tissue and cell homeostasis during embryo development, but also a complementary platform for various known or ongoing zebrafish studies in early embryo developments.

Z6040A Immune cell-independent elimination of signaling-perturbed cells support robustness of early vertebrate embryogenesis. *T. Ishitani*, Y. Akieda. Kyushu University, Fukuoka, Fukuoka, JP.

Developmental systems possess robustness, which is the ability to maintain phenotypic stability in the face of diverse perturbations arising from environmental changes, stochastic events, and genetic variation. But molecular signaling systems that control embryonic development are sensitive to these perturbations. Therefore, developing embryos should equip the mechanisms for overcoming the signaling perturbation during development. However, such mechanisms have been well understood. Here we show that early vertebrate embryonic tissues have an ability to eliminate signaling-perturbed cells. To investigate how embryonic tissues overcome the signaling perturbation, we mosaically induced Wnt signaling-hyperactivated cells in early zebrafish embryos. Interestingly, these mosaically-induced abnormal cells underwent apoptosis in wild-type embryos, but not in APC- or GSK3-inhibited embryos in which Wnt signaling is hyperactivated in entire tissues, indicating that signaling-perturbed cells are eliminated from normal embryonic tissues and this elimination requires the existence of normal cells. These results also suggest the possibility that embryonic cells may sense the difference of signaling activity in neighboring cells and make decision whether the neighboring cells should be eliminated. This cell elimination must be immune cell-independent event because early embryos don't have immune cells. Importantly, Wnt signaling-hyperactivated cells-introduced normal embryos grew up almost normally, while inhibition of abnormal cell elimination severely disturbed embryonic development. It is suggesting that the signaling-perturbed cell elimination supports robustness of early vertebrate embryogenesis. Now we are exploring the detailed mechanisms that regulate the signaling-perturbed cell elimination. We'd like to show our recent progress of these studies.

Z6041B *unc119* genes are required for cilia function in zebrafish. *F. Jean*, D. Pilgrim. University of Alberta, Edmonton, Alberta, CA.

Unc119 proteins form a structurally distinct family composed of proteins that are strikingly conserved among all metazoans. Intriguingly, this

small family is implicated in diverse activities, including initiating the cellular immune response, promoting neural development in *C. elegans*, and coordinating signal transduction in mice. Despite these seemingly distinct roles, orthologues appear to be functionally interchangeable, suggesting that a common biochemical mechanism underlies each of these cellular processes and that redundancies may arise where paralogues exist.

A compelling unifying mechanism is intraflagellar transport (IFT), which is a protein trafficking system initially described in cilia. Cilia are microtubule-based protrusions from the cell body and are critically important for vertebrate organ function, body patterning and signalling pathways. IFT factors are additionally required for cellular processes unrelated to cilia, like those that involve Unc119 proteins. Since Unc119 proteins appear to be required for all cellular processes that rely upon IFT and have been shown to be required for trafficking, I propose that they are novel IFT factors. I hypothesize that the highly conserved Unc119 family proteins are previously unrecognized ciliary factors that promote proper cellular trafficking via the IFT machinery. To test this hypothesis, I am studying the cellular and phenotypic consequences of a loss of *unc119* genes using zebrafish.

Zebrafish have four paralogues of *unc119*, none of which have previously been studied in great detail in terms of cilia function. To begin, I wanted to determine whether any of the paralogues have a potential role in cilia by performing *in situ* hybridization. I found that each paralogue has a distinct expression pattern, although there is a large degree of overlap particularly in ciliated organs. To assay for ciliary phenotypes, I have been using CRISPR-Cas9 technology to create deletion mutations in each of the zebrafish paralogues. My preliminary results using morpholinos to target one of the paralogues (*unc119b*) have revealed that there are ciliary phenotypes (hydrocephaly, kidney cysts, l-r asymmetry defects), but these are milder and less penetrant than expected, which is consistent with redundancy between paralogues. Given the conserved nature of these genes and the overlap in expression between paralogues, it's possible that there is functional redundancy, which we will be testing using rescue assays. Thus far, we have shown that at least one of the paralogues is involved in cilia but it is likely that the other paralogues act similarly molecularly to affect cilia function.

Z6043A Cartilage development requires the function of Estrogen-related receptor alpha that directly regulates sox9 expression in zebrafish. *Y. Kim*, S. Bhandari, I. Nam, K. Yoo, R. Park, S. Choe. center for metabolic function regulation, Wonkwang Univ, Iksan, Jeonbuk, KR.

Estrogen related receptor alpha (ESRRa) regulates a number of cellular processes including development of bone and muscles. However, direct evidence regarding its involvement in cartilage development remains elusive. In this report, we establish an *in vivo* role of ESRRa in cartilage development during embryogenesis in zebrafish. Gene expression analysis indicates that *esrra* is expressed in developing pharyngeal arches where genes necessary for cartilage development are also expressed. Loss of function analysis shows that knockdown of *esrra* impairs expression of both *sox9* and *col2a1* and thus induces abnormally formed cartilage in pharyngeal arches. Importantly, we identify putative ESRRa binding elements in upstream regions of *sox9* to which ESRRa can directly bind, indicating that ESRRa may directly regulate *sox9* expression. Accordingly, ectopic expression of *sox9* rescues defective formation of cartilage induced by the knockdown of *esrra*. Taken together, our results indicate for the first time that ESRRa is essential for cartilage development by regulating *sox9* expression during vertebrate development.

Z6044B Characterisation of tail mutants in the self-fertilising mangrove killifish. *T. Kudo*[1], Hussein Saud[1], Brian Ring[2]. 1) University of Exeter, Exeter, Devon, GB; 2) Valdosta State University, Valdosta, Georgia, USA.

The mangrove killifish, Kryptolebias marmoratus and another related killifish species are only two known self-fertilising vertebrates. By making use of the self-fertilising ability, it is possible to isolate both zygotic and maternal mutant lines from this fish with one generation earlier than zebrafish and medaka allowing, much smaller scale and shorter process of mutant screening and maintenance. We have isolated two mutant lines that shows abnormalities in the tail development, R109 (short-tail/stl) and R228 (ball-tail/btl). At the somitogenesis stage, both mutants show normal length of the tail, but in the stl mutant, gene expression in the tail including sox3 (neural tube), col9a1b (notochord) and hsp90 (muscle) all reduce. The tail stop growing at late somitogenesis and regress. In the btl mutant, sox3 and col9a1b expression is normal but hsp90 is strongly suppressed suggesting the defect is specific to the tail somite muscle. We are currently mapping these mutations in the genome to identify the mutations and will report the progress of the mutation analyses in the meeting.

Z6045C Vitamin D receptor signaling is required to modulate BMP signaling during cranial cartilage development in zebrafish. *H.-J. Kwon*[1], B. B. Riley[2]. 1) Princess Nora University, Riyadh, Kingdom of Saudi Arabia; 2) Texas A&M University, College Station, TX.

Vitamin D, an essential regulator of calcium and phosphate homeostasis, is necessary for skeletal development. The active hormonal form of vitamin D functions through the vitamin D receptor (VDR), a member of the nuclear receptor family of transcription factors. The *vdr* mRNA and VDR protein are present in several cartilages of the skull in zebrafish embryos. To investigate the role of VDR on craniofacial cartilage development, *vdr* depletion experiments were performed. In zebrafish embryos, two distinct VDR genes (*vdra* and *vdrb*) have been identified. Knockdown of *vdra* has little effect on cartilage elements, whereas disrupting *vdrb* gene causes reduction and malformation of head cartilages. Remarkably, depletion of both *vdra* and *vdrb* produces more severe defects including complete loss of cartilage. These results strongly indicate that VDR signaling is essential for cartilage development in zebrafish. Previous studies in zebrafish revealed correct BMP signaling is required for cartilage formation in the skull. Interestingly, we show that knockdown of VDRs lead to increased expression of *fsta*, a BMP antagonist, in pharyngeal region around 36 hours post-fertilization. Taken together, these findings suggest that VDR may control cranial cartilage development by modulating BMP signaling.

Z6046A Development of left-right asymmetries in the vertebrate brain. *I. Lekk*, A. Faro, C. Stern, S. Wilson. University College London, London, GB.

While most of the bilaterian body is symmetrical with respect to the midline, a few functions have evolved to be concentrated on one (left or right) side. Although long thought to be a human-specific characteristic, it is now clear that lateralisation of the brain is an evolutionarily conserved mechanism to add layers of complexity to brain function and enable lateralised behaviour within the population. Here, to shed light on how brain asymmetries arise during vertebrate development and to what extent underlying molecular mechanisms are conserved within different species, zebrafish and chick embryonic epithalamus has been studied. By two-photon laser ablations, cell transplants and high-resolution imaging, we find that the exact timing of regulation by the parapineal – a small left-sided group of cells in the zebrafish epithalamus – is essential for the establishment of overt molecular and anatomical asymmetries described in the the adjacent habenulae. However, whole-mount *in situ* hybridisation, RT-PCR and qPCR experiments show no apparent asymmetries in gene expression patterns of chick epithalamus. Since no region equivalent to parapineal has yet been discovered in birds, it is likely that the anatomical and functional asymmetries found in the epithalamus of vertebrates such as fish, reptiles and amphibians, result from the emergence of a parapineal or a parapineal-like organ during evolution.

Z6047B Roles of PGE2 signaling pathway in ciliogenesis and organ development. *Wenyan Li*[1], Quan Zhao[1], Daqing Jin[1], Tao P. Zhong[1,2]. 1) State Key Laboratory of Genetic Engineering, Department of Genetics, School of Life Sciences, Fudan University, Shanghai, 200438, China; 2) Department of Medicine, Vanderbilt University School of Medicine, Tennessee 37232, USA.

ABSTRACT: Cilium, a microtubule-based organelle protruding from the cell surface, has crucial roles in embryonic development and human physiology. Our lab reveals that Prostaglandin E2 plays a significant role in ciliogenesis and organ development. Based on these results, we hypothesize that key components in PGE2 signaling pathway participate in cilia formation and function. We have generated *ptgs1*, *ptgs2a*, *ptger4a* and *ptger4b* mutants using CRISPR/Cas9 technique in zebrafish. These homozygous mutants can survive into adulthood. We are currently in the process of constructing double mutants to eliminate functional redundancy in ciliogenesis. To understand the conserved ciliary roles of PGE2 signaling in mammalian models, we analyzed cilia-associated phenotypes of *ptger4*-deficient mouse. Our study shows that mouse embryonic fibroblasts (MEF) display deficiency in *ptger4* receptor. Furthermore, *ptger4*-deficient mouse exhibit ciliogenesis defects and abnormal development of lungs, kidneys and tracheas. Together, these findings indicate the conserved regulation of PGE2 in mammalian ciliogenesis.

Key words: Prostaglandin E2 ciliogenesis *ptger4* zebrafish mouse .

Z6048C Probing how cell sorting refines developmental patterning. *Z. Liu*, O. Weiner. University of California, San Francisco, San Francisco, CA.

The final developmental pattern of multicellular organisms is extraordinarily precise, with sharp boundaries between cell types that can be as fine as a single-cell layer. Although morphogen gradients that direct cell fates can be quite defined, development operates on a background of substantial variability due to intrinsic stochasticity in cell fate specification and active migration of cells within the morphogenic field. Cell sorting is thought to enable systems with initially noisy fate specification to generate robust final patterns. In the early zebrafish embryo, an initially mixed mesendodermal population ultimately resolves into distinct mesodermal and endodermal cell layers, but how this precise patterning is achieved is not understood. Previous studies demonstrated that induced endodermal cells transplanted to ectopic locations can still sort into the endogenous endodermal domain. Here, we aim to understand how this sorting behavior is achieved and how cell sorting enables pattern refinement during endodermal morphogenesis.

We experimentally introduced positional errors by first overexpressing the constitutively activate Nodal receptor TARAM-A* to induce endoderm fate and then transplanting these cells to the animal pole of a wild type embryo, which normally gives rise primarily to ectoderm. We then used whole embryo imaging to track the transplanted cells. Consistent with previous reports, we found that these ectopically introduced endodermal cells preferentially migrate to the correct endodermal layer, but surprisingly, these cells did not follow the normal endodermal migration pattern of epiboly and involution. Instead, they appeared to take a short-cut by radially ingressing into the inner layer. We propose that this ingression ensures that cells that may arrive at the margin late can still find a path into the inner layer, thus increasing the precision of the first step of endoderm formation. Currently we are exploring the informational sources of such directionality. By examining contractility based extrusion of the surrounding cells and membrane protrusions of ectopic endodermal cells, we will be able to determine the underlying mechanisms of the ingression based sorting behavior, and thus further our understanding of the largely unexplored frontier of how cell sorting facilitates the robustness of multicellular development.

Z6049A The requirement of cell-matrix interactions for planar cell polarity and convergence and extension. *A. Love*, J. Jessen. Middle Tennessee State University, Murfreesboro, TN.

Integral to convergence and extension movements during development, the planar cell polarity (PCP) pathway is a non-canonical Wnt pathway that regulates the coordinated movement of cells within a tissue plane. During zebrafish gastrulation, defects in core PCP proteins such as Vangl2, encoded by the *trilobite* gene, result in failed convergence and extension cell movements. As a result, *vangl2*/*trilobite* mutant embryos have hallmark phenotypes, including a shortened and broadened dorsal body axis. Previous work from the Solnica-Krezel lab showed that mesodermal cells in *vangl2*/*trilobite* mutant embryos do not follow the intended path of collective migration to the dorsal axis for proper embryonic development, instead these cells lack directionality. In other work, the Jessen lab demonstrated that *vangl2*/*trilobite* mutant embryos also exhibit an increase in matrix metalloproteinase activity and a decrease in the extracellular matrix (ECM) protein fibronectin. Given

that cells in *vangl2/trilobite* mutant embryos are characterized as having inappropriate membrane protrusive activity, we hypothesize that these embryos have defective cell-matrix interactions necessary for proper protrusion formation. Integrins are transmembrane receptors for many ECM proteins, including perhaps the most prevalent ECM protein in the zebrafish gastrula, fibronectin. Integrins control dynamic interactions between the cell's actin cytoskeleton and its surrounding matrix. It is well known that mesenchymal cell migration begins with protrusion at the leading edge, adhesion to ECM, and stabilization; at which time, cell body movement occurs as the rear cell adhesions are released. Using whole-mount in situ hybridization and confocal microscopy, we are characterizing the relationship between cell-matrix interactions and the PCP pathway. We have shown that *vangl2/trilobite* mutant embryos are sensitive to loss of integrins a5, aV, and b1 function. Moreover, we find that loss of fibronectin in *vangl2/trilobite* mutant embryos results in only a subtly enhanced convergence and extension phenotype, indicative of decreased sensitivity to loss of fibronectin. These preliminary results are consistent with the notion that *vangl2/trilobite* mutants have decreased total fibronectin. Ongoing experiments address the effects of disrupted cell-matrix interactions on the establishment of planar cell polarity at the cellular level.

Z6050B Sox2 and canonical Wnt signaling co-regulate multipotent tailbud progenitors. *B. L. Martin*, Yu-Jung Tseng, Richard Row. Stony Brook University, Stony Brook, NY.

Tailbud progenitor cells contribute to multiple tissues of the lengthening post-gastrula vertebrate embryo. Recent evidence shows that these cells continue to make germ layer decisions after gastrulation, contrary to long-held dogma. Wnt signaling and the transcription factor Sox2 are known regulators of these cells, and we have found an unexpected interaction between these two pathways.

The tailbud is a conserved structure in vertebrate embryos, formed at the posterior-most end of the body axis after gastrulation completes. Multiple progenitor cell pools are maintained in the tailbud, and these cells are continuously specified to different germ layer fates. In zebrafish the posterior-most progenitors make an ectoderm/mesoderm fate decision, primarily contributing to the neural tube or somites respectively. Wnt signaling and the transcription factor Sox2 are potent regulators of this fate decision. Undifferentiated progenitors express *sox2* and reside in a region of active Wnt signaling. Cell fate is specified when a cell receives only one of the two signals. Sox2 is considered to have a pro-neural function in this context while Wnt specifies a mesodermal fate. Both signals can antagonize the activity of the other, likely serving to lock in a fate decision when the balance tips to one side within a cell. We use transgenic zebrafish that allow inducible overexpression of *sox2* or manipulation of Wnt signaling to understand the role of these signals in tailbud progenitors.

We found an unexpected role for Sox2 in regulating the behavior of mesoderm-fated cells: Sox2 prevents the maturation program of these cells from progressing. A further surprising result is that Sox2 can respecify some mesoderm-fated cells to a neural fate. Precise regulation of Sox2 and Wnt levels is crucial both for maintenance of tailbud progenitors and for proper maturation of mesoderm-fated cells.

Z6051C Zebrafish *ambra1a* and *ambra1b* silencing affects heart development. *G. Meneghetti*[1], T. Skobo[2], N. Facchinello[1], P. Bonaldo[2], F. Cecconi[3], L. Dalla Valle[1]. 1) Department of Biology, University of Padua, Italy; 2) Department of Molecular medicine, University of Padua, Italy; 3) Department of Biology, University of Rome Tor Vergata, Italy.

In zebrafish two paralogous genes, *ambra1a* and *ambra1b*, both required for the autophagic process and during development, encode the protein Ambra1, a positive regulator of early steps of autophagosome formation. As evidenced by whole mount *in situ* hybridization (WMISH), both transcripts are expressed in the heart-forming region. In this work, we analyse the *ambra1a* and *ambra1b* knockdown effects on heart development by means of morpholino oligonucleotides (MOs). Silencing of the two proteins by ATG-MOs affects heart morphogenesis resulting in a small, string-like heart with pericardial edema, whereas treatment with SPLIC-MOs does not result on clear cardiac phenotypes, indicating the importance of maternally supplied *ambra1* transcripts. Co-injection of both ATG-MOs determines a more severe phenotype with a prominent pericardial edema. Cardiac defects are effectively rescued by co-injection of MOs with human *AMBRA1* mRNA showing the conservation of Ambra1 functions during evolution. WMISH of *myosin light chain 7* (*myl7*) transcripts as well as confocal analysis of *ambra1a* and *ambra1b* morphants of the transgenic line Tg-myl7:egfp reveal, at 24 hpf, defects in the heart jogging process followed by imperfect cardiac looping with a high percentage of 48 hpf embryos in which the heart is organized as a linear tube. Moreover, WMISH of *pitx2* transcripts, which at the 21-somite stage are expressed on the left plate mesoderm, reveals both bilateral or reversed expression of this gene in both *ambra1* morphants indicating that *ambra1* silencing interferes on heart laterality.

3-dpf WT and morphants purified hearts were used for expression analysis of developmental heart markers by means of qPCR. This analysis shows a differential expression not only between WT and morphants hearts but also between *ambra1a* and *ambra1b* morphants. In fact, while *atrial* and *ventricular myosin heavy chain*, as well as *gata5* transcripts present a general down regulation in both types of morphants, the expression of other cardiac markers, such as *nkx2.5* and *hand2,* is up regulated only in *ambra1b* morphants. Although both *ambra1a* and *ambra1b* genes appear to be involved in heart development, the different expression pattern suggests the possible acquisition of specific functions by the two paralogous genes.

Z6052A Eph-ephrin signaling maintains the boundary of the embryonic left-right organizer during laterality development in fish. *A. Meng*, J. F. Zhang, Z. Jiang, X. Liu. Tsinghua University, Beijing, CN.

The kupffer's vesicle (KV) is the so-called left-right organizer in teleost fishes. KV is formed from dorsal forerunner cells (DFCs) and generates asymmetrical signals for breaking symmetry of embryos. It is unclear how DFCs or KV cells are prevented from intermingling with adjacent cells. In this study, we show that the Eph receptor gene *ephb4b* is highly expressed in DFCs while the ephrin ligand genes including *efnb2b* are expressed in cells next to the DFCs cluster during zebrafish gastrulation. *ephb4b* knockdown or mutation and *efnb2b* knockdown cause dispersal of DFCs, a smaller KV and randomization of laterality organs. The DFCs often dynamically form blebs, filopodia and lamellipodia at the

interface, which attempt to invade but are bounced back by adjacent non-DFC cells during gastrulation. Upon inhibition of Eph-ephrin signaling, however, the repulsion between DFCs and non-DFC cells is lost, allowing DFCs to migrate away. Ephb4b/efnb2b signaling by activating RhoA activity mediates contact and repulsion between DFCs and neighboring cells during gastrulation, preventing intermingling of different cell populations. Therefore, our data uncover an important role of Eph/ephrin signaling in maintaining DFCs boundary and KV boundary for normal left-right asymmetrical development.

Z6053B Transcriptional Regulation of Neural Plate Patterning by Wnt Signaling through the Sp1 family of Transcription Factors. *Saurav Mohanty*, Arne Lekven. Texas A & M University, College Station, TX.

Wnt signaling has a conserved role in anterior-posterior (A/P) patterning of the remarkably complex vertebrate brain from a very basic primordium, the neural plate, by differentially expressing downstream target genes to induce distinct neural fates. Wnt proteins originate from the blastoderm margin and diffuse anteriorly to re-pattern the default state of the anterior neural tissue to more posterior fates. How this graded Wnt ligand in the neural plate is translated by responding cells to establish specific cell fate zones along the A/P axis is not well established. In order to understand neural posteriorization by Wnt better, we need to identify the downstream components of this pathway, for instance, the transcriptional targets of wnt signaling and their subsequent roles. We are currently focussing on two direct targets of Wnt/b-catenin signaling in Zebrafish, the *sp5* and *sp5-like* genes. These paralogous genes potentially play an important role in mediating Wnt-dependent posterior neural plate patterning.

The *sp5* genes encode zinc finger transcription factors and transcriptional regulation of *sp5* orthologs by Wnt signaling is conserved in vertebrates. In spite of the compelling evidence of a significant and evolutionarily conserved relation between the *sp5* genes and Wnt signaling in vertebrates, function of these genes and how they induce changes downstream of the Wnt pathway to pattern the vertebrate brain is not understood properly. We are currently studying the in vivo functions of *sp5* genes by mutant analysis, utilizing the CRISPR-Cas9 genome editing tool to generate zebrafish *sp5* and *sp5l* mutant lines. We are examining the regulation of these genes by Wnt signaling and other signaling pathways through analysis of transgenic zebrafish sp5 reporter lines developed in our lab. Finally, we want to understand how Wnt signaling regulates the transcription of several target genes downstream through the sp5 transcription factors, to pattern the neural plate during embryogenesis.

Z6054C Biomechanics of zebrafish gastrulation. *A. Mongera*, E. Shelton, D. Kealhofer, A. Lucio, P. Rowghanian, F. Serwane, O. Campas. UCSB, Santa Barbara, CA.

Morphogenesis is traditionally regarded as the implementation of multiple genetic programs regulating cell differentiation rather than a complex biomechanical process involving cell-generated forces and tunable mechanical properties of the tissue. It has been shown that mechanics controls not only massive cellular rearrangements, as observed during gastrulation, but also pathological changes in tissue shapes such as those found in neoplastic transformation. *In vivo* measurements of endogenous forces and local mechanical properties during gastrulation are crucial to understanding how collective cellular movements arise during morphogenesis. Employing novel techniques that use fluorescent oil micro-droplets as *in vivo* force transducers and actuators, we quantify the spatiotemporal patterns of both forces and mechanical properties (tissue elasticity and fluidity) in different cell populations of the zebrafish embryo. Furthermore, we show that cell adhesion, cortical tension, and extracellular matrix composition affect different mechanical parameters in the tissue. The quantitative description of the embryonic force fields and the local mechanical properties will enable a better understanding of the interplay between signaling and mechanics, which in turn will facilitate the development of treatments for diseases, such as cancer, by restoring normal mechanical conditions within the tissues of interest.

Z6055A Role of Snail1b in migration of Posterior Lateral Line primodium. *U. M. Neelathi*[1], A. B. Chitnis[2]. 1) NIH, Bethesda, MD; 2) NIH, Bethesda, MD.

Authors Uma M Neelathi and Ajay B Chitnis

Collective cell migration, is an important event in embryonic development, wound healing and cancer metastasis. Posterior Lateral line primodium (PLLp) presents an excellent model for studying collective cell migration. Its a group of 100 cells, from ectodermal placodes behind the otic vesicle migrates along the horizoantl myoseptum depostiing neuromasts at regular intervals, which constitutes the lateral line sensory system. While the leading PLLp cohort maintain mesenchymal cellualrity thorugh Wnt signaling. The trailing epithelial cell types are conferred via a Wnt dependant Fgf signaling, which are organised into rossettes that are deposited as neuromasts. Factos, which make the pseudo epithelial to mesemchymal transition of the primodium cells during migration, are not known. Expression analysis of various mesenchymal genes by RNA in situ, identified Snail1b in the leading doamin at 22hpf when primodium initiates its migration. Snail1b is positively regualted by Sdf1a in the leading domain and negatively by Fgf singaling in the trailing domain. Morpholino based loss of function of Snail1b cause a delay in initiation and reduced speed of migration of PLLp compared to the controls. The delay in initiation and rate of migration is though imbalance between the Wnt and Fgf signaling systems in the PLLp. Absence or a total loss of migration of PLLp could possibley due to partial knockdwon of Snail1b or by other compensatory complementary genes. Our data suggest that Snail1b is necessary in breaking the symmetry of Wnt signaling and giving in way for Fgf signaling to estbalsih and initiation of the primodium migration.

Z6056B The formation of dorsal axial structures in zebrafish requires the activity of a homolog of the *Drosophila* gene *squid*, which regulates dorsal patterning in flies. *M. L. O'Connell*, L. J. Pasick, N. Maglakelidze, D. M. Ferrer. The College of New Jersey, Ewing, NJ.

In *Drosophila melanogaster* the hnRNA binding protein Squid is required during oogenesis and early embryogenesis for the earliest patterning

events in the embryo. In particular, specific isoforms of the Squid protein bind to and regulate the localization and translation of the *gurken* mRNA, which codes for a TGFa-like growth factor required for proper dorsal/ventral and anterior/posterior patterning of the egg and future embryo (Kelley, R., *Genes & Dev* 7:948, Norvell, A. et al., *Genes & Dev* 13:864). While the orientation of the dorsal/ventral versus anterior/posterior and left/right axes in invertebrate embryos is reversed relative to that of vertebrate embryos, at the molecular level there is clear conservation of many regulatory genes and paradigms. In fact, the expression of key genes required for dorsal/ventral patterning is exactly reversed in the two groups of embryos. In light of the tremendous conservation this demonstrates in the molecular regulation of bilateral symmetry across divergent taxonomic groups, we have analyzed a family of four genes in zebrafish that are the most closely related fish genes to fly *squid*, based on an NCBI BLAST search. In zebrafish, the mRNAs for all four fish genes (zsqdA-zsqdD) are maternally provided, suggesting that – like their fly counterpart – the fish proteins likely play a role in embryogenesis. Furthermore, we provide evidence that the mRNA for zsqdA (gene symbol: hnrnpaba), which shares the greatest sequence homology to fly *squid*, is translationally controlled via cytoplasmic polyadenylation at a particularly interesting stage during embryogenesis. This would result in the zsqdA protein being produced soon after the MBT and just before the formation of the earliest dorsal tissues (the shield). Finally, inhibition of zsdA translation via morpholino injection specifically disrupts early neurogenesis and the formation of axial structures such as the somites. Therefore, it appears that the zsdaA gene in zebrafish is not only homologous in sequence to *Drosophila squid*, but also in function.

Z6057C Development of the second pharyngeal pouch in zebrafish; Interface of discrete developmental systems. *K. Okada*[1,2], S. Takada[1,2,3]. 1) OIIB, Okazaki, Aichi, Japan; 2) NIIB, Okazaki, Aichi, Japan; 3) SOKENDAI, Okazaki, Aichi, Japan.

Pharyngeal Arches (PA) are segmental structures, which are bilaterally arranged in the pharynx of all vertebrate embryos. During the segmentation of PAs, the pharyngeal endoderm plays a prominent role to organize each PA by means of reiterative outpocketings called Pharyngeal Pouches (PPs). In gnathostome (jawed vertebrate), the first and second pouches (PP1 and PP2) are concurrently formed earlier than the posterior PPs. On the other hand, the PPs posterior to PP2 are sequentially formed in an anterior to posterior order. Additionally, previous studies in several vertebrate species have clearly shown the mechanisms controlling PP1-2 and PP3-6 development are different. Thus, distinct segmentation systems for the anterior and posterior PPs appear to cooperate in the generation of the complete set of PPs. However, it remains to be elucidated how these distinct systems are coordinated to integrate properly in PP development. Here, we focus on the development of PP2, which develops near the interface point of the two systems. Time-lapse recording and lineage tracing of endodermal cells in transgenic zebrafish showed that the prospective rostral and caudal halves of PP2 are generated in separate locations in the endoderm and then fused together forming PP2. In concordance with morphological observations, the pattern of gene expression throughout PP development supports the idea that there exists two distinct cell populations in PP2. For example, *nkx2.3*, which is a common PP marker gene, was expressed separately in the rostral and caudal halves before PP2 maturation. We also examined the PP phenotypes of zebrafish mutants for several genes, which were generated using the CRISPR/Cas9 system. Interestingly, we found that in some cases, in PP2, only the rostral- or caudal-half development is impaired. Furthermore, the caudal-half development of PP2 was disrupted by the inhibition of retinoic acid signaling, which is required for the segmentation of PP3-6, in spite of this the rostral half of the PP2 was almost normal. These results suggest that discrete segmentation systems are independently established, and subsequently fused to generate the PP2. This hybrid type of PP organization may have contributed to the evolution of the oropharyngeal system in gnathostomes by segregating the feeding component (mandibular and hyoid arches) from the gill region. Our findings also illustrate the drastic alteration of the developmental mechanisms of the PA, which is the most conserved phylotypic structural characteristic within vertebrates.

Z6058A The role of TGFβ member Gdf3 in left-right patterning. *J. Pelliccia*, R. Burdine. Princeton University, Princeton, NJ.

The proper placement of organs in vertebrates is dependent on the expression of *nodal* in the left lateral plate mesoderm (LPM). For this to occur in zebrafish, the expression of the nodal ortholog *spaw* needs to be activated in the left LPM and inhibited in the right LPM. Bilateral expression of nodal (i.e. expression in both the left and right LPM) or expression in the right LPM leads to aberrant organ placement. We have focused on understanding the role of Gdf3 in the proper expression of *spaw*. Gdf3 is a TGFbeta ligand whose mRNA is present during the stages of mesoderm and endoderm patterning and during the stages of left-right patterning in zebrafish embryonic development. Using morpholinos against *gdf3*, we have identified a dual role of *gdf3* in left-right patterning. Both in the expression of *spaw* and in the proper development of the laterality organ in zebrafish called Kupffer's vesicle (KV). Our data suggest that *gdf3* is required for proper temporal expression of *spaw* in the lateral plate mesoderm and for the proper asymmetric expression of *dand5*, a *spaw* inhibitor needed to prevent *spaw* expression in the right lateral plate mesoderm. The aberrant development of KV in these *gdf3* morphants was likely the cause of the irregular expression of *dand5*. Additionally, our attempts at making mutants using CRISPR/Cas9 technology have been rewarded with potential *gdf3* zebrafish mutants that exhibit defects in mesoderm and endoderm formation. These defects are also attributed to previously identified mutations in two other *nodal* orthologs called *cyclops* and *squint*. Collectively, our initial findings indicate that *gdf3* is important for mesoderm and endoderm patterning in early development and it may then be subsequently required for proper left-right patterning .

Z6059B The zebrafish *specter* mutant: a role for Cyclin B1 in early embryogenesis. *T. Petrachkova*, L. Bakke, A. Bard, J. Singh, R. M. Warga, D. A. Kane. Western Michigan University, Kalamazoo, MI.

Cell division is controlled by genes that regulate the cell cycle. Here we show that the zebrafish mutant *specter* (*spr*) is a mutation in *cyclin B1*, a gene necessary for the G2 to M transition of the cell cycle. The *spr* mutant phenotype becomes visible by the 7-somite stage as subtle differences in the head-to-tail ratio. Initial studies showed that the *spr* mutant arrests with a body shape at roughly midsegmentation and eventually exhibits signs of massive cell death in the nervous system. Investigation of the nervous system reveals that *notch1b* and *deltaA* are

more weakly expressed in neural stem cells that give rise to fewer, but bigger neuronal precursors. Likewise blood markers reveal mutant embryos also have fewer but bigger blood cells.

The *spr* mutation was mapped to the zebrafish *cyclin B1* gene. Sequencing showed a transition (C139T) that causes a nonsense mutation in exon 2 of the *cyclin B1* gene. *In situ* analysis revealed that *cyclin B1* mRNA is absent in the mutant embryo by the end of gastrulation suggesting that *spr* is a loss of *cyclin B1* function. Phospho-histone-3 antibody staining showed that the cell cycle is abnormal in the mutant embryo. The expression of the Dual FUCCI transgene in live *spr* mutant embryos confirmed that cells are delayed in the S/G2/early M phase of the cell cycle. We confirmed that *spr* is a mutation in *cyclin B1* using CRISPR/Cas9 mediated germline mutagenesis. This produced a mutation that failed to complement the original mutant allele confirming that the absence of CyclinB1 causes the mutant phenotype. Interestingly, the CRISPR *cyclin B1* mutation expresses morphological changes that are more severe and can be detected earlier. Phospho-histone-3 antibody staining and the expression of the Dual FUCCI transgene in the live CRISPR *spr* mutant embryos suggested that the cell cycle abnormalities are more severe: very few cells enter mitosis, and a majority of the cells remains in the S and G2 phase of the cell cycle. *In situ* hybridization of *cyclin B1* revealed that mRNA transcripts are still present in the CRISPR mutant and reverse transcriptase/PCR showed variable mRNA sizes. These results suggest that the new mutation is possibly a splicing variant that causes a gain-of function mutation, although we have yet to identify an obvious dominant phenotype.

We conclude that the *spr* mutant phenotype is caused by the mutation in the cell cycle gene *cyclin B1*, an essential regulator of the cell cycle progression from the G2 to M phase of the cell cycle. This leads to mitotic abnormalities, such as delayed cell cycle progression, developmental arrest, and activation of apoptotic pathways.

Z6060C Investigating the role of cadherin-mediated cell adhesion during planar cell polarity. *D. Prince*, J. Jessen. Middle Tennessee State University, Murfreesboro, TN.

Planar cell polarity (PCP) describes the coordinated polarization of cells or cell structures within the plane of a tissue and is an essential process of embryogenesis. For zebrafish gastrula cells engaged in directed cell migration, PCP is defined as the elongation and mediolateral alignment of each cell with respect to the dorsal embryonic axis. Loss of PCP protein function disrupts membrane protrusive activity, cell elongation and orientation resulting in misshapen embryos. Mutations in the Wnt co-receptor *glypican4/knypek* cause embryos to have a pronounced convergence and extension phenotype characterized by a shortened and broadened body axes. For many types of collective or group cell migration events, it has become clear that the movement of polarized cells requires regulation of cell-cell adhesion. Rapid remodeling of cadherin-mediated cell adhesion allows dynamic changes in cell coupling while maintaining coherent movement of cell populations. However, though cadherins are often required for collective cell migration, their molecular relationship with the PCP pathway is not well understood. Previous research by the Jessen laboratory showed that *glypican4/knypek* mutant embryos have increased cell surface expression of cadherin. Moreover, *glypican4/knypek* mutant embryos have increased cell-cell adhesion and increased fibronectin fibrillogenesis. We have now shown that loss of Glypican4 function affects the cell surface level of both E-cadherin (Cdh1) and N-cadherin (Cdh2). We hypothesize that Glypican4 function and Wnt/Frizzled PCP signaling regulate the endocytosis of Cdh1 and Cdh2. Indeed, we find that *glypican4/knypek* mutant embryos are sensitive to *rab5c* knockdown. Current efforts are aimed at further understanding the molecular mechanism connecting Glypican4 function and cell surface cadherin levels and determining the role of cadherin-mediated cell adhesion in establishing planar cell polarity.

Z6061A Tbx20 is an essential regulator of cardiomyocyte proliferation in zebrafish. *L. Raphel*[1], W. Rottbauer[2], S. Just[2]. 1) University of Ulm, Ulm, Baden Wuettemberg, DE; 2) University Clinic Ulm, Clinic for Internal Medicine 2, Albert Einstein Allee 23, Ulm, Germany.

Cardiac diseases continue to be one of the major causes of death worldwide. Ischemic heart disease such as myocardial infarction can result in an irreversible replacement of healthy, contractile heart tissue with a non-contractile, collagen-rich fibrotic scar. As the intrinsic proliferative capacity of adult cardiomyocytes is limited, future therapeutic approaches to mend ischemic heart diseases can hugely benefit from a better understanding of the regulatory mechanisms involved in cardiomyocyte proliferation.

Searching for novel regulators of cardiomyocyte proliferation, we isolated the mutant zebrafish line *weiches herz (whz)* in an ENU mutagenesis screen. *Whz* mutant embryos exhibit a cardiac phenotype, with a thin ventricular myocardial layer. Positional cloning identified a missense mutation in the *tbx20* gene as cause for the *whz* phenotype.

Although, *tbx20* mRNA was not found to be down-regulated in *whz* mutant embryos, we found a severe reduction in Tbx20 protein levels. This suggests that the *whz* mutation leads to destabilization and subsequent degradation of Tbx20 in *whz* zebrafish embryos. Furthermore, injection of a morpholino antisense oligonucleotide against *tbx20* mimicked the *whz* phenotype, confirming that the *whz* mutation causes a loss of Tbx20 function.

Interestingly, counting of cardiomyocytes in *whz* mutant ventricles revealed significantly reduced numbers of cardiomyocytes compared to wild-type littermates at 72 hours post fertilization. To assess whether decreased cardiomyocyte numbers is due to less cardiomyocyte precursors in the Anterior Lateral Plate Mesoderm, we evaluated the cardiac precursor cell population by *cmlc2*-specific *In situ* hybridization at 14 and 20 somite stages. We found no reduction of cardiomyocyte precursors in *whz* mutant embryos, suggesting that impaired cardiomyocyte proliferation later during cardiogenesis might account for reduced cardiomyocyte numbers in *whz* mutants. To address this question, we performed EdU staining at 72 hours post fertilization to measure proliferating cardiomyocytes in *whz* embryos and found that indeed cardiomyocyte proliferation was severely reduced in the mutant embryos compared to the wild-type littermates. We also performed TUNEL staining to analyze the apoptosis in our *whz* mutants and found no difference in cell death compared to the wild-type littermates.

Taken together, our data indicate that the loss of *tbx20* in zebrafish embryos result in a reduced number of cardiomyocytes caused by impaired proliferation of cardiomyocytes.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

Z6062B Cytoskeletal regulation by *racgap1*: required for more than just cytokinesis. *S. E. Richards*, R. M. Warga, D. A. Kane. Western Michigan University, Kalamazoo, MI.

Cytokinesis is tightly regulated by the actions of a key protein complex known as centralspindlin. Composed of two dimers of mitotic kinesin-like protein MLKP1 and the Rho family GTPase-activating protein RacGAP1, centralspindlin positions and assembles the actomyosin ring driving furrow ingression and coordinates abscission. Like in files and worms, cells in the zebrafish *racgap1* mutant*, ogre* (*ogr*), become binucleate and even quadnucleate during early development. Although cytokinesis fails, development proceeds albeit somewhat abnormally. Here we present evidence that RacGAP1 plays significant roles in other cellular processes independent of cytokinesis during development. Using confocal microscopy, we show that cell junctions in the mutant are disorganized; further inspection of the membrane reveals that β-catenin is no longer sequestered at the plasma membrane and instead is mislocalized to the cytoplasm. These results suggest that *racgap1* may have a function in cell adhesion. To investigate this hypothesis further, we created double mutants with *half baked* (*hab*), carrying a mutation in the cell adhesion protein E-cadherin. Previously, we have shown that *hab* mutants have a zygotic maternal dominant effect that is expressed when both zygotic and maternal genomes are heterozygous for the mutant locus. These *hab*(-/+) embryos display an epiboly phenotype that is less severe than that of its homozygous mutant siblings; unlike the homozygous mutants, the heterozygous population is able to recover. Embryos that are *ogr(-/-);hab(-/+)* show a delay in epiboly that is more marked than *hab*(-/+) heterozygotes alone; interestingly, these double mutants display a distinctive, repetitive start and stop pattern that is unlike either the heterozygous or homozygous *hab* mutant. Supporting the idea that like E-cadherin, RacGAP1 regulates cell behavior by maintaining cell adhesion, we analyzed the collective migration of the dorsal forerunner cells (dfc) and found that *ogr*(-/-);*hab*(-/+) embryos have a greater tendency than *hab*(-/+) embryos for the dfc to break apart into three or more clusters. Therefore cells appear to be less adhesive when both *racgap1* and E-cadherin are compromised. Our results also indicate that RacGAP1 may have a role in regulating the cytoskeletal framework surrounding the nucleus for when *ogr*(-/-)*;hab*(-/+) embryos eventually reach somatogenesis, they develop a unique nuclear morphology. We conclude that the effects on cytokinesis have obscured the pleiotropic roles of RacGAP1 in mediating other cellular processes and that in particular it may be required for regulating the cytoskeleton at the cell surface and possibly at the nuclear membrane.

Z6063C Roles of RyR-mediated intracellular calcium mobilization in tissue patterning during development. *Erin Ritchie*, Alex Chagovetz, David Grunwald. University of Utah, Salt Lake City, UT.

The Ryanodine Receptor (RyR) intracellular calcium release channels (CRC) regulate release of calcium from intracellular stores in the ER/SR. Though expressed in many cell types, they are best known for their roles in muscle contraction; indeed loss-of-function mutations are associated with myopathy in humans. We propose this narrow view of the RyR function fails to account for the full range of phenotypes observed in humans carrying *RYR1* mutations, which include slow muscle cell defects. We are interested in understanding how each of the *ryr*s contributes to development and behavior. We have generated null *ryr* mutants in each of the genes that contribute to skeletal muscle function in zebrafish. Our results show *ryr1a* appears to be specific to slow muscle fibers, while *ryr1b* and *3* seem to be fast muscle specific. Surprisingly, both *ryr1a* and *ryr3* are dispensable, and we are interested in how calcium release during muscle contraction and behavior are affected. We have used GCaMP6 to visualize calcium fluctuations in both slow and fast muscle fibers for null *ryr* mutants. Muscle contractions are electrically stimulated and imaged using a SPIM microscope. Behavioral assays have also been used to characterize the phenotypes of single and combinations of *ryr* mutants. Our preliminary results indicate: First, animals lacking the RyR isoform specific to slow muscle fibers have no functional slow muscle, exhibit altered movement behaviors as embryos and adults, and are fully viable in the laboratory. This has given us a probe to investigate the role of this subset of muscle. Second, *ryr* mutants have defects in muscle cell differentiation in addition to defects in muscle contraction. Third, triple mutants that are completely paralyzed have distinct malformations in craniofacial development that are wholly secondary to loss of muscle contractility.

Z6064A Gpr15 Adhesion GPCR is an essential component of the Wnt/Planar cell polarity signaling during zebrafish early development. *I. Roszko*[1], X. Li[2], D. Sepich[1], F. Marlow[2], L. Solnica-Krezel[1]. 1) Washington University School of Medicine, Saint Louis, MO; 2) Albert Einstein college of Medicine, Bronx, NY.

Planar cell polarity (PCP) signaling has been shown to be essential for the polarization of mesenchymal cell behaviors during vertebrate gastrulation. Convergence and extension gastrulation movements narrow the embryonic tissues mediolaterally while extending them in the anteroposterior direction. These movements are driven by polarized directed migration and intercalation of mediolaterally elongated cells. Zebrafish mutants carrying mutations in or overexpressing the core Wnt/PCP proteins present impaired mediolateral cell polarization and movements leading to phenotypically shorter and broader embryos. We have identified the G-protein coupled receptor protein Gpr125/Adgra3 as a novel modulator of the Wnt/PCP pathway. We showed that Gpr125/Adgra3 protein is able to recruit the core Wnt/PCP component Dishevelled (Dvl) to cell membrane subdomains and to promote the localization of Frizzled7 (Fzd7) and Glypican 4 into these subdomains. Molecular analyses demonstrated a direct interaction of Gpr125 intracellular domain with Dvl. Using TALEN technology, we generated *gpr125* mutants. Whereas zygotic indel *gpr125* mutants do not exhibit any visible gastrulation defects, mutants lacking both maternal and zygotic *gpr125/adgra3* function show mild C&E defects and consequently shorter and wider embryonic bodies. *gpr125/adgra3* mutants show a strong genetic interaction with the *vangl2* and *scribble1* mutants. These results indicate an essential role for Gpr125 during early embryogenesis. Gpr125/Adgra3 is a seven pass transmembrane protein with a long N terminal and C-terminal fragment. The specific functional domains of Gpr125/Adgra3 regulating particular morphogenetic behaviors as well as the downstream signaling partners are unknown to date. The results of ongoing analyses of the MZ*gpr125/adgra3* mutants and the Gpr125/Adgra3 protein's functional domains will be presented.

Z6065B Loss of SET- and MYND-domain-containing protein 1a (SMYD1a) leads to sarcomeric disorganization in zebrafish. *S. Rudeck*, W. Rottbauer, S. Just. University Hospital, Ulm, Baden-Württemberg, DE.

Assembly, maintenance and renewal of sarcomeric units require highly organized and balanced folding, transport, modification and degradation of sarcomeric proteins. However, mechanisms that regulate these fundamental processes are only poorly understood, but of great clinical importance, since many cardiac and skeletal muscle diseases are associated with defective sarcomerogenesis.
The mutant *flatline* (*fla*) shows disturbed sarcomere assembly exclusively in heart and fast-twitch skeletal muscle. We identified a nonsense mutation within the *SET- and MYND-domain-containing protein 1* gene (*smyd1b*) to be responsible for the *fla* phenotype. Smyd1b localizes to the sarcomeric M-line, where it associates with myosin. Moreover, the zebrafish genome contains a second highly conserved *smyd1*-orthologue, *smyd1a*, which has minor importance for myofiber organization. We found that *smyd1a* mRNA, similar to *smyd1b*, is strongly expressed in skeletal muscle cells and to a weaker extent in the embryonic heart. To characterize the *in vivo* function of *smyd1a*, we performed a morpholino-mediated knock-down of *smyd1a*, which leads to a cardiac defect accompanied by progressive reduction of ventricular contractility. To investigate whether the observed cardiac defects are caused by defective specification of cardiomyocytes, we performed a MF20/S46 immunostaining and found normal specification of atrial and ventricular cardiomyocytes. Furthermore, MF20 staining of *smyd1a* morphants revealed disorganization of skeletal muscle sarcomeres. To assess whether the observed sarcomeric disorganization is caused by impaired skeletal muscle development, we further investigated the expression of myoG and myoD by performing *in situ* hybridization and found no alteration compared to controls, indicating that loss of Smyd1a function does not affect early skeletal muscle development. To compare the localization of the different *smyd1*-orthologues, we generated transgenic lines using a heart and skeletal muscle specific *unc45b*-promoter followed by *smyd1a*- or *smyd1b-gfp* fusion constructs. Interestingly, both orthologues, Smyd1a and Smyd1b localize in an alternating pattern to α-actinin, indicating M-band localization, and implying that both proteins might have redundant functions. To evaluate this hypothesis, we performed rescue experiments by injection of *smyd1a*- or *smyd1b*-mRNA into *fla* mutant oocytes. Remarkably, *smyd1b* deficiency was functionally and structurally rescued by ectopic expression of either *smyd1a*-mRNA or *smyd1b*-mRNA, demonstrating redundant roles for Smyd1a and Smyd1b regarding the control of sarcomere organization in zebrafish.

Z6066C Identification of neuromast disruptor compounds through *in vivo* screening in zebrafish . *Rachna Sachanandani*[1], Savini Thrikawala[1], Cliff Stephan[2], Nghi Nguyen[2], Mary Sobieski[2], Richard Judson[3], Jan-Åke Gustafsson[1], Maria Bondesson[4]. 1) Department of Biology and Biochemistry, CNRCS, UH, Houston, TX; 2) Center for Translational Cancer Research, Texas A&M Health Science Center, IBT, Houston, TX; 3) National Center for Computational Toxicology, Office of Research and Development, US EPA, Research Triangle Park, NC; 4) Department of Pharmacological and Pharmaceutical Sciences, UH, Houston, TX.

Many industrial chemicals, such as pesticides, are released into the environment without a full assessment of their adverse effects on human life and the ecosystem. We aim to understand the developmental toxicity effects of environmental pollutants by using the vertebrate zebrafish (*Danio rerio*) as a model to identify neuromast disruptors. The zebrafish lateral line responds to the flow of water via receptors called neuromasts, which consist of a cluster of hair cell surrounded by a ring of supporting cells and innervated by sensory neurons. Neuromasts are positioned along the zebrafish body through migration of the neuromast primordium formed in the head region. The same family of chemokine receptors that are responsible for neuromast migration also guide metastasis of certain cancer cells (Galardo et al., Disease Models & Mechanisms (2015) 8, 565-576). Transgenic zebrafish embryos expressing fluorescent markers in neuromasts were used to visually monitor neuromast development under normal and perturbed conditions using the control compounds AG1478 and VPA. Next, these embryos were used in a primary high throughput screen (HTS) of 309 (294 unique) compounds of the ToxCast phase I chemical inventory, primarily consisting of pesticides and antimicrobials. This generated a list of 48 hits that altered neuromast development and/or migration. These hits were re-screened with a higher number of replicates to characterize effects and to determine the lowest effect levels (LELs). A univariate analysis was performed to identify ToxCast *in vitro* assays that significantly correlated with the identified *in vivo* neuromast disruptors. In addition, the effect of IT1t, which has been shown to inhibit the metastasis of MDA-MB-231 cells *in vivo* (Tulotta, C. et al. Disease models & mechanisms (2016) 9, 141-153), was tested on neuromast migration. To further investigate the connection between cancer metastasis and neuromast migration, we investigated the effects of neuromast disruptors on the migration of MDA-MB-231 cells. In conclusion, our results identified several environmental pollutants with neuromast disrupting capacity, suggesting that the developing zebrafish embryo is an efficient *in vivo* model that can be used for identification of compounds interfering with cell migration.

Z6067A Intracellular calcium release by Ryanodine Receptors is required for Hh-dependent cell formation and gene expression. *D. K. Shaw*, M. Jurynec, K. Hoshijima, D. Gunther, D. J. Grunwald. University of Utah, Salt Lake City, UT.

The Hedgehog (Hh) pathway has indispensable functions in development and tissue homeostasis. Hh ligand acts as a morphogen, signaling across developmental fields creating a gradient that leads to patterned cell differentiation. Proper differentiation depends on slight differences in ligand concentration; therefore, small fluctuations in ligand or modulations of signaling efficiency have significant impacts on development and homeostasis. This work focuses on characterization of a new and unexpected modulator of this pathway, the intracellular mobilization of calcium via Ryanodine Receptors.

Ryanodine receptors (RyRs) mediate release of calcium from intracellular stores contained in the endoplasmic/sarcoplasmic reticulum and the nuclear envelope. We have found that disruption of RyR function in the zebrafish embryo causes loss or reduction of Hh-dependent muscle, neurons, spinal cord precursors and fins in a manner consistent with reduced Hh signaling. Furthermore, blocking RyR function reduces Hh-dependent gene expression. **My hypothesis is that intracellular calcium release from RyRs is necessary for proper Hh signal transduction.**

In future experiments, we will visualize ciliary and cytoplasmic calcium dynamics during periods of Hh signaling *in vivo* in zebrafish. Additionally, we are currently using novel gene targeting in zebrafish to generate a *Smo-mCherry* targeted knock-in fusion protein that will

allow us to visualize Hh component trafficking in real-time. We will visualize Smo trafficking defects upon depletion of RyR function. Our ultimate goal is to determine where in the Hh pathway RyR function is required.

Z6068B Zebrafish Marcksb regulates dorsoventral axis formation by controlling BMP secretion. *Yonghua Sun*, Xiaosi Wang, Ding Ye, Changyong Wei, Houpeng Wang. Institute of Hydrobiology, CAS, Wuhan, China.

Bone Morphogenetic Proteins (BMPs) plays a pivotal role in dorsoventral (DV) patterning during early embryogenesis. Myristoylated alanine-rich C kinase substrate (MARCKS), a major substrate for protein kinase C, has been shown to regulate cell motility and membrane trafficking. Here, we show the essential role of Marcksb in zebrafish DV patterning. There are 4 MARCKS family genes, *marcksa*, *marcksb*, *marcksl1a* and *marcksl1b* in zebrafish genome, and *marcksb* is the only one showing maternal expression. Knockdown of *marcksb* but not the other 3 MARCKS family members resulted in dorsalization, and the dorsalization defects could be partially rescued by overexpression of each of the 4 genes. The overall BMP signaling activity was strongly reduced in the *marcksb* morphants, as revealed by analysis of BMP targets and phosphorylated Smad1/5/8 levels. Furthermore, Bmp2b-induced ventralization was blocked by knockdown of *marcksb*, indicating that Marcksb is required for proper BMP signaling. Mechanically, we found that the mCherry-fused Bmp2b proteins were largely reduced at ex-cellular space in *marcksb* morphants comparing to wildtype embryos, suggesting that the proper secretion of Bmp2b was disrupted in *marcksb* morphants. We generated maternal-zygotic mutants of *marcksb* (MZ*marcksb*) using TALEN technology. Although *marcksb* is transcriptionally silent in MZ*marcksb*, defects of DV patterning was not observed in those embryos. In contrast to the *marcksb* morphants, the MZ*marcksb* embryos showed strong upregulation of *marcksa*, *marcksl1a* and *marcksl1b*. This suggests that genetic compensation effects occurred when *marcksb* was genetically absent. Therefore, our findings reveal a novel function of *marcksb* in DV patterning possibly by regulating the secretion of BMP ligands.

Z6069C Role of MK2/TTP pathway in early development and innate immunity in zebrafish. *B. Tandon*, GM. Young, SGDLT. Canny, MC. Gustin, DS. Wagner. Rice University, Houston, TX.

The p38 mitogen-activated protein kinase (MAPK) pathway is involved in many important cellular processes, including cell growth, cell survival and immune response. Previous work done in our lab shows that p38 pathway plays an important role in early development in zebrafish. p38 MAP kinase activates MAP-kinase-activated protein kinase (MK2)/*betty boop (bbp)*. Embryos from mutant *mk2a*/*bbp* females undergo dramatic constriction and lysis of yolk cell at 50% epiboly. Preliminary data from this project suggests that *mk2a* negatively regulates the activity of zebrafish homolog of an RNA binding protein Tristetraprolin (TTP), which in turn regulates mRNA degradation and translational repression of target mRNAs by binding to AU-rich elements (AREs) in the 3' untranslated region (UTR). We have identified putative TTP targets expressed in the yolk cell and have developed an in vivo assay to investigate the regulation of gene expression by elements in the 3' UTR.

MK2/TTP pathway is not required for early development in mammals. Instead, it's involved in inflammatory response by regulating production of important immune modulators like TNF- α and GM-CSF. Embryonic zebrafish offers unique advantages over other systems, including the transparency of larvae and the lack of adaptive immune responses for the first few weeks after hatching. Here, we use zebrafish as a model to explore the role of MK2/TTP in regulation of different components of innate immunity in their interaction with the fungus *Candida albicans.*

We hypothesize that the ancestral role of MK2 in innate immunity is conserved and that a novel function in regulating yolk cell gene expression evolved within the actinopterygian linage to regulate gene expression in the newly evolved yolk cell.

Z6070A The regulatory subunits of calcineurin differentially direct zebrafish brain development. *Robert Thorn*, Danielle Clift, Robbert Creton. Brown University, Providence, RI.

Calcineurin, a serine/threonine phosphatase that is integral for immune function has been a target for immunosuppressant drugs for decades. Use of calcineurin inhibition drugs during pregnancy is of particular concern since little is known about the effects of cyclosporine during brain development. We have previously shown that exposing zebrafish embryos to cyclosporine, a calcineurin inhibitor, has led to decreased brain size in larval zebrafish. The current studies have examined the specific role of calcineurin in the developing zebrafish brain. Zebrafish embryos were injected with varying levels of morpholinos against one of the two regulatory subunits of calcineurin (ppp3r1a or ppp3r1b). Brain sizes were assessed at 3 days post fertilization (dpf) and behavioral tests were performed at 5 dpf. At 'medium' injection levels the ppp3r1a morpholino showed a decrease in all measured brain regions, the ppp3r1b morpholino injected embryos showed only a decrease in the midbrain size. In addition, we found a significant decrease in zebrafish brain size when embryos were injected with 'low' levels of both morpholinos together, but separate injections of each morpholino showed no difference at these levels. 'Low' injection embryos underwent behavioral testing and showed abnormal social behavior in the ppp3r1a and ppp3r1b injected larvae. Additionally, ppp3r1b injected larvae were hypoactive compared to controls and ppp3r1a injected, while the ppp3r1a exhibited less thigmotaxis behavior compared to the controls and the ppp3r1b injected larvae. These results suggest an integral role of calcineurin during early brain development in zebrafish. The results also suggest that the calcineurin regulatory subunits have different roles in behavioral development during early zebrafish development.

Z6071B Regulation of cell shape changes during brain morphogenesis. *M. R. Visetsouk*, R. Garde, S. Sahu, C. Kwas, J. H. Gutzman. University of Wisconsin-Milwauke, Milwaukee, WI.

In the developing vertebrate brain, the boundary separating the midbrain from the hindbrain forms through a highly conserved folding of the neuroepithelial tissue, known as the midbrain-hindbrain boundary (MHB) constriction. During MHB formation, cells at the constriction shorten and narrow before basally constricting and apically expanding to form a sharp fold in the tissue. We previously determined that two isoforms of

the actin cytoskeletal motor protein, non-muscle myosin II (NMII), differentially regulate cell shape, with non-muscle myosin IIA specifically regulating cell length and non-muscle myosin IIB specifically regulating cell width. Non-muscle myosin II proteins are tightly regulated via the phosphorylation of their associated myosin regulatory light chains; however, the upstream signaling pathways that initiate differential regulation of NMII-mediated cell shape during midbrain-hindbrain boundary morphogenesis are not known. Our current studies have revealed that calcium signaling is critical for the regulation of cell length, but not cell width, during midbrain-hindbrain boundary formation. In particular, manipulation of cytosolic calcium levels resulted in abnormal midbrain-hindbrain boundary cell length and inhibition of cytosolic calcium rescued the cell length phenotype observed in embryos with over activation of non-muscle myosin II. In addition, we found that calcium signals mediate phosphorylation of myosin light chain in the midbrain-hindbrain boundary region. In addition, we have found that *wnt5b,* which is expressed specifically at the MHB during the time of morphogenesis, is an upstream regulator of cell width. 2D Differential Gel Electrophoresis also revealed that Wnt5b potentially regulates tubulin levels, implicating a novel role for microtubules in this process. Together our findings suggest that modulation of myosin activity by calcium and Wnt5b are critical for proper regulation of cell length and cell width at the MHB to determine embryonic brain shape. We further hypothesize that these mechanisms may be conserved and critical for shaping other epithelial cells and tissues throughout development.

Z6072C Fascin actin-bundling protein 1 is required for trafficking and signaling of TGF-β type I receptors during endoderm formation. *Qiang Wang*[1], Zhaoting liu[1], Guozhu Ning[1], Ranran Xu[1], Yu Cao[1], Anming Meng[2]. 1) Institute of Zoology, Chinese Academy of Siences, Beijing, Beijing, CN; 2) School of Life Sciences, Tsinghua University, Beijing, Beijing, CN.

Microtubules function in TGF-β signaling by facilitating the cytoplasmic trafficking of internalized receptors and the nucleocytoplasmic shuttling of Smads. However, nothing is known about whether actin filaments are required for these processes. Here, we report that zebrafish actin-bundling protein *fscn1a* is highly expressed in mesendodermal precursors and its expression is directly regulated by TGF-β superfamily member Nodal signaling. Knockdown or knockout of *fscn1a* leads to a reduction of Nodal signal transduction and endoderm formation in zebrafish embryos. Fscn1 specifically interacts with TGF-β type I receptors, and its depletion disrupts the association between receptors and actin filaments and sequesters the internalized receptors into clathrin-coated vesicles. Therefore, Fscn1 acts as a molecular linker between TGF-β type I receptors and the actin filaments to promote the trafficking of internalized receptors from clathrin-coated vesicles to early endosomes during zebrafish endoderm formation.Our findings indicate that *fscn1a* and Nodal signaling promote endoderm formation through a positive feedback loop and may allow for a better understanding of how TGF-β signaling is elevated in Fscn1 overexpressed metastatic tumors.

Z6073A Bmp3 is a novel regulator of neural crest cells and ocular fissure closure. *S. A. Widen*, P. Desai, O. J. Lehmann, A. J. Waskiewicz. University of Alberta, Edmonton, AB, Canada.

Proper development of the vertebrate embryo requires fusion of epithelial cell sheets, resulting in closure of the developing neural tube, palate and retina. Within the eye, failure of the ocular fissure to close results in ocular coloboma. Together with the etiologically related disorders microphthalmia (small eyes) and anophthalmia (no eyes), coloboma represents up to 11% of all pediatric blindness. Previous work from our laboratory and others has defined a key role for Bone Morphogenetic Protein (BMP) signaling within the retina in regulating ocular fissure closure. Outside the retina, a population of neural crest cells known as periocular mesenchyme (POM) migrates to the ocular fissure sites and is required for fissure closure, although our understanding of the mechanism remains limited. Here we identify a novel regulator of ocular fissure closure: *bmp3*. Exome and Sanger sequencing of 480 patients with coloboma has identified five with variants in *BMP3*, three of which are in the mature domain and predicted to damage protein function. The high degree of conservation at these residues, and absence of such variants in exome databases and control samples, demonstrates the likely pathogenicity of these mutations. Studies in cultured cells demonstrate that the identified BMP3 variants have altered activity *in vitro*. In zebrafish, morpholino inhibition of Bmp3 results in small eyes and fissure closure defects, implicating this ligand as a key regulator of eye morphogenesis. *bmp3* is an intriguing candidate as it is not expressed within the eye, but in cells immediately anterior to the eye, unlike any previously identified BMP ligand. Intriguingly, knockdown of Bmp3 activity causes defects in cranial neural crest cell development and fewer POM cells surrounding the eye. We hypothesize that *bmp3* is a novel factor that governs neural crest cell development. Consistent with this, *bmp3* has been previously implicated in craniofacial development. To test this hypothesis in a *bmp3* null, we have used the CRISPR-Cas9 system to generate a frameshift mutation that results in a premature stop codon upstream of the mature domain of Bmp3. Current studies are focused on defining neural crest and ocular phenotypes in a *bmp3* mutant background and elucidating the molecular mechanisms underlying Bmp3 function in neural crest development.

Z6074B MiR-145 regulates liver development through Progranulin A signaling in zebrafish. *Jen-Leih Wu*, Ya-Wen Li, Yen-Hsing Li. Academia Sinica, Taipei, TW.

The liver is an essential metabolic organ that maintains numerous vital functions in the body. The regulatory mechanisms of hepatogenesis are complex, involving many growth factors and transcription factors across three stages- hepatic specification, differentiation and outgrowth. miRNAs are known to be important genetic regulators in development. Less is known about which miRNAs participate in embryonic hepatic outgrowth. Dysregulation of miR-145 has been shown to promote pathological liver growth. However, the regulatory mechanism of miR-145 in embryonic liver development remains unclear. In this study, we demonstrated a significant decrease in miR-145 expression during hepatogenesis. We modulated miR-145 expression in zebrafish embryos using a miR-145 mimic or a miR-145 hairpin inhibitor. Impaired embryonic liver morphogenesis was observed in response to altered miR-145 expression. We further confirmed a critical role of miR-145 in hepatic outgrowth, but not in liver specification and differentiation, by whole-mount *in situ* hybridization. Loss of miR-145 expression

promoted hepatic cell proliferation, and vice versa. Our previous studies identified a growth factor, Progranulin A (GrnA), which regulates zebrafish hepatic outgrowth through MET signaling. We further revealed that GrnA is a target of miR-145 and MET signaling is also regulated by miR-145, which was verified by luciferase reporter assay and gene expression analysis. In addition, co-injection of GrnA mRNA with miR-145 mimic or MO-grnA with miR-145 inhibitor could restore the liver defects caused by dysregulation of miR-145 expression. In conclusion, our findings identified a functional role of miR-145 in zebrafish hepatic outgrowth through regulating GrnA signaling. This mechanism may be a new therapeutic target for liver failure and liver cancer.

Z6075C A family of FOX genes determines precise spatial patterns of growth and differentiation within craniofacial skeleton. *P. Xu*, B. Balczerski, A. Ciozda, G. Crump. University of Southern California, Los Angeles, CA.

FOX genes encode a large family of winged helix/forkhead transcription factors that have been shown to play multiple roles during development. While mutations in a number of FOX genes are known to cause craniofacial defects, how members of this large family coordinate development of the craniofacial skeleton remains unclear. In situ analyses in mice have led to the proposal that FOX genes form a complex expression code, much like the Dlx or Hox genes, that pattern the craniofacial primordia, yet this model remains to be tested at the functional level.

In this study, we find that the homologous FOX genes of zebrafish (*foxc1a*, *foxc1b*, *foxd1*, *foxd2*, *foxf1*, *foxf2a, foxf2b*, *foxl1 and foxl2*) are also expressed in distinct patterns within the neural-crest-derived pharyngeal arches that are the precursors to the facial skeleton. By manipulating major signaling pathways, we show that these distinct expression patterns result from differential sensitivity of FOX enhancers to Hh, Fgf, Bmp and Edn1 signaling. This suggests that FOX genes act as integrators of multiple signaling cascades in the cranial preskeletal mesenchyme. Next, we use knockout mutants and conditional transgenic misexpression approaches to show that FOX genes are required in patterning and growth of cartilage in distinct regions of the developing face, suggested that FOX genes act in region-specific manners to regulate the development of craniofacial primordia.

In summary, our evidence in zebrafish supports a model in which different members of the FOX family act very locally to specify cartilage elements of the facial skeleton. Our work indicates that expression and function of FOX genes specify major patterning domains of zebrafish pharyngeal arches.

Z6076A The molecular mechanism for the termination of segmentation clock during zebrafish somitogenesis. *T. Yabe*, S. Takada. National Institute for Basic Biology (NIBB), Okazaki, Aichi, JP.

Somites are metameric structure transiently formed beside the neural tube during vertebrate development and give rise to various tissues including vertebral bone, skeletal muscle and dermis in the adult body. During somitogenesis, somites are sequentially and periodically generated from the anterior end of presomatic mesoderm (PSM). The periodical generation of somite is known to be explained by "Clock and Wavefront" model, in which the spatio-temporal information provided by the cyclic activation of "segmentation clock" and posterior regression of "wave front" are integrated and converted to the morphological pattern of somite at the anterior PSM. In vertebrate somitogenesis, the wave of the activation of segmentation clock propagates from the posterior to anterior PSM and ceases at the anterior end of PSM, in which future segmentation boundary is formed. Previously the termination of segmentation clock was considered to be caused by the gradual decrease of oscillation frequency of segmentation clock, because wave length of segmentation clock became narrow before the termination of segmentation clock. However, recent time-lapse analysis suggested that termination of segmentation clock regulated in the manner independent to the decrease of oscillation frequency. Thus the molecular mechanisms to coordinate the formation of segmentation boundary and the termination of segmentation clock still remains to be elucidated.

In this study, we found Ripply, a groucho interacting protein, negatively regulated expression of the clock genes, *her1* and *her7*, resulting in termination of the oscillatory expression of *her1* and *her7* in the anterior PSM. Conversely, Her1 and Her7 negatively regulate expression of *ripply1* and *ripply2* in the anterior PSM, suggesting that mutual repression between ripply and her is important for segmentation clock termination. Since *ripply1* and *ripply2* are required for the formation of somite boundaries, the mutual repression is important for coupling of the timing of the boundaries formation and the clock termination in zebrafish somitogenesis.

Z6077B Foxc1a plays essential roles in zebrafish cardiogenesis. *Y. Yue*, L. He, Q. Zhang, C. Gu, Q. Zhao. Nanjing University, Nanjing, Jiangsu, CN.

Foxc1 is a highly conserved transcription factor during evolution. It plays dual roles by acting as a transcriptional activator or transcriptional repressor. Foxc1 is involved in regulating mouse out flow tract development. The mutated *FOXC1* is associated with the congenital heart diseases in human. However, its functions and the molecular mechanisms underlying the vertebrate cardiogenesis remain largely unknown. To study the function of Foxc1 in early heart development, we had generated knockout zebrafish mutants of *foxc1a*, an orthologue gene of human *FOXC1*, using TALEN. We found that the *foxc1a* null embryos exhibited severe cardiac edema from 48 hours post fertilization and died at 9-10 days post fertilization. Further analyses revealed that *foxc1a* null mutants displayed serious defects of cardiac structures and heart function. And the data from transcriptomic analysis on the mutated embryos further demonstrated that the expressions of the key genes that control heart development were greatly changed in *foxc1a*$^{-/-}$ zebrafish embryos during cardiogenesis. The roles of the gene in mediating *foxc1a* in zebrafish cardiogenesis are being investigated.

Z6078C Optical control of physiological processes in Zebrafish: the case of fgf8a. *W. Zhang*, B. Ducos, D. Bensimon. ENS PARIS, Paris, FR.

Somitogenesis is a robust mechanism of pattern formation in the development of vertebrates. Although many key factors of somitogenesis

such as fgf8a have been characterized, a quantitative analysis of their action remains poorly documented. Such investigations require the control of the concentration, timing and location of the relevant molecules, which can't be achieved simultaneously by conventional genetic knock-in/out approaches. To fill this gap our lab has developed an optical method combining the use of Gal4-ERT/UAS:fgf8a system and a caged ligand, namely caged-cyclofen. Upon illumination with UV light (at 370nm) or with a two-photon laser beam, cyclofen is released in the illuminated cell(s). It binds to the hormone binding domain of a truncated estrogen receptor (ERT) fused to the Gal4 transcription factor and then releases the Gal4-ERT from the complex it forms with cytoplasmic chaperones. The active Gal4 diffuses to the nucleus, binds to its UAS promoter and turns on the fgf8a gene. This method allows us to over-express fgf8a in live zebrafish in a controlled manner at given time and spatial location.

We have thus analyzed the effect of global over-expression of the fgf8 activated at 70% epiboly and 5 somites stage. The expression of fgf8a was measured using RT-qPCR and its action on somitogenesis was quantitated by time lapse microscopy. While the period of the somitogenetic clock was unaffected by the over-expression of fgf8a, we observed different shortening levels of the somites upon different expression levels and timing of fgf8a over-expression.

Z6079A BATCH-GE: Batch analysis of Next-Generation Sequencing data for genome editing assessment. *A. Boel*, W. Steyaert, N. De Rocker, B. Menten, B. Callewaert, A. De Paepe, P. Coucke, A. Willaert. Ghent University, Ghent, BE.

The CRISPR/Cas9 system recently emerged as the golden standard for targeted genome editing. The simplicity of this technique has enabled the high-throughput set-up of CRISPR/Cas9-based experiments. Analysing these experiments however, is a challenging and time-consuming task. Next-Generation Sequencing (NGS) has been replacing standard techniques such as the T7 Endonuclease I assay, due to its high capacity, sensitivity and ever-decreasing cost. However, the analysis of large amounts of NGS data is a significant hiatus in the otherwise advanced field of CRISPR/Cas9. In this work, we present and evaluate a straightforward tool, BATCH-GE, that performs batch analysis of NGS data for the assessment of knock-out and knock-in genome editing experiments.

BATCH-GE shows a number of important advantages over the current NGS-based genome-editing analysis methods. The tool's most striking asset and improvement, is that it allows for batchwise analysis of a large number of samples. Furthermore, BATCH-GE is implemented as a freely available script, and is therefore available for further optimization by the user. Besides, it can run either within a server environment or on a stand-alone computer, providing an ensured availability. In addition, the user is only required to complete two simple input files, containing easy-to-determine variables that provide the user with the necessary flexibility. Lastly, BATCH-GE enables the assessment of both indel generation as well as HDR-mediated precise genome editing experiments.

BATCH-GE generates four comprehensive text files. The first file lists genomic region, type, length and frequency of every detected indel variant, providing a detailed overview of all sequence alterations. In the second file, a distinctive analysis of full and partial HDR events is carried out. Third, general indel and repair rates are summarized, enabling a quick and straightforward evaluation of the overall mutation efficiency. In the fourth file, URLs are provided to visualize the reads in the UCSC genome browser, offering the possibility to look into the specific sequence alterations.

To evaluate the performance of BATCH-GE, the tool was used for genome editing assessment in two zebrafish experiments, covering sgRNA efficiency testing and precise genome editing via HDR, validating that BATCH-GE is a new and reliable tool for the analysis of NGS-derived genome editing data and contributes to a faster, more informative and flexible analysis of multiple genome editing experiments.

Z6080B Programming the Third Genome Through Mitochondrial DNA Editing. *J. M. Campbell*, E. Perales Clemente, H. Ata, T. J. Nelson, S. C. Ekker. Mayo Clinic, Rochester, MN.

Of the three major genomes that contribute to our health - the nuclear genome, the mitochondrial genome and the bacterial genomes of our microbiome – only the mitochondrial genome has yet to be programmed. The highly conserved mitochondrial DNA (mtDNA) genome consists of 37 genes essential for electron transport chain function, and mutations in mtDNA can cause disease. Much is not known about these diseases because of a lack of sufficient models, and there currently is no long-term treatment for these patients. The ability to edit the mtDNA genome would enable both the interrogation of gene function as well as facilitate the study of mtDNA-based genetic diseases.

Here, we describe to our knowledge the first evidence of mtDNA programming using site-specific mitochondrial DNA enzymes. We use zebrafish as our model system to test these tools. Zebrafish mitochondrial DNA are similar in gene number and order to mammalian models and are readily microinjected to deliver editing tools. We initially deleted a 5kb fragment of DNA between *mt-nd5* and *mt-atp8* by using novel custom enzyme tools. This corresponding deletion is found in humans to cause the disease Kearns-Sayre Syndrome (KSS). Approximately 35% of injected animals carried a detectable deletion, with no deletion detectable in uninjected animals. The resulting deletion was sequence-verified to be mtDNA. To develop mitochondrial DNA carrying a single gene deletion, we then demonstrated that a single 2kb region encoding *mt-nd4* could also be deleted using the same strategy.

We next asked whether we could improve the efficiency of editing by selecting for deleted mtDNA using a site-specific nuclease that targets only the non-deleted genomes. Double-strand breaks are not repaired efficiently in mitochondria, and enzymes that cause DSBs degrade their target rather than inducing NHEJ repair. This represents a major technical challenge in editing mtDNA using standard approaches deployed in the nucleus. By coinjecting the *mt-nd5* and *mt-atp8* custom editors along with a nuclease targeting *mt-nd4,* we were able to detect deletions in over 90% of injected animals, a 2.5-fold improvement. The respiration capacity of these animals were measured, and those injected with custom mitochondrial editors and a nuclease were 4 times lower than uninjected animals and 3 times lower than nuclease-alone injected

animals.

This method of using custom mitochondrial DNA editors combined with nucleases to program the mtDNA genome enable more precise mtDNA disease modeling and single gene function studies.

Z6081C Influences of the gut microbiome on behavioral and stress responses in isogenic mice and zebrafish populations. *D. J. Davis*, H. M. Doerr, A. K. Grzelak, A. C. Ericsson, E. C. Bryda. University of Missouri, Columbia, MO.

The gut microbiota (GM) represents a dynamic microbial community whose collective set of genes encodes a vast array of functions. These microbes play a major role in many physiological processes within the host and are essential for health and well-being. Ongoing research suggests that the GM is not only involved in gut physiology but may also have significant effects on many other aspects of health and disease. However, many researchers are unaware of the impact the GM can have on their animal models, which can hinder reproducibility. Here, we demonstrate the ability of the GM to influence a broad array of behavioral phenotypes in both mice and zebrafish. First, we show that isogenic mice with divergent GM exhibit different, sex-specific neurobehavioral phenotypes. Specifically, male mice with a less diverse GM spent significantly more time in the open arms of an elevated plus maze and displayed less time immobile during a forced swim test. Opposing effects were observed in female mice, wherein a more diverse GM resulted in reduced anxiety- and depression-related behavior. Moreover, we demonstrate that the GM mitigates anxiety-related behavior and is required for characteristic stress responses in zebrafish larvae. Germ-free zebrafish larvae displayed significantly less thigmotactic behavior and exhibited a blunted response to an osmotic stress test compared to conventionally raised larvae. Lastly, we show that alterations of the GM via *Lactobacillus plantarum* supplementation can reduce anxiety-related behavior and protect from stress-induced dysbiosis in adult zebrafish. These findings underscore how changes in GM that can occur during model development may contribute to phenotypic differences of various animal models. Identifying and maintaining a consistent GM will not only facilitate in providing a robust phenotype for particular animal models but also aid in generating reproducible results between experiments.

Z6082A Cdk5-mediated kinase cascade regulates morphogenesis of the intrahepatic biliary network. *M. Dimri*, C. Bilogan, T. Sakaguchi. Cleveland Clinic, Cleveland, OH.

Biliary atresia (BA) is a life threatening liver disorder in infants characterised by the disruption of the biliary system. Various types of BA are associated with the alterations in the intrahepatic biliary network, a complex three dimensional network of conduits lined by biliary epitheilal cells. However, the lack of methodologies for accurately and consistently quantitate the difference in three dimensional branching patterns has impeded the study. We thus designed a computer based algorithm that quantitatively computes the three dimentional structure of the network. We have used the computational algorithm in combination with the systematic screening of small molecule inhibitors and identified that inhibiting cyclin dependent kinase 5 (Cdk5) led to a biliary branching defect. Inhibition of Cdk5 did not alter the total number of biliary epithelial cells; however, decreased the connections of the intrahepatic biliary network. We further identified a downstream kinase cascade regulated by Cdk5, which in turn regulates the activity of Cofilin, an actin severing protein. We showed that chemical manipulations to the kinase cascade changed the activity of Cofilin and hence influenced the actin dynamics in the biliary epithelial cells. Our results provide a new insight into the Cdk5-mediated kinase cascade in actin remodeling and the branching morphogenesis of the intrahepatic biliary network.

Z6083B Optimizing CRISPR/Cas9 rates of mutagenesis and germ-line transmission. *B. Feldman*, C.-H. Tsai-Morris, W.-C. Tseng, F. Porter, G. Trivellin, C. Stratakis, M. Miller, B. Weinstein, D. Martinelli, S. Kaler, S. Krispin. NIH - NICHD, Bethesda, MD.

Adapting published protocols of gRNA synthesis and lesion detection[1-2], we have used CRISPR/Cas9 mutagenesis to target more than 30 genomic loci. Most of the gRNAs we used were taken from the CRISPRscan track[3] and >75% induced a degree of target disruption that we find to be sufficient for the generation of loss-of-function mutations that are germ line transmittable. However, with the exception of the highest-magnitude prediction scores, we observe no strong correlation between the magnitude of CRISPRscan's gRNA-efficiency prediction scores and our quantification of gRNA efficiency outcomes. Thus, most listed CRISPRscan gRNAs are sufficient for mutant allele generation, but if particularly high cutting rates are needed, as may be for F0 phenotyping or homology-directed repair projects, gRNAs with the very highest CRISPRscan prediction scores may be preferable. We will present prediction-outcome correlations for our most up-to-date set of gRNAs, including correlations, where available, with scores from other gRNA design resources that employ distinct prediction algorithms.

Leveraging the ability to multiplex gRNAs in a single injection[2] we are also exploring a strategy of including an extra gRNA, *tyr*-gRNA[4], which targets the *tyrosinase* gene locus, in our microinjections. *tyr*-gRNA causes an observable loss of pigment in embryos and adults >48 hours post-fertilization and accordingly serves as an internal control for injection quality, Cas9 efficiency, and selection of F0 adults with extensive albinism, which presumably indicates they are more likely to transmit germ-line mutations. We have tested this strategy for a limited number of F0 adults, leading to successful egg production in 3 of 4 lines tested. With more *tyr-gRNA*-injected F0s in our pipeline, more definitive data is forthcoming, but we currently hypothesize that inclusion of *tyr*-gRNA facilitates our mutant-generation pipeline with no significantly adverse effects on viability or breeding.

[1]Varshney GK et al., (2015). Genome Res., 25(7):1030-42.
[2]Carrington B et al., (2015) Nucleic Acids Res., 43(22):e157
[3]M. A. Moreno-Mateos et al., (2015). Nature Methods 12,982–988
[4]L.E. Jao et al., (2013) Proc. Natl. Acad. Sci. U.S.A., 110:13904–990.

Z6084C Intraspecific susceptibility to environmental toxicant PCB 126 mediated by variation in xenobiotic metabolism gene *cyp1a* in zebrafish. *L. Holden*, P. Vu, K. Brown. Portland State University, Portland, OR.

Understanding the hypervariable effects of environmental toxicant exposure within and between species is vital for ecosystem modeling and remediation. Our aim is to discern the genetic components underlying intraspecific variation in environmental toxicant susceptibility. Zebrafish, *Danio rerio*, exhibit variable susceptibility across lab strains to 3,3',4,4',5-pentachlorobiphenyl (PCB 126), a ubiquitous aromatic hydrocarbon with a high toxic equivalence factor. SNP haplotypes explain 24% of the phenotypic variation in the PCB-susceptibility trait, but the remaining 76% is unknown. Interestingly, 15% of the zebrafish genome is comprised of genomic copy number variants (CNV)—large deletions or duplications—and CNV loads differ between zebrafish strains. We hypothesize that CNV are significant contributors to variation in the PCB 126-susceptibility phenotype.

We assessed CNV across two biomarker genes, aryl hydrocarbon receptor 2 (*ahr2*) and cytochrome P450 1A (*cyp1a*), using publicly available microarray data (GEO: GSE28328) and measured post-exposure mRNA expression levels of the same genes in three strains of zebrafish (AB, TU, WIK). CNV presence was determined using mean log2 ratios over three consecutive probes. Hepatic mRNA expression levels in adults exposed to environmentally relevant levels of PCB 126 for 0, 3, 6, 9, 12, 24, and 48 hours (n=10/timepoint) were compared with time-matched vehicle controls. *ahr2* and *cyp1a* expression levels were assessed in total mRNA via RT-qPCR, normalized to *β-actin*, and quantified using the relative fold change ΔΔCt method.

We found no evidence of CNV across *ahr2*, but found evidence of a deletion across WIK *cyp1a* exons 2-5 of 7 compared to AB and TU strains. PCR confirmation across the identified deletion in *cyp1a* exposed intact gene region frequencies of 0.64 in AB, 1.00 in TU, and 0.40 in WIK (n=25/strain). Post-exposure mRNA levels support increased expression in both *ahr2* (4-fold) and *cyp1a* (14-fold) mRNA in WIK as compared to AB and TU strains. An increase in *cyp1a* expression in WIK may indicate that the identified deletion may be across an intronic repressor region. These results support our hypothesis that CNV are important contributors to interspecific variation in a receptor-mediated xenobiotic metabolism pathway. Alternatively, we may have identified a novel *cyp1a* subtype in zebrafish lacking exons 2 and 3, similar to *cyp1a2* found in some mammals.

Z6085A Zebrafish Genomics Resources – What's There and What's Next? *K. Howe*, J. Loveland. Wellcome Trust Sanger Institute, Cambridge, GB.

For the last decade we have been holding hands-on workshops at the European and International Zebrafish Conferences to introduce zebrafish researchers to web resources for zebrafish genomics data. These workshops provided talks and exercises about the generation, evaluation and improvement of the genome reference sequence assembly, the reference gene set and state-of the art web resources used for data access, analysis and interpretation. In the absence of a framework to provide such a workshop this year, we will present a summary of the usual topics and recent updates.

Both the reference genome assembly, curated by the Genome Reference Consortium, and the gene set, curated by the Havana group, have reached a level of quality that is comparable to that of other model organisms, for instance mouse. This enables us to now embark on sequencing and analysing additional zebrafish strains, and identifying notable variation to be included in future assembly releases.

As always, we welcome community input to alert us to remaining issues and focus on their resolution. Please visit us at the poster and/or write to us at zfish-help@sanger.ac.uk. The latest gene sets can be accessed at vega.sanger.ac.uk and the assembly evaluation browser gEVAL (geval.sanger.ac.uk) allows the assessment of individual genomic regions to support further improvements.

Z6086B Leveraging comparative genomics for zebrafish annotation. *Jane Loveland*, Sarah Donaldson, Deepa Manthravadi, Jen Harrow. Wellcome Trust Sanger Institute, Cambridge, GB.

A high quality reference genome and gene set is essential. In the Human and Vertebrate Analysis and Annotation (HAVANA) team we use our Otter/Zmap annotation tools to manually annotate the zebrafish genome, GRCz10, and collaborate closely with ZFIN to provide an accurate, dynamic and distinct resource for the zebrafish community. We annotate multiple biotypes, so as well as protein coding genes, we annotate pseudogenes and long non-coding RNAs (lncRNAs). Within these main biotypes we also have over 50 controlled vocabulary terms to further categorise and describe our genes. Our annotation tools also allow us annotate multiple species at the same time and unlock the power of comparative genomics, which can tell us a great deal about how genes and genomes evolve.

Gene clusters are a particular target for manual annotation as they are difficult to annotate with automated methods, such as Ensembl. One such cluster is the Olfactory receptors, which consists of protein coding genes and pseudogenes, whose numbers and biotypes vary hugely between organisms, and are the largest multigene family in vertebrates. We are manually annotating these genes and have found them to be greatly expanded in the mouse genome (~1500 genes/pseudogenes) relative to human (~900 genes/pseudogenes), and much fewer in the zebrafish genome (~135 genes, of these only 3 are pseudogenes).

LncRNAs show an absence of sequence homology between different organisms. Despite this many exhibit positional synteny, such as the lncRNA gene *sox2ot,* which suggests functional conservation. The *sox2ot* gene has been manually annotated and shown to be highly conserved between human, mouse and zebrafish and is thought to be important during embryo development and is deregulated in cancer.

Our experience of annotating across several species is proving to be a powerful tool for gene discovery in model organisms.

All of the manual annotation is publicly available from the Vertebrate Genome Annotation database (VEGA), and this is merged with the Ensembl gene set quarterly. Annotation is a continuous process and so between database updates all new annotation is made available for all of our whole genome species in the update track in VEGA: http://vega.sanger.ac.uk/info/data/frequent_update.htm

Our annotation software is freely available via our website: http://www.sanger.ac.uk/resources/software/otterlace/.

Z6087C The Status of Line Rederivation At The Zebrafish International Resource Center (ZIRC). *A. Nasiadka*, J. Hwang-Shum, J. Matthews, K. Core, E. Loucks, D. Marston, J. Murphy, E. Williams, D. Lains, A. Freeman, R. Holland, M. Westerfield, Z. M. Varga. ZIRC, University of Oregon, Eugene, OR.

As the zebrafish research community grows, an increasing number of shared resources are generated. At the ZIRC, adjustments are continually made to accommodate these changes and maintain a balance between efficient resource regeneration and effective distribution. For example, the inventory of mutant and transgenic lines at the ZIRC has increased by over 1000% within the last five years. This increase impacted greatly the strategies undertaken for line identification, regeneration, quality control, and distribution. Previously, most fish lines were mono-allelic. A large number of these lines were maintained as stocks consisting of identified transgene and mutation carriers. The lines were distributed as genotyped adult fish or embryos obtained from natural crosses. Now, most of the ZIRC lines are multi-allelic, and they arrive as frozen sperm. Distribution of these lines involves shipment of embryos generated by in vitro fertilization (IVF) using thawed sperm. We rederive lines to amplify samples in the ZIRC sperm bank. This
process depends strictly on the demand. Lines frequently requested are regenerated more often than lines that are seldom ordered. Regeneration of multi-allelic mutant lines required a significant increase of the genotyping throughput at the ZIRC. We handled this in part by using Kompetitive Allele Specific PCR (KASP) genotyping (LGC Genomics). With this technique, genotypes are determined based on a fluorescence detection system rather than time-consuming restriction enzyme digestion and gel electrophoresis. Due to the immense increase in the number of imported lines, we perform quality control for new imports primarily during line regeneration rather than at the time of importation as previously. Thus, the ZIRC staff no longer confirms all of the distributed genotypes. To reflect the status of ZIRC sperm samples with regard to line identification, we now provide sperm sample
categories. These include a category for samples derived from males whose genotypes were confirmed at the ZIRC as well as a category for samples with genotypes confirmed elsewhere. Only lines with genotypes verified at the ZIRC are distributed without a disclaimer. Lines genotyped elsewhere or whose genotypes have not been confirmed are offered with specific disclaimers.

Z6088A NCBI's Zebrafish Genome Resources. *N. A. O'Leary*, T. D. Murphy, K. D. Pruitt. National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, MD.

Genomic research on model organisms such as zebrafish (*Danio rerio*) increasingly relies on the availability of high-quality annotated reference genomes to facilitate consistent reporting and mapping of genetic data. The Reference Sequence (RefSeq) project (https://www.ncbi.nlm.nih.gov/refseq/) at the National Center for Biotechnology Information (NCBI) provides a comprehensive annotation of the zebrafish Tuebingen strain reference genome assembly (GRCz10) maintained by the Reference Genome Consortium (GRC). The zebrafish RefSeq dataset is generated by a combination of computational analysis and manual curation that results in an annotation that focuses on representation of all full-length, non-redundant transcripts. The primary sources of data used in this annotation pipeline include mRNAs, expressed sequence tags (ESTs), protein data, RNA-seq data, and protein homology. Zebrafish is one of a select group of vertebrates that are the major focus of RefSeq's manual curation efforts, which involves the in-depth review of sequence data to define new transcript variants, resolve sequence errors, and remove inaccurate information. We also collaborate with expert groups, including the Zebrafish Information Network (ZFIN) and UniProtKB, to provide appropriate annotation and nomenclature for both genes and proteins. In addition to zebrafish, NCBI provides stable reference genome annotation for other fish species with high-quality genome assembly data submitted to NCBI's Assembly resource (https://www.ncbi.nlm.nih.gov/assembly/). To date, 26 other fish species have RefSeq annotated genomes, providing a valuable resource for comparative genomic research. In this poster presentation we will provide an overview of NCBI's zebrafish genome resources and highlight the utility of these resources to the zebrafish research community. We will also provide practical guidance on how to access RefSeq data and tools for analysis of individual genes as well as whole genome datasets.

Z6089B Improving Homology-Directed Repair efficiencies in zebrafish. *Andy Willaert*, Annekatrien Boel, Hanna De Saffel, Anne De Paepe, Paul Coucke. Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium.

The recent emerge of site-directed genome editing technologies such as CRISPR-Cas9 is of great interest for research in medicine. In multiple organisms, it has been shown extensively that the technology can be used to generate knock-out disease models with high efficiencies, enabling the study of gene function. However, to generate disease-relevant models, the introduction of specific base pair alterations (knock-in) is desired, since numerous diseases are caused by specific point mutations, leading to amino acid substitutions or splicing defects. Also in zebrafish, CRISPR-Cas9 has been successfully applied to generate knock-out models. However, results from a limited number of small scale studies, showed that the efficiency of CRISPR-Cas9 mediated knock-in approaches to introduce point mutations, is relatively low. These approaches are based on CRISPR-Cas9 stimulated homology-directed repair (HDR), using either single-stranded oligodeoxynucleotide (ssODN), double stranded DNA (dsDNA) or plasmid templates with varying lengths of homology arms. Because there is no clear consensus concerning the most optimal approach, we assessed the suitability of different types of donor templates (ssODNs, dsDNA and plasmids) to introduce specific point mutations at multiple sgRNA target sites in the zebrafish genome by means of CRISPR-Cas9 stimulated homology-directed repair (HDR). Furthermore, we evaluated the influence of homology arm length of the different templates on mutagenesis efficiency and we tested if there is a difference in mutagenesis efficiency between sense and antisense ssODNs at each of the target sites. Finally, we assessed the influence of several chemical compounds, which either block the non-homologous end joining (NHEJ) pathway or stimulate the HDR pathway, on HDR-based knock-in efficiency.

Z6090C CRISPR-Cas9 based knock-in in zebrafish to facilitate streamlined visual genotyping. *Roland S. Wu*[1], Ian I. Lam[1], Thomas O. Auer[2], Filippo Del Bene[2], Shaun R. Coughlin[1]. 1) Cardiovascular Research Institute and Department of Medicine, University of California San Francisco, San Francisco, CA; 2) Institut Curie, Centre de Recherche, Paris, France.

CRISPR/Cas9-based genome editing has greatly facilitated the ease of generating zebrafish genetic deletions. However, genotyping often requires significant investments in resources and remains a significant bottleneck in maintaining mutant zebrafish lines. Furthermore, there are currently no broadly applicable methods to identify the genotype of a living, unperturbed zebrafish embryo. Here we describe a cloning-free genotyping system to facilitate convenient, high-throughput genotyping of living zebrafish mutant lines. We utilize a knock-in strategy using homology-independent DNA repair to insert a Gal4 reporter into an endogenous locus, allowing live interrogation of gene expression. This knock-in harbors a cassette conferring lens fluorescence, and generation of mutant lines with lens fluorescence in different channels allows unambiguous genotyping of compound heterozygotes by visual examination of living embryos. We use this system to disrupt the *s1pr1* gene locus. Sphingosine-1-phosphate (S1P) has been found to play an important role in vertebrate development and effects of S1P are mediated by a family of five G protein-coupled receptors, sphingosine-1-phosphate receptors 1-5 (S1PR1-5), which confer the diversity of responses to S1P. We have found that disruption of the *s1pr1* gene leads to partial lethality in zebrafish larvae. Analysis of *s1pr1* knock-in embryos reveals *s1pr1* expression in the developing nervous system, caudal hematopoietic tissue, and vasculature. We exploited live embryo genotyping to interrogate the effects of *s1pr1* gene disruption in these tissues. This strategy of gene disruption requires generating two new alleles instead of one per knockout and hence more initial investment than standard CRISPR/Cas9-based gene deletion. However, these knock-in lines allow visual genotyping of live embryos, greatly increasing the efficiency of maintenance and phenotypic evaluation, including identification of null embryos before the onset of phenotype.

Z6091A Determining the functional significance of variant human alleles using zebrafish. *X. Xing*[1], U. Broeckel[2], R. Blank[2], P. Giampietro[3], M. Pickart[1]. 1) Concordia University Wisconsin, Mequon, WI; 2) Medical College of Wisconsin, Milwaukee, WI; 3) University of Wisconsin, School of Medicine and Public Health, Madison, WI.

Assigning function to sequence variants observed in association with clinical studies remains a key priority in the post-genomic era. Numerous reports over the last decade demonstrate that in many instances, overexpression of functional human alleles are sufficient to rescue loss of function (lof) orthologous knockdowns and knockouts in zebrafish. The inability of polymorphic and variant human alleles to rescue lof zebrafish models is thus an efficient platform to assign functional significance to these alleles. To explore this potential further, our collaborative team is investigating known sequence variants in *Tyrosinase* (*Tyr*) and *T* (*Brachyury*) and applying what is learned through this study to inform an ongoing study of sequence variants of unknown significance from patients with underlying vertebral malformations. Human ORFs are cloned into pT3TS or pKTol2BA RNA and DNA expression vectors, respectively. ORFs are modified using pcr mediated overlap extension to create variant alleles and cloned similarly. Both human functional and potential lof variant alleles are then tested for their capacity to rescue zebrafish lof phenotypes. Initial studies of two families with likely inherited vertebral malformations have been examined using whole exome sequencing. Resulting sequences have been processed by applying bioinformatics filters for stop gain/loss and non-synonymous coding changes to ensure high relevance of the 30 variant alleles identified and include genes such as *Polr1d*, *Tle4*, *and Ppib* for example. Functional studies of these alleles are commencing following initial technical progress demonstrating that overexpression of human *Tyr* rescues an orthologous *Tyr* lof zebrafish model. These results support the use of zebrafish for functionally validating pathogenic sequence variants and ultimately improving clinical relevance of association studies for complex disease.

Z6092B A comprehensive map and comparative analysis of cis-regulatory elements in the zebrafish genome. *H. Yang*[1], T. Liu[1], D. Balciunas[2], G. Gerhard[3], F. Yue[1]. 1) Penn State Univ College of Medicine, hershey, PA; 2) Temple University College of Science and Technology; 3) Temple University Lewis Katz School of Medicine.

Zebrafish has been widely used for the study of human diseases, as ~70% of the protein-coding genes are conserved between the two species. Moreover, zebrafish embryos are transparent and thus can serve as an ideal model for genetic studies in animal development. Surprisingly, the functional annotation of zebrafish genome itself has been severely lagging when compared with other model systems such as mouse and drosophila. Here we took a similar approach adopted by the ENCODE and Roadmap Epigenomics projects, and performed RNA-Seq and ChIP-Seq for several histone modifications to generate a comprehensive map of transcriptomes and regulatory elements in a variety of zebrafish tissues, including brain, heart, liver, skeletal muscle, kidney and several embryonic tissues. We predicted over 100,000 cis-regulatory elements in the zebrafish genome, the most comprehensive functional annotation effort in zebrafish so far to our knowledge. We also identified tissue-specific and developmental stage-specific regulatory elements. By comparing the data generated by the ENCODE and Roadmap Epigenomics projects, we also defined a set of functionally conserved and species-specific regulatory sequences among zebrafish, mouse and human. In summary, we generated a great genomics/epigenomics resource for the functional annotation in the vertebrate genomes and further expanded the value of zebrafish as a model of human disease.

Z6093C Establish a Zebrafish genetic mosaic system for single-cell resolution phenotypic analysis of mutant cells. *Guoxin Zhang*, Laura Fontenas, Sarah Kucenas, Hui Zong. University of Virginia, Charlottesville, VA.

Genetic mosaicism is defined by the existence of cell populations with different genotypes in an individual organism. Many human diseases, such as cancer, are caused by genetic mosaicism since cancerous cells harbor genetic mutations that are absent in normal cells within the same person. In research labs, studying mosaic animals reveals **cell-autonomous functions** of a given gene, particularly suitable for polarity, migration, cell-cell interaction problems. Obviously, the unequivocal labeling of rare mutant cells is the prerequisite for one to analyze them in

a genetic mosaic animal. Previously we generated the MADM (mosaic analysis with double markers) system in mice, which generate GFP-labeled mutant cells and RFP-labeled sibling WT cells through site-specific recombinase mediated inter-chromosomal mitotic recombination (Zong 2005 Cell). By comparing green and red cell behaviors in vivo, one can detect even the subtlest phenotypes at the **single cell resolution**. MADM has been broadly adopted in many fields such as neurobiology (Hippenmeyer 2010 Neuron), developmental biology (Packard 2013 Developmental cell), and cancer biology (Liu 2011 Cell). While we have learned a lot of fascinating biology with the mouse MADM system, Zebrafish model carries great advantages in terms of the transparency of its body and the ease to generate a large, genetically identical population. Based on our experience in establishing the mouse MADM system, we are now in the process of establishing the MADM system in Zebrafish. We envision that the system would not only help deepen our understanding of cellular behaviors in normal development and adult functions, but also enable the establishment of disease models and drug-screening platforms.

Z6094A Validation of a cost-effective method to record electrographic activity in larval zebrafish brain. *M. C. Gonsales*, P. G. Barbalho, A. S. Vieira, I. Lopes-Cendes, C. V. Maurer-Morelli. University of Campinas, Brazil.

Purpose: Being able to record zebrafish brain activity is essential to investigate and characterize the abnormal electrical discharges during seizure-like responses in epilepsy studies. The current procedure employed to record extracellular field potentials in the larval zebrafish forebrain is an adaptation of conventional extracellular recording techniques, using a patch clamp amplifier. In the present work, we aim to establish a protocol to obtain electrographic recordings of zebrafish with a simpler and more cost-effective setup for laboratories intending to implement this technique in their routine. **Methods:** Each zebrafish 7 dpf larvae was anesthetized and paralyzed with 400µM tricaine and 10µM d-tubocurarine. After complete loss of movement, the animal was placed in a lid removed from a 1.0 ml eppendorf tube filled with 1% low-melting agarose prepared with aquarium water. Fish was positioned horizontally, with the dorsal side exposed to the surface. Quartz/platinum-tungsten microelectrodes were attached to a pair of connectors and fitted onto a MN-153 Narishige micromanipulator. One electrode was placed slightly in front of the forebrain of the animal, and another in the agarose. Electrical activity was recorded using the RHD2000 Evaluation System (Intan Technologies®). We used a setup that includes an interface board connected to a host computer via standard USB cable, and to a small amplifier board via a 0.9m serial peripheral interface cable. To prevent external electromagnetic interferences, the setup was placed inside a Faraday cage. To validate the system, we performed preliminary recordings of larvae with and without addition of 30 mM pentylenetetrazol (PTZ). **Results:** To date, we have built a setup to record extracellular field potentials in zebrafish brain using a system that transform weak electrode signals directly into a digital data stream. Our pilot study showed high amplitude electrographic discharges in the animal exposed to PTZ compared to the control, starting approximately after 30 minutes of exposition to PTZ, with intervals of 5-10 minutes. **Conclusion:** In the present work, we described a cost-effective protocol for electrographic recordings on immobilized zebrafish larvae, as it does not require a patch clamp amplifier. We have accomplished stable long-term monitoring of brain activity in immobilized zebrafish larvae and we are currently working towards a characterization of the epileptiform discharges in order to study the seizure-like responses evoked by the convulsant agent PTZ. Since studies using zebrafish to elucidate the basis of seizure generation are still scarce, this study provides new tools to study the mechanisms underlying seizures in this model. Supported by Fapesp.

Z6095B Myomesin2 - a potential candidate gene for congenital heart defects. *K.de L. Troelsen*, P. R. Lundegaard, M. Moennich, L. A. Larsen. University of Copenhagen, København N, DK.

Congenital heart defects (CHD) are the most common congenital malformations in newborns and are a major cause of infant morbidity and mortality. Despite many genetic studies, much of the genetic mechanisms behind normal and abnormal heart development remain to be elucidated.

The Myomesin 2 gene (*MYOM2*) encodes a protein located in the M-band of the sarcomere in cardiac and skeletal muscle. A potential role for MYOM2 in the assembly of sarcomeric Titin has been proposed. The assembly of the sarcomere is of tremendous importance for a fully functional skeletal and cardiac muscle and mutations in sarcomere genes like *ACTC1*, *MYH6* and *MYH7* have previously been associated with CHD and rare *MYOM2* variants have been reported in CHD patients. This study focuses on the role of MYOM2 during heart development. The zebrafish genome encodes a *myom2* gene with a 55% homology to the hyman MYOM2. Therefore the zebrafish was used to model the expression of *myom2* during development and to explore the role of gene knockdown in live developing zebrafish hearts.

In situ hybridization showed that *myom2* is expressed specifically in heart and skeletal muscle during zebrafish embryonic development. We also found *myom2* to be duplicated, with similar expression patterns. Two approaches to knock down the gene in zebrafish were used. First zebrafish carrying a nonsense mutation in the *myom2a* gene was obtained from the Zebrafish Mutation Project (Sanger Institute, UK). No obvious cardiac or muscle phenotype was present in homozygous mutants, suggesting that mutants compensate for the loss of myom2 function. Whereas the development of zebrafish embryos injected with morpholinos targeting *myom2*awas delayed and developmental defects were observed. Targeting myom2b with morpholinos did not differ from control injected wild type embryos. Interestingly the *myom2b* morpholino did show developmental changes in *myom2a* mutants.

The preliminary data indicates a possible role of *myom2* in heart development, but further experiments are needed.

Z6096C The zebrafish *prox1a* controls liver development by regulating Wnt signaling pathway. *B. Zhang*, Y. Hu, Z. Luo, M. Wang. Peking University, Beijing, CN.

Prox1, encoding a homeobox transcription factor, has been shown to be essential for liver development in mice, though its molecular mechanism needs further investigation and its function in zebrafish hepatogenesis has not been reported. In this study, we generated *prox1a* knock-out zebrafish by using CRISPR/Cas9 system. *prox1a* homozygous mutants exhibited severe edema around the gut upon 4-5 dpf, with

smaller liver and abnormal intrahepatic structure. The number of mature hepatocytes was reduced, whereas early endoderm development and hepatic bud formation was not affected, as shown by the *in situ* hybridization results of the corrresponding marker genes. *prox1a* ablation resulted in defective hepatocyte proliferation but not apoptosis in the liver outgrowth stage. Moreover, cholangiocyte differentiation was totally blocked in the mutant liver, as shown by using both Notch-reporter transgenic fish and cytokeratin staining. Endothelial cells also failed to invade into the mutant liver to form vascular networks. As a result, the *prox1a* mutants lack intrahepatic biliary ducts and blood vessels. Liver-specific RNA seq was performed to explore downstream effectors of *prox1a*. About 1000 genes were identified as significantly differentially expressed in the mutant liver. Functional clustering analysis on these genes revealed that, majority of the downregulated group was involved in metabolic process, while Wnt signaling was highlighted in the upregultated pathways. Dissecting different components of this pathway uncovered bifurcating outcomes. Upon *prox1a* ablation, excessive expression of Wnt ligands, receptors, as well as inhibitors integrate to activate non-canonical Wnt signaling but suppress canonical Wnt signaling. Reduced expression of canonical Wnt target genes *cmyc* and *ccnd1* led to defective hepatocyte proliferation, rendering smaller liver size. However, enhanced non-canonical Wnt signaling activity interfered with cell polarity, displayed by aberrant subcellular localization of aPKC, an apical membrane protein. Lack of correct cell polarity resulted in loose organization and irregular orientation of hepatoblast/hepatocyte, and may influence interations between different cell types, on which both cholangiocyte differentiation and endothelial cell invasion are dependent. In conclusion, our data illustrated that *prox1a* controls zebrafish liver development at multiple levels, including cell proliferation, polarity and differentiation, through regulating and integrating Wnt signaling pathway.

Z6097A Global identification of the genetic networks and cis-regulatory elements of the cold response in zebrafish. *Liangbiao Chen*, Peng Hu, MingLi Liu, Jinfeng Wang, Hongbo Niu, Yimeng Liu, Zhichao Wu, Bingshe Han, Wangying Zhai, Dong Zhang. Shanghai Ocean University, Shanghai, CN.

The transcriptional programs of ectothermic teleosts are directly influenced by water temperature. However, the cis- and trans-factors governing cold responses are not well characterized. We profiled transcriptional changes in eight zebrafish tissues exposed to mildly and severely cold temperatures using RNA-Seq. A total of 1943 differentially expressed genes (DEGs) were identified, from which 34 clusters representing distinct tissue and temperature response expression patterns were derived using the k-means fuzzy clustering algorithm. The promoter regions of the clustered DEGs that demonstrated strong co-regulation were analysed for enriched cis-regulatory elements with a motif discovery program, DREME. Seventeen motifs, ten known and seven novel, were identified, which covered 23% of the DEGs. Two motifs predicted to be the binding sites for the transcription factors Bcl6 and Jun, respectively, were chosen for experimental verification, and they demonstrated the expected cold-induced and cold-repressed patterns of gene regulation. Protein interaction modeling of the network components followed by experimental validation suggested that Jun physically interacts with Bcl6 and might be a hub factor that orchestrates the cold response in zebrafish. Thus, the methodology used and the regulatory networks uncovered in this study provide a foundation for exploring the mechanisms of cold adaptation in teleosts..

Z6098B Evolutionarily Conserved Functional Compatibility of The Lysosomal Symporter Spin/Spns1 over One-Billion Years across Species. *S. Kishi*[1], S. Lian[1], T. Sasaki[1], A. Khan[1], W. Ja[1], K. Jia[2], M. Gill[1], J. Choe[3]. 1) The Scripps Research Institute, Jupiter, FL; 2) Florida Atlantic University, Jupiter, FL; 3) Rosalind Franklin University of Medicine and Science, North Chicago, IL.

Is phylogenetic distance between worms and humans negated in some cases? If a certain gene function is conserved throughout such organisms, our naïve question would be how often the gene itself can be intactly replaceable between them beyond some 990 million years. As a group, teleost fish are phylogenetically well positioned for comparisons to other vertebrates, having diverged from the amniote lineage (mammals, birds, reptiles) some 400 million years ago. The comparison with human genomes also provides an opportunity to examine the dynamics of fish chromosomal evolution. The genomes of zebrafish (*Danio rerio*) species have been sequenced with annotations and display remarkable structural similarity with the human genome, meaning that findings from zebrafish provide insights into many human conditions and diseases. While many molecules have been considered for their functional conservation among invertebrate and vertebrate species, as far as we know, little has been demonstrated for truly reciprocal compatibility between them. Here, for the first time, utilizing the zebrafish model system, we show that the putative lysosomal sympoter Spinster (Spin)/Spinster homolog 1 (Spns1) is evolutionarily conserved and replaceable across wide variety species from worms through humans beyond approximately one-billion years' evolutionary distance. We demonstrate that human Spns1, *Drosophila* Spin and *C. elegans* Spin-1 are functionally compatible to zebrafish Spns1 and transgenic expressions of any of these Spin/Spns1 can rescue zebrafish *spns1* mutants with successful generations of the stable transgenic lines. Homology-scanning mutagenesis of Spin/Spns1 further confirmed the biologically functional and potential ligand-binding capabilities of the proteins *in vivo* and *in vitro*, respectively. We further demonstrate that a sugar-derived molecule, rather than a conventional sugar, is a common ligand for both human and zebrafish Spns1. Finally, primary and tertiary structure analysis reveal that the Spin/Spns1 proteins have unique substrate- and proton-binding sites as symporters, suggesting that these proteins may represent a novel, as yet unclassified, type of the solute carrier family.

Z6099C Tracing the Evolutionary History of the SLC1 Gene Family. *André Lehnherr*, Lucia Cadetti, Matthias Gessemann, Stephan C. F. Neuhauss. University of Zurich, Zürich, CH.

Efficient removal of glutamate from the synaptic cleft is essential to end synaptic transmission protecting neurons from excitotoxicity. This is achieved by excitatory amino acid transporters (EAAT), which belong to the Solute Carrier Family 1 (SLC1) gene family. Besides glutamate and associated cation transport, EAATs induce a chloride conductance upon glutamate binding.

Phylogenetic analysis of the SLC1 gene family revealed duplication and deletion events during evolution leading to varying number of SLC1

genes in different species. While the zebrafish genome contains thirteen SLC1 genes, amphibians have retained nine and mice and humans only seven SLC1 genes.

In this study, we compare expression and function of selected vertebrate SLC1 genes across species (Zebrafish, *Xenopus*, anole lizard, chicken and mouse) in the retina. Retinal expression of SLC1 RNA provides an ideal system to compare the abundance of SLC1s in different vertebrate species, identifying potential sub-, non or neofunctionalization events. In fact we found striking differences in RNA expression between different species.

For further examination of these differences, we elaborate the biophysical properties of the SLC1 family members by expression of the transporters in Xenopus oocytes and subsequent two electrode voltage clamp recordings. This allows us to correlate expression and function across species and thereby to follow the evolutionary history of the SLC1 gene family.

Z6100A Transcriptional landscape of the major pancreatic cells reveals conserved expression patterns amongst distant vertebrate species. *Bernard PEERS*, Estefania Tarifeno, Arnaud Lavergne, Keerthana Padamata, Alice Bernard, Marianne Voz, Isabelle Mainfroid. Ulg, liege, liege, BE.

Defining the transcriptome and the genetic pathways of pancreatic endocrine and exocrine cell types is crucial for elucidating the molecular attributes of disorders such as diabetes and pancreatic cancer. The comparison of these transcriptomes amongst distant vertebrate species highlight the genes under strong evolutionary constraints which have maintained their selective expression in pancreatic cells due to their crucial function in these cells.

We took advantage of zebrafish transgenic lines to isolate by FACS the major pancreatic cell types and we obtained highly purified preparations of endocrine α-, β- and δ cells as well as the exocrine acinar and ductal cells. Transcriptomic profiling by RNA-seq identified the transcriptomic signature of each cell type and highlighted novel cell-specific markers including transcription factors, signaling pathways components and lincRNAs. By performing interspecies comparisons, we identified hundreds of genes with conserved enriched expression in endocrine or in exocrine cells amongst human, mouse and zebrafish. This list includes many regulatory genes known as crucial for the differentiation and the function of endocrine and exocrine cells, but also pinpoints previously unrecognized regulators. While the transcriptomic signature of pancreatic endocrine and exocrine cells is well conserved amongst vertebrates, the signatures distinguishing the endocrine cell subtypes, and notably alpha (glucagon+) and beta (insulin+) cells, are much less conserved between zebrafish, mouse and human, with only a few genes displaying conserved cell subtype specific expression. This suggests that endocrine cell subtype identity may be determined by one or a few regulatory genes. The function of some identified endocrine regulators is presently addressed through CRISPR mutagenesis.

In conclusions, this study provides the molecular signature of the major zebrafish pancreatic cell types and identifies sets of genes with selective and evolutionary conserved expression in pancreatic endocrine or exocrine cells from fish to mammals, likely important in pancreas physiology and relevant to pancreatic diseases such as diabetes and cancer.

Z6101B The Functional Studies of miR-7132 on the Erythropoiesis. *Qianghua Xu*[1,2,3,4], Xingxing Hu[1]. 1) College of Marine Sciences, Shanghai Ocean University, Shanghai, China,201306; 2) Key Laboratory of Sustainable Exploitation of Ocean Fisheries Resources, Ministry of Education, Shanghai, China, 201306; 3) National Distant-water Fisheries Engineering Research Center, Shanghai Ocean University, Shanghai, China, 201306; 4) Collaborative Innovation Center for Distant-water Fisheries, Shanghai, China, 201306.

MicroRNAs are small non-coding RNAs, playing roles at post-transcriptional level by binding the 3'UTR of the target gene. Some studies indicated that microRNAs play important roles in the process of erythropoiesis. *Chionodraco hamatus* is a white-blooded Antarctic icefish, the only known vertebrate with a very few non-functional erythrocytes. Previous studies showed that highly upregulated expression of about one hundred microRNAs in the *C. hamatus* head kidney might inhibit the erythropoiesis of the Antarctic icefish. In our study, by using zebrafish microinjection technique, dual-luciferase reporter, and target prediction methods, we studied the functions of the miR-7132 in the process of erythropoiesis, which was highly expressed in the head kidney of the icefish. Results showed that, after being micro-injected with miR-7132, zebrafish embryos showed dramatic reduced expression level of hemoglobin by o-dianisidine staining, which indicated that overexpression of miR-7132 repressed the erythropoiesis process in zebrafish embryos. The dual-luciferase vector containing the 3'UTR of ALAS2, the limited enzyme in the process of hemoglobin production, was constructed and co-transfected with miR-7132, and the activity of luciferase was detected. The expression vector containing the GFP fluorescent protein was constructed. Zebrafish embryos were then micro-injected with the mixture including miR-7132 and the GFP plasmid. The expression of GFP protein was then detected by western blot analysis. Result showed that miR-7132 significantly reduced the relative activity of luciferase in 293T cells, The GFP fluorescent intensity was significantly decreased when injected with miR-7132. The study indicated that miR-7132 regulated erythropoiesis in the Antarctic icefish by repressing the expression of ALAS2.

Z6102C Zebrafish as a model to comparatively study male and female meiosis and sexually dimorphic responses to meiotic perturbations. *Yana P. Blokhina*, An D. Nguyen, Hester E. Roberts, Trent A. Newman, Daniel B. Chu, Bruce W. Draper, Sean M. Burgess. University of California, Davis, Davis, CA.

Aneuploidy is the leading cause of birth defects and spontaneous abortions in humans. The majority of aneuploidies are maternally derived, and most of the clinically relevant cases arise from segregation errors in meiosis I. During meiosis I, key steps leading to the formation of a stable bivalent must be undertaken with precision in order to ensure proper segregation of homologous chromosomes. In our research we use the zebrafish, *Danio rerio*, to comparatively study early meiotic processes in males and females with the goal of understanding which factors

contribute to aneuploidy generation in the two sexes. A thorough examination of these processes has been hindered in mammals by the limited accessibility of female oocytes going through early prophase I. In zebrafish, however, the gonad is transparent and easily accessible, and germ cells in both males and females undergo meiosis throughout their lives (Draper, McCallum and Moens, 2007). While previous studies have shown telomere clustering and Sycp3 loading in zebrafish males (Saito K et al., 2011 and 2014), there are still many unanswered questions. Most notably there has not been a thorough study of how zebrafish male and female meioses differ, and how the two sexes respond to perturbations in meiosis. We have created loss of function mutations in genes with key roles in meiotic chromosome dynamics. The first is in spo11, which encodes the nuclease that generates programmed double strand breaks which initiate homologous recombination. The second is in rad21l1 that encodes a meiosis-specific cohesin subunit found only in vertebrates. Both *spo11* and *rad21l1* mutants have sexually dimorphic phenotypes. While *spo11* males are completely infertile and lack sperm, the *spo11* females are fertile but most of their offspring are highly deformed and aneuploid. The *rad21l1* mutants by contrast have a defect in maintaining the female phenotype with over 97% of homozygotes developing into males which appear to be fully fertile. Interestingly, in *rad21l1/tp53* double mutants, the defect in maintaining femaleness is rescued but most of the double mutant females' offspring are deformed. This phenotype is distinctly different from the *spo11* aneuploid offspring, suggesting the defects may be due to some other lesion not related to aneuploidy. Together, our results support zebrafish as a coherent model that demonstrates both similarities and differences between meiosis in the two sexes.

Z6103A Igf3 and Amh, two Fsh-responsive growth factors, regulate spermatogonial differentiation in a concerted manner. *J. Bogerd*[1], R. D. V. S. Morais[1], R. H. Nóbrega[2], D. Crespo[1], H. J. G. van de Kant[1], R. Male[3], R. W. Schulz[1,4]. 1) Utrecht University, Utrecht, The Netherlands; 2) São Paulo St. University, Botucatu, Brazil; 3) University of Bergen, Bergen, Norway; 4) Institute of Marine Research, Bergen, Norway.

Follicle-stimulating hormone (Fsh) modulates fish spermatogenesis in a steroid-independent manner by modulating the expression of growth factors controlling spermatogonial proliferation and differentiation. Here, we find that two of these Fsh-responsive growth factors influence each other's activity on zebrafish spermatogonia: anti-Müllerian hormone (Amh) and the insulin-like growth factor 3 (Igf3). Fsh-time and -dose response experiments *ex vivo* demonstrate that *igf3* transcript levels are rapidly up-regulated, remain elevated, and respond to lower Fsh concentrations than are required to decrease *amh* mRNA levels. Immunofluorescence studies suggest that Fsh also decreases Amh protein levels while increases in Igf3 protein levels were comparatively small. Zebrafish Amh compromised Igf3-induced proliferation of type A undifferentiated (A_{und}) and type A differentiating (A_{diff}) spermatogonia. Proliferation of Sertoli cells associated with type A_{und} spermatogonia was also reduced by Amh. To better understand the inhibitory effects of Amh on germ cell development, we investigated Amh-induced changes in zebrafish testicular gene expression by RNAseq. Analyzing the differentially expressed genes demonstrates that several signaling pathways are potentially involved in mediating the inhibitory activity of Amh. The majority of the genes identified were down-regulated, such as the transcript levels of *igf3*, *insl3* and of steroidogenesis-related genes, all three stimulating germ cell differentiation. At the same time, Amh increased the expression of inhibitory signals, such as *inha* and *id3* transcript levels. Altogether, our results provide new information on how Fsh regulates zebrafish spermatogenesis by orchestrating the actions of different growth factors and other paracrine signaling systems, including the control of the multiple inhibitory effects of Amh.

Z6104B An improved method for gynogenesis in zebrafish produces fertile males. *Thomas A. Delomas*, Konrad Dabrowski. School of Environment and Natural Resources, The Ohio State University, Columbus, OH.

Analyzing recessive mutations typically requires the production of three generations: an original heterozygous carrier, an F_1 cross, and multiple F_2 crosses. This can be shortened to two generations by producing uniparental progenies from the heterozygous carrier through gynogenesis. Gynogenesis is typically performed using UV-irradiated zebrafish sperm, but this limits its use to females carrying an easily identifiable recessive mutation, so as to recognize any contamination from sperm that was not deactivated. This could be overcome by using UV-irradiated heterologous sperm that is capable of initiating development but, if inactivation is not complete, results in either inviable or distinguishable hybrids. Koi carp *Cyprinus carpio* was evaluated as a candidate for this purpose.

Hybridization was performed by artificially crossing 18 zebrafish females with one koi carp male. Embryonic development was successfully initiated by koi carp sperm. There was high progeny mortality in all tested pairs over the first 24h and complete mortality prior to the swim-up stage. Several distinct and consistent deformities were observed in the hybrid embryos: blastomeres of unequal size, short bodies, and edema.

Gynogenesis was performed using UV-irradiated koi carp sperm and either an early pressure or a heat shock treatment was used to restore diploidy. Survival to 21 days post fertilization was similar between progenies from the two types of shocks, averaging 2.2% and 2.5% for early pressure (n=3) and heat shock (n=5), respectively. Thus far, only males have been present in gynogenetic progenies produced (n=8). Samples of males resulting from 2 separate early pressure treatments were spawned naturally with non-sibling females. Control males (half-siblings of the gynogenetic males) were also spawned with the same females. The proportion of males that were fertile, fertilization rates and survival from fertilization to swim-up larvae was recorded. Fertilization rates were significantly lower than the control in one gynogenetic progeny ($p<0.10$), but not in the second. Survival from fertilization to the swim-up stage was not significantly different from the control in either progeny. This demonstrates that gynogenetic males could be used as broodstock for the rapid development of homozygous mutant lines. Males from a third early pressure shock progeny and from five heat shock progenies will be tested for fertility and these results will be presented.

Z6105C Wnt4a is expressed in the early gonad and is required for normal female sex determination. *Michelle Kossack*[1], Samantha High[2], Anastasia Utkina[1], Bruce Draper[1], John Postlethwait[2]. 1) Molecular and Cellular Biology, University of California Davis; 2) Institute of Neuroscience, University of Oregon, Eugene OR 97403.

Sex determination in zebrafish is not well understood. In AB and TU laboratory strains, sex determination occurs in the absence of a typical

sex chromosome, but animals directly from nature have a strong sex determinant on LG4. Early stage oocytes are necessary for primary female sex determination, and therefore it is hypothesized that oocytes produce a signal that act on the somatic gonad that maintains female sex or inhibits male sex differentiation. In mammals, Wnt4 is a signaling ligand that is essential for female development. We therefore asked if *wnt4a,* the single copy ortholog of *WNT4* in zebrafish*,* has a similar role in zebrafish primary sex determination. We found, using qPCR and *in situ* hybridization, that *wnt4a* is expressed in the somatic cells of juvenile gonads by 12 days post fertilization and continues in the follicle cells of adult ovaries. Thus *wnt4a* is a plausible candidate for the oocyte-produced sex determining factor. Fish homozygous for *wnt4a*(*fh295*), a TILLING allele that encodes a C-terminally truncated protein lacking residues shown to be important in WNT signaling, develop almost exclusively as male. Because the existing *wnt4a* TILLING alleles are C-terminal truncations and are therefore suspected dominant-negatives, we used CRISPR/Cas9 to produce two loss-of-function mutant alleles, *wnt4a(uc55) and wnt4a(uc56),* which result from a 17 and a 26 base pair insertion where in the protein is truncated to 68 or 33 amino acids, respectively. We found that homozygous adult mutants for both *wnt4a(uc55) and wnt4a(uc56)* develop predominantly as males. We further found that mutant males are sterile because reproductive ducts do not grow as rapidly toward the vent as wild-type ducts, and never connect to the genital orifice. However, sperm from macerated testes of *wnt4a* mutants produced normal offspring. Tissue surrounding the genital orifice in wild types strongly expressed *wnt4a*. These results show that Wnt4 is necessary for normal male reproductive duct development, but not for male sex determination. To further define the role of WNT signaling for female development and male duct development, we created CRISPR/Cas9 mutants in *rspo1*, which encodes a component of the canonical WNT signaling pathway. Our results strongly support the hypothesis that Wnt4a plays an important role in female sex determination and male reproductive duct development.

Z6106A Polycystic ovarian syndrome in zebrafish mutants for the TGF-beta signaling molecule Gsdf. *J. Postlethwait*[1], Y.-L. Yan[1], T. Desvignes[1], R. BreMiller[1], C. Wilson[1], M. Kossack[2], B. Draper[2]. 1) Univ Oregon, Eugene, OR; 2) Univ of California, Davis, CA.

In gonad development, somatic cells support germ cell development, and reciprocally, germ cells help sustain the development and function of somatic gonad support cells. Currently we lack a full understanding of mechanisms and molecules that perform these functions. To learn about gonad ó soma signaling, we used TALENs to knock out *gsdf* (gonadal soma derived factor), which encodes a ray-fin fish-specific TGF-beta signaling molecule. Gsdf is the major sex determinant in the Luzon medaka, but whether it performs this role in zebrafish is unknown. Several deletion alleles were isolated and all shown to produce similar female sterile phenotypes. Results showed that in wild types, gonadal expression of *gsdf* starts in the somatic cells surrounding the primary germ cells in indifferent gonads before 12 days post fertilization and continues into adulthood in granulosa cells in ovaries and Sertoli cells in testes. Mutant ovaries and testes both showed substantial down-regulation of *gsdf* transcript. Mutant ovaries never developed follicles beyond stage III and did not sequester yolk protein, and instead accumulated thousands of early stage oocytes, leading to sterility. Gene expression studies showed that ovarian follicle cells were abnormal in mutants and failed to express *cyp19a1a* (*aromatase*), *amh* (*antiMüllerian hormone*) and *gata4* normally. Mutant testes were also enlarged, but males were fertile. Mutant testes had normal expression levels of *sox9a* but elevated expression of *amh*. Zebrafish *gsdf* mutant females had aberrant accumulation of lipid in several organs, abnormal expression of the insulin receptor and several lipid metabolism genes. In addition, qPCR confirmed that estrogen receptor and vitellogenin were misexpressed in the livers of *gsdf* mutant females. Mutant ovaries down-regulated many genes involved in sex differentiation, steroid biosynthesis, hormone signaling, cell signaling, and apoptosis and proliferation. These experiments show that in zebrafish, *gsdf* does not have a major effect on sex determination, but rather provides a signal from the gonadal soma to the germ line that regulates oocyte maturation but not germ cell proliferation. Downstream consequences of the lack of Gsdf activity result in a phenotype that mimics human females with polycystic ovarian syndrome (PCOS), with accumulation of immature ovarian follicles, diminished expression of genes encoding steroid hormone biosynthesizing enzymes, obesity, diabetes, and female sterility, thus suggesting a role of TGF-beta signaling molecules in the etiology of PCOS.

Z6107B Zebrafish liver diurnal gene expression and comparative transcriptomics. *G. Breton*[1], G. Boyle[1], J. Foster[1], H. Priest[2], T. Mockler[2], K. Richter[3], D. Traver[3], S. Kay[3]. 1) Univ Texas Health Sci Center Houston, Houston, TX; 2) Donald Danforth Plant Science Center, St-Louis, MO; 3) Univ California at San Diego, La Jolla, CA.

From photosynthetic bacteria to mammals, the circadian clock evolved to track diurnal rhythms and enable organisms to anticipate daily recurring changes such as temperature and light. It orchestrates a broad spectrum of physiology such as the sleep/wake and eating/fasting cycles. While we have made tremendous advances in our understanding of the molecular details of the circadian clock mechanism and how it is synchronized with the environment, we still have rudimentary knowledge of its connections to diurnal physiology. One reason for this lack of understanding is the sheer size of the output network. Transcriptomic studies have identified more than 2000 clock-controlled genes (CCGs) with rhythmic expression patterns. Toward exploring this network in vertebrates, we selected *Danio rerio* as model system. As an initial step, by combining liver tissue sampling in a 2-days time series, transcription profiling using oligonucleotide arrays and bioinformatics analysis, we profiled rhythmic genes and identified several thousands rhythmic genes including ~200 clock-controlled transcription factors (CCTFs). Comparative transcriptomics between Zebrafish, mice and human datasets revealed interesting features of the output network. The results from a global analysis of the union and intersection between the datasets suggest that a large portion of the CCTFs may be involved in circadian gating. Undoubtedly, the Zebrafish model system will help identify new vertebrate outputs and their regulators and provide leads for further characterization of the cis-regulatory network.

Z6108C Profiling the active genomic elements of progenitor cells in the zebrafish optic tectum and telencephalon. *R. ESPOSITO*[1,2], A. Heuzé[1], L. Bally-Cuif[2], JS. Joly[1]. 1) CNRS Neuro-PSI, CASBAH group, Gif-sur-Yvette, FR; 2) CNRS Neuro-PSI, Zebrafish Neurogenetics group, Gif-

sur-Yvette, FR.

Zebrafish, as other teleost fish, display a remarkable capacity to produce neurons throughout life. This process arises from neurogenic niches distributed along the rostro-caudal axis of the brain, responsible for both constitutive neurogenesis and neuronal repair after injuries. Different progenitor subtypes, some exhibiting Neural Stem Cells (NSC)-like features, have been distinguished in adult neurogenic zones: self-renewing radial glial cells (RG; *i.e.* in the dorsal telencephalon, hypothalamus) and neuroepithelial-like cells (*i.e.* lateral and ventral telencephalon, optic tectum, cerebellum), which can also contribute to the generation of RG pools. The main interest of our laboratories is to dissect the genetic and molecular mechanisms controlling adult neurogenesis in the zebrafish telencephalon and optic tectum. To this aim, one approach consists in investigating the epigenetic state characterizing cell proliferation, quiescence and differentiation within the aforementioned neurogenic niches. Indeed, epigenetic mechanisms changing chromatin compaction and its nucleoprotein structure are part of processes controlling cell state, as biologically active genomic elements (*e.g.* enhancers, promoters, etc) need to be accessible to the transcription machinery, whereas inactive regions are sequestered in the hierarchical chromatin packaging. Therefore, the analysis of chromatin accessibility can provide information on the state or the potential of a cell in a given context. We are profiling the active genomic elements involved in zebrafish neurogenesis using ATAC-seq, a relatively new chromatin accessibility assay, which can be applied also to a very limited amount of starting material (5K cells). In order to isolate by FACS the cells to analyse, we are using fish lines in which specific regulatory regions drive the expression of GFP and/or RFP in the appropriate cells: glial and neuroepithelial progenitors, proliferating cells and differentiated neurons, for epigenomic profiling in the optic tectum, and quiescent RG, activated RG and transient progenitors, for the telencephalon. Our work should allow identifying *in vivo* the regulatory logic that specifies the identity and/or the different cell states/types (from progenitors to neurons) operating in two important neurogenic zones in the zebrafish adult brain, and will also permit a comparison between these two territories.

Z6109A Transcriptional Regulation of Heart Development in Zebrafish by ZNF143. *L. E. Huning*, Gary Kunkel. Texas A&M University, College Station, TX.

Regulation of transcription in eukaryotes is an intricate interplay between activator/repressor proteins and co-regulator proteins that bind together to form a molecular machine. This regulation is tightly controlled to ensure that cells produce the correct amount of proteins needed for development. An important activator protein that binds over 2000 mammalian promoters is Zinc Finger Protein 143 (ZNF143). ZNF143 binds to promoter regions of both small RNA genes along with protein coding genes, and has been implicated in the regulation of promoters important for cell cycle progression. Knockdown experiments of this protein caused several pleiotropic effects during early embryogenesis in zebrafish including heart, blood, and brain phenotypes. Although ZNF143 is involved in many different cellular processes, the overall function and mechanism of molecular control at promoter regions of developmental genes is still largely unknown. Phenotypic analyses following knockdown of ZNF143 via antisense morpholinos reveal developmental defects in expression patterns of known heart marker genes at different stages during cardiogenesis. Preliminary ChIP-seq analysis with 24 hour embryos demonstrated that ZNF143 binds to over 500 promoter regions of genes, with 8% of these being bidirectional. Gene ontology analysis with these promoters showed that ZNF143 binds to genes involved in an assortment of biological processes including metabolism, cellular transport and organization, and developmental genes. Current studies involve a targeted CRISPR/Cas9 knockdown of ZNF143 and its effect on cardiogenesis. In addition, zebrafish transgenic lines are being constructed with several ZNF143 target promoters and binding-deficient versions to investigate the molecular mechanism of ZNF143. Hypothesized mechanistic activities of ZNF143 that will be assessed include the amount and timing of RNA polymerase II recruitment, different histone modifications, and nucleosomal patterns that occur at target ZNF143 bound promoter regions.

Z6110B Program number not assigned.

Z6111C New insights into the role of DNA methylation in development and disease from a zebrafish model of ICF syndrome. *S. Rajshekar*[1,2], M. G. Goll[1]. 1) Memorial Sloan Kettering Cancer Center, New York, NY, USA; 2) Weill Cornell Graduate School of Medical Sciences, Cornell University, New York, NY, USA.

The modified base, 5-methylcytosine (5mC) is enriched at repetitive sequences such as satellite repeats that are located in the vicinity of centromeres. Loss of methylation at these repeats correlates with chromosomal instability and is detected in many cancers and the rare genetic disease Immunodeficiency, Centromeric instability and Facial abnormalities (ICF) syndrome. *ZBTB24* is mutated in ICF syndrome Type-2. However, the function of this gene is unknown and animal models have not been described. To understand how *ZBTB24* regulates DNA methylation near centromeres and to determine how centromeric hypomethylation contributes to disease pathology, we have developed a zebrafish model for ICF syndrome by generating loss of function mutations in the zebrafish ortholog of *ZBTB24* using TAL Effector Nucleases (TALENs). *Zbtb24* mutant zebrafish recapitulate features characteristic to ICF patients including distinct cranio-facial anomalies, slow growth and reduced lifespan. We demonstrate that *zbtb24* mutation results in a progressive, rather than acute, loss of 5mC at satellite repeats and that this hypomethylation correlates tightly with the onset of phenotypic abnormalities. Intriguingly, we detect an inflammatory RNA signature in *zbtb24* mutants prior to the onset of disease phenotypes. This observation suggests the presence of an endogenous inflammation based surveillance system that detects loss of centromere DNA methylation. We propose that during normal development this system protects against malignancy by eliminating affected cells before they accumulate substantial DNA damage.

Z6112A Identifying interacting ligands of human RORγ using transgenic zebrafish. *Rachel Sung*[1], Henry Krause[1,2]. 1) Department of Molecular Genetics, Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto; 2) InDanio Bioscience, Toronto.

The retinoic acid receptor-related orphan receptor gamma, or RORγ, is a member of the nuclear receptor ROR family and is involved in

important physiological processes including development, reproduction and metabolism. Its activity is regulated by the binding of small compounds, called ligands, which can induce conformational changes, which in turn, can influence the binding of cofactors, including transcriptional coactivators and corepressors,. There are two isoforms of RORγ. The first is RORγ, which is expressed in the liver, adipose tissue, skeletal muscle and kidneys, and is involved in glucose and lipid metabolism and regulating the circadian rhythm. The second is RORγt, which is expressed in the immune system, and is involved in thymopoiesis and secondary lymphoid organ development. It has been shown that RORγ is involved in the progression of various diseases, including metabolic and autoimmune diseases. These diseases can be controlled through natural or synthetic ligands that can either agonize or antagonize RORγ.

Previous *in vitro* screens have identified interacting ligands of RORγ, but many of these fail in humans due to metabolic inactivation, failure to reach target tissues and off-target toxicity; therefore, it will be much more effective to develop an *in vivo* screening system that can ensure that the ligand is interacting with the receptor in the correct *in vivo* setting. Our lab has developed an *in vivo* GFP reporter system, incorporated into the zebrafish genome, that allows high throughput screening to identify ligands that target RORγ. A drug screen using a compound library consisting of ~4000 natural compounds and approved drugs is being performed to identify new interacting ligands of RORγ. Using new identified ligands of RORγ, cofactors and target genes that are associated with RORγ can also be discovered, which will allow for further understanding of the role and function of RORγ as well as its involvement in diseases. Ultimately, the active drugs identified during the drug screen can be tested on various disease models.

Z6113B Regulation of brain and heart development in zebrafish by the autism risk factor CHD8. *J. A. Tracy*, G. Kunkel. Texas A&M University, College Station, TX.

Accessibility of the transcription machinery to regulatory elements upstream of genes is reliant on the location and stability of nucleosomes within the section of DNA. Remodeling these regions to allow binding of transcriptional regulators depends on the ATP-dependent movement of nucleosomes within the chromatin structure. The focus of this work is the ATP-dependent chromodomain helicase DNA-binding protein 8 (CHD8). CHD8 interacts with several factors that are relevant to the regulation of transcription and modulation of expression of genes implicated in crucial developmental processes and mutations within CHD8 have in multiple patients with autistic spectrum disorder. In addition to chromatin remodeling, CHD8 contains an A-kinase anchoring protein (AKAP) domain. AKAP proteins anchor protein kinase A (PKA), a kinase activated the pathway of gene regulation, to subcellular structures, thus allowing for spatial control and specialization of the PKA phosphorylation. Defects in AKAPs lead to various types of heart disease. Through the use of morpholino oligonucleotides and CRISPR/Cas9 to disrupt CHD8 expression, we have determined that CHD8 necessary for proper brain and heart development in zebrafish embryos. Reduction in CHD8 leads to disruption of expression patterns of several genes known to in brain and heart development. There are at least two isoforms of CHD8 that result from alternative splicing, and CHD8L. Interestingly, while CHD8S lacks the essential helicase domain required for nucleosome remodeling, we have that both isoforms of CHD8 are capable of activating gene expression at a ZNF143 targeted promoter but during zebrafish development.

Z6114C MicroRNA regulation of BMP signaling and its effects on vascular smooth muscle cells. *C. G. Watterston*, L. Zeng, A. Onabadejo, S. J. Childs. University of Calgary, Calgary, Alberta, CA.

Stroke is the third leading cause of death in North America and hemorrhagic stroke accounts for 12% of all stroke. As blood vessels develop it is essential that they are physically supported to ensure to supply blood to peripheral tissues. Vascular smooth muscle cells (vSMC) support the endothelial lining of blood vessels and can switch their phenotypic expressions from contractile (differentiated) to synthetic (undifferentiated). Synthetic vSMCs cannot properly support blood vessels the effects of which can lead to uncontrolled bleeding (hemorrhage). We aim to uncover the relationship between microRNAs (miRNAs) and their target genes using a zebrafish model in vascular stabilization. microRNA26 (miR26) targets smad1 downstream of BMP signaling *in vitro.*We show that knockdown of *miR26* leads to brain hemorrhage in developing zebrafish and that Smad1 is upregulated. Concomitantly overexpression of *miR26* leads to decreased Smad1.Overexpression of Smad1 in wild type fish also leads to hemorrhage and double knockdown of *miR26* and Smad1 rescues loss of *miR26*, suggesting that *miR26* mediates vascular stabilization via targeting of Smad1. We observe increased pSmad1/5/8 suggesting increased activation of the BMP pathway. A range of other phenotypes are also observed suggesting alterations in the BMP pathway including axial patterning defects.*miR26* is expressed in the vicinity of brain blood vessels and loss or gain of *miR26* leads to alterations in genes critical for vascular mural cell development including *pdgfrβ*, *acta2*, and *nmhc-b*. We also show decreased *acta2* positive cell coverage of blood vessels in *miR26* knockdown embryos.To test the role of BMP in vascular smooth muscle development, we applied the BMP receptor kinase inhibitor DMH1 to wild type and *miR26* knockdown. DMH1 treatment leads to a reduction in *acta2* positive smooth muscle cells in wild type embryos. DMH1is unable to rescue loss of *miR26*, which is expected if increased Smad1 resulting from *miR26* knockdown is unable to be phosphorylated by the BMP receptor.Taken together our results indicate that both loss and gain of pSmad1 leads to defects in vascular smooth muscle differentiation *in vivo*, and that *miR26* controls this fine balance to promote vascular maturation.

Z6115A Efficient CRISPR/Cas9 genome editing for heat shock-mediated conditional regulation in zebrafish. *Y. Wu*, I. Wang. National Taiwan University Hospital, Taipei, TW.

Purpose: Conditional mutation is crucial for determining stage- and tissue-specific functions of genes. Herein, we developed and characterized an inducible knockout platform composed of the heat-inducible hsp70l promoter and clustered regularly interspaced shot palindromic repeats (CRISPR)/Cas9 system, an easy and efficient strategy for generating gene-modified cells and organisms. **Materials and methods:** We generated a heat shock-dependent Cas9 zebrafish embryo by inserting an hsp70l promoter-driven Cas9 transgene expression

cassette through the Tol2 transposon system. To overcome the difficulty in identifying zebrafish germline integrations and facilitate observation of Cas9 expression, this cassette contains heart-specific *cmlc2*:EGFP and a selectable marker, P2A-mCherry. Real-time PCR and immunoblotting were performed to characterize heat-dependent, constitutive expression of Cas9 in zebrafish after heat shock. Furthermore, we designed and constructed plasmids containing zU6 promoter-driven *tyr* guide RNA and *cmlc2*:mCherry, which is a distinguishable marker used to confirm expression of gRNA. This plasmid was injected into one-cell fertilized Cas9 embryos. After heat shock, we estimated the efficiency of targeted mutagenesis of injected embryos through T7 endonuclease I assay and real-time PCRs. **Results:** Heat treatment of Cas9 transgenic embryos resulted in expression of mCherry fluorescence throughout the whole body without morphological abnormalities, and Cas9 protein was expressed 1 hr after the onset of heat shock, with high levels of Cas9 gene expression observed by 6-12 hr post-heat shock. In testing endogenous Tyr locus, we found that the mutagenesis efficiency reached approximately 50%. Mosaic phenotypes were observed in the injected embryos. **Conclusions:** The results indicate that our developed mutagenesis approach is efficient and could be used to generate conditional alleles throughout the zebrafish genome.

Z6116B Characterization of the *Meis2* locus. *Ted Zerucha*, Megan Tennant, Cody Barrett, Caroline Cochrane, Tyler Ferrara, Brantley Graham, Tray Neilson, Kyle Nelson, Zach Williams, Brandon Carpenter. Appalachian State University, Boone, NC.

The *Meis* genes are a member of the homeobox super-family of genes that code for the production of transcription factors. Homologs of the *Meis* genes have been identified in all animals studied and have been found to be expressed in similar patterns during the embryonic development of those animals. The products of the *Meis* genes are able to directly regulate the expression of target genes but are most well-known for functioning as cofactors, directly interacting with other transcription factors as well as DNA to facilitate transcriptional regulation. Most notably, they appear to act as co-factors of the evolutionarily well-conserved Hox proteins and have also been described as acting with other transcription factors on DNA. Despite being fairly well-characterized in terms of their molecular function and expression during development, little is known concerning how their expression is regulated. We have identified four highly conserved noncoding elements associated with the vertebrate *Meis2* gene and named them m2de1-4 (for *Meis2* downstream element). While M2de2-4 have to date only been found in land vertebrates, m2de1 is also found in teleosts including zebrafish. Interestingly these elements are found in the introns of an adjacent gene, *zgc:154061* in zebrafish, whose orthologs are always found in an inverted convergently transcribed orientation directly downstream of *Meis2* in vertebrates. Our hypothesis is that the genomic organization of these two linked genes has been preserved due to them sharing *cis*-regulatory elements. To test this, we have examined the expression patterns of *meis2a* and *zgc:154061* as well as the expression of a reporter transgene directed by m2de1 in zebrafish.

Z6117C Precise levels of the transcription factor *gata2*, modulated through a conserved *cis*-element, are required for generation of definitive hematopoietic stem cells. *T. Dobrzycki*[1,3], R. Patient[1,2], R. Monteiro[1,2]. 1) Weatherall Institute of Molecular Medicine, Oxford, UK; 2) BHF Centre of Research Excellence, Oxford, UK; 3) Wellcome Trust DPhil Programme in Chromosome and Developmental Biology, Oxford, UK.

Hematopoietic stem cells (HSCs) maintain the vertebrate blood system throughout life. They arise during embryogenesis from the hemogenic endothelium (HE) located in the floor of the main embryonic artery, the dorsal aorta. Our understanding of the mechanisms underlying HE specification remains incomplete, but precise regulation of the transcription factor Gata2 is crucial for the generation of HSCs. For example, CRISPR/Cas9-mediated deletion of a conserved intronic enhancer (i4 enhancer) leads to a decrease in *gata2a* expression and subsequent loss of HSCs. By contrast, knockdown of the Lim-domain-only protein Lmo4, previously identified as a binding partner of Ldb1, results in loss of HSCs and massively increased *gata2a* expression in the HE. HSC formation in *lmo4a* morphants can be rescued by inhibiting Gata2 activity. Thus, the dose of *gata2a* in HE is critical for *de novo* production of HSCs, and *gata2a* levels are controlled by a critical negative input from Lmo4. We have found that the zebrafish i4 enhancer drives GFP expression in endothelial cells, including the HE, and have established a transgenic zebrafish line to monitor its activity *in vivo*. This line provides high temporal resolution for monitoring changes in Gata2a activity in the hematopoietic lineage and we could detect increased i4 enhancer activity upon Lmo4 loss-of function. To unravel the molecular basis for the loss of HSCs upon altered Gata2a expression we have generated deletion mutants for the i4 enhancer and for *lmo4*a, in order to respectively lower and elevate the levels of Gata2a in the embryo. Our current focus includes transcriptome-wide gene expression analysis and genome-wide analysis of chromatin structure in both mutant backgrounds in endothelial cells, including the HE. This will determine the global impact on gene regulation caused by modulation of *gata2a* levels and will help us understand how the precise control of *gata2* specifies the HE and generates HSCs.

Z6118A *bif* modulates the BMP pathway to pattern lateral plate mesoderm into primitive red blood cells. *J. GHERSI*, J. BERTRAND. CMU, GENEVA, CH.

During embryonic development, gastrulation leads to the specification of mesoderm that will contribute to the cardiovascular system, the muscles, and many other tissues. In zebrafish, mesoderm appears at 6 hours post fertilization (hpf), and the first blood progenitors can be detected as early as 12hpf in the lateral plate mesoderm (LPM). To better understand the mechanisms involved in LPM patterning into blood, we performed transcriptome analysis to compare the first erythrocyte progenitors with the first angioblasts, sorted at 12hpf. We identified a new gene, *bif (BMP-inhibitory factor)* that is specifically expressed in the posterior LPM that will give rise to primitive red blood cells. *bif* is expressed as early as 11hpf in the LPM and then remains specifically expressed in erythrocytes. *bif* is a transcription factor of unknown function. Gain-of-function experiments resulted in an expansion of the blood marker *gata1* in the posterior LPM at 12hpf, in a similar way that was observed when BMP signalling is perturbed. Moreover, overexpression of *bif* resulted in the duplication of the tail axis at 24hpf, and the mis-expression of cloaca markers, two phenotypes also observed in BMP-impaired embryos. Further analyses by qPCR and in situ hybridization

showed that *bif* expression diminishes the expression of BMP target genes.

We thus conclude that *bif*, specifies the LPM towards blood fate by down-regulating BMP signalling. We are now building new transgenic tools, as well as mutants, in order to understand the full spectrum of *bif* actions.

Z6119B Cardiac lymphatic development in the adult zebrafish. *Michael Harrison*[1], Xidi Feng[1], Stefan Schulte-Merker[2], Ching-Ling Lien[1,3]. 1) Children's Hospital Los Angeles, Los Angeles, CA; 2) Cells-in-Motion, Cluster of Excellence, University of Muenster, Germany; 3) Keck School of Medicine, University of Southern California, CA.

An intricate network of cardiac blood and lymphatic vessels provide cardiac tissue with oxygen and nutrients and eliminate excess fluid from the interstitium. Lymphatic vessels are required to maintain normal heart function and their blockage or destruction leads to cardiac edema, fibrosis and inflammation. Despite their critical roles in physiology and pathology, little is known about lymphatic vessel growth and development in the heart. Building on our recent work on coronary vessel development, we have described the formation of the lymphatic vasculature system in adult zebrafish. Lymphatic endothelial cells reside on the bulbus arteriosus until 3 months of age when they sprout on to the ventricle, preferentially migrating down the coronary arteries. Over several weeks these initial sprouts form vessels that are in close proximity to the coronary arteries and extend branching vessels that partially cover the ventricle. We are currently investigating the role of CXC-signaling in guiding this process and the potential role lymphatic vessels play in heart development and regeneration.

Z6120C *foxc1a* and *foxc1b* exhibit distinct compensatory requirements during brain and trunk angiogenesis and haematopoietic stem cell formation in zebrafish. *Z. Jiang*[1,2], T. Evans[3], M. Loose[3], T. J. A. Chico[1,2], R. N. Wilkinson[1,2]. 1) Department of Infection, Immunity & Cardiovascular Disease, University of Sheffield, Medical School, Sheffield, UK; 2) The Bateson Centre, University of Sheffield, Sheffield, UK; 3) Medical School, University of Nottingham, Queen's Medical Centre, Nottingham, UK.

The cardiovascular system is essential for all aspects of tissue growth and physiology. Haematopoietic stem cells (HSCs) are derived from the embryonic artery during early development and give rise to all adult blood cell lineages throughout life. The shared origin of arterial endothelial cells (ECs) and HSCs means these cells share common mechanisms which regulate their formation. Our understanding of these mechanisms remain incomplete. In mammals, the transcription factors *foxc1* and *foxc2* are required for both cardiovascular and haematopoietic development, however, how these genes interact with endothelial signalling pathways to regulate these processes remains unclear.

We have generated novel zebrafish mutants in orthologues of mammalian *foxc1* (*foxc1a* and *foxc1b*) to determine the function of these genes during blood vessel and HSC formation. *foxc1a* mutants display aberrant cranial blood vessel formation and defective blood-brain barrier formation with almost total loss of central arteries (CtAs). *flt4* expression is drastically reduced within the primordial hindbrain channel (PHBC) of *foxc1a* mutants, indicating *foxc1a* may regulate the ability of ECs within the PHBC to receive VEGF. Arteriovenous differentiation is disrupted within the trunk of *foxc1a* mutants. While the dorsal aorta and posterior cardinal vein are present in *foxc1a* mutants, arterial gene expression is substantially reduced and venous expression is increased, indicating *foxc1a* is required for correct specification of arteries and veins. Segmental arteries (SeA) are correctly specified and sprout normally in *foxc1a* mutants, however *foxc1a; foxc1b* double mutants display ectopic secondary SeA branching indicating *foxc1a* and *foxc1b* genetically interact during angiogenesis. In keeping with observed reductions in arterial gene expression, *foxc1a* mutants exhibit reduced HSC numbers and HSC progeny. Interestingly, *foxc1a* mutants also display defective somite patterning as has been reported previously. Furthermore, somitic Wnt16 and Dlc/Dld signalling are known to instruct HSC formation non-cell-autonomously and *wnt16* and *dlc* expression are reduced in *foxc1a* mutants. Collectively, this indicates *foxc1a/b* play compensatory and pleiotropic roles in co-ordinating both blood vessel and HSC formation and may influence these processes via distinct pathways.

Z6121A Vegfa signaling promotes zebrafish intestinal vasculature development through endothelial cell migration from the posterior cardinal vein. *Andrew L. Koenig*[1], Kristina Baltrunaite[1], Neil I. Bower[2], Andrea Rossi[3], Didier YR Stainier[3], Ben M. Hogan[2], Saulius Sumanas[1]. 1) Division of Developmental Biology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA; 2) Division of Genomics of Development and Disease, Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD 4073, Australia; 3) Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, Bad Nauheim 61231, Germany.

The mechanisms underlying organ vascularization are not well understood. The zebrafish intestinal vasculature forms early, is easily imaged using transgenic lines and in-situ hybridization, and develops in a stereotypical pattern thus making it an excellent model for investigating mechanisms of organ specific vascularization. Here, we demonstrate that the sub-intestinal vein (SIV) and supra-intestinal artery (SIA) form by a novel mechanism from angioblasts that migrate out of the posterior cardinal vein and coalesce to form the intestinal vasculature in an anterior to posterior wave with the SIA forming after the SIV. We show that vascular endothelial growth factor aa (*vegfaa*) is expressed in the endoderm at the site where intestinal vessels form and therefore likely provides a guidance signal. Vegfa/Vegfr2 signaling is required for early intestinal vasculature development with mutation in vegfaa or loss of Vegfr2 homologs causing nearly complete inhibition of the formation of the intestinal vasculature. Vegfc and Vegfr3 function, however, are dispensable for intestinal vascularization. Interestingly, ubiquitous overexpression of Vegfc resulted in an overgrowth of the SIV, suggesting that Vegfc is sufficient to induce SIV development. These results argue that Vegfa signaling directs endothelial cells to migrate out of existing vasculature and coalesce to form the intestinal vessels. It is likely that a similar mechanism is utilized during vascularization of other organs.

Z6122B DLC1 is a negative regulator of directed endothelial cell migration during embryonic vascular development. *T. Linnerz*, J. Y. Bertrand. Centre Médicale Universitaire, Geneva, CH.

The integrity of the vascular system is established during development. During vertebrate embryogenesis, the first endothelial precursors

arise in the lateral plate mesoderm (LPM) as early as 12hpf. At mid-somitogenesis stages, angioblasts migrate towards the midline, merge and organize into nascent cords giving rise to the major blood vessels (vasculogenesis). Subsequently, primary sprouting events occur from the pre-existing vessels (angiogenesis). We use the zebrafish model to investigate how the endothelial program initiates.

We are interested in *dlc1*, a gene encoding a RhoGTPase activating protein and tumor suppressor gene, which plays a role in the regulation of RhoA, B and C activity, hence influencing several cell processes such as migration or adhesion. *dlc1* is first expressed in the primordial angioblasts of the LPM, consistent with the high motility of these cells. Several gain-of-function approaches led to similar phenotypes: although arterio-venous specification was not impaired, angiogenic sprouting is greatly affected, resulting in poorly lumenized or hypersprouting intersegmental vessels (ISV) in the trunk of the embryo. As a result, blood circulation is disturbed leading to cardiac edemas and brain hemorrhages.

To further elucidate *dlc1*'s role in vascular development, we generated a mutant line with the CRISPR/Cas9 technology. Whereas *dlc1* knock-out mice die in utero preventing the study of any vascular phenotype, the *dlc1* null zebrafish survives until 5 days, allowing a better insight into its role in angiogenesis. The mutant fish has severe vascular defects that manifest in impaired primary blood circulation in aorta and vein, which is resulting in massive cardiac edemas. We will now use this new tool to investigate the angiogenic processes. Altogether, we will understand the physiological role of *dlc1* during development and reveal new mechanisms controlling angiogenesis in vertebrates.

Z6123C Heparin Receptor Involvement in Zebrafish Angiogenesis. *L. J. Lowe-Krentz*, S. L. N. Farwell, M. K. Iovine. Lehigh University, Bethlehem, PA.

Studies employing model systems from cell and tissue culture to zebrafish have determined critical role(s) for heparan sulfate chains of proteoglycans in vascular development and regeneration. Heparan sulfate chain interactions with growth factors and their receptors have been proposed to explain these requirements. Recently, our studies have confirmed the identification of a membrane protein (TMEM184A) that acts as a receptor for heparin (and heparan sulfate, by extension). This receptor plays a role in signal transduction and heparin uptake in cultured vascular cells. To examine the role of this receptor in a vertebrate organism, we employed zebrafish, and the specific *TG(fli1-EGFP)* transgenic line to evaluate the presence of the receptor and its function in angiogenesis. We have identified this protein (TMEM184A) in ontogenic and regenerating zebrafish vasculature through co-immunofluorescence with GFP in *Tg(fl1-EGFP)* zebrafish. The heparin receptor co-localizes with GFP in both arteries and veins in ontogenic tail fin rays from the fish. After amputation to approximately 50%, immunofluorescence confirms localization of the heparin receptor matching the GFP pattern in regenerates. In tissue culture systems, cell proliferation is increased in cells where TMEM184A is knocked down. Knock-down fin rays have larger numbers of GFP containing vascular cells, and the cells are more disorganized than either untreated or control morpholino treated fins. This effect is reversible. Transient knockdown using morpholinos results in significantly less TMEM184A staining in regenerating vessel cells within 24 hours of morpholino injection. These studies indicate expression of the heparin receptor in zebrafish endothelial cells and a modulatory role in angiogenesis. Coupled with information from cultured cells where heparin treatment decreases cell proliferation and receptor knockdown allows more extensive proliferation, these studies suggest that heparan sulfate chains from proteoglycans interact with the heparin receptor in this modulatory function.

Z6124A Effect on lymphoid transcriptional regulation factors correlate with the downregulation of *lmna* during hemotopoiesis. *l. shu*, F. Liu, H. Huang, H. Zhang, X. Wu, J. Wang, Z. He. Guizhou Medical University, guiyang, guizhou, CN.

Li-Ping Shu [*] Feng Liu [*] Hui-Min Huang, Heng-Lu Zhang, Xi-Jun Wu, Jun Wang, Zhi-Xu He [#](* co-author [#] correspongding author(hzx@gmc.edu.cn))

Abstract:objective Lamins are the major components of nuclear lamina underneath the inner nuclear membrane (INM). Lamins express in most cell and involve in the whole process of growth, which play a major role in cell stability and embryo development. While the effect on lymphoid transcriptional regulation factors correlate with the downregulation of *lmna* during hemotopoiesis remains unknown. **method** MO(Morpholino Oligomer) tagged to zebrafish *lmna* gene was microinjected into zebrafish embryos of different phases to downregulate the expression of *lmna*. The expression level of different transcriptional regulation factors such as *lmo2*, *scl*,*c-myb* and *runx1* were detected by Q-PCR. **result** The Q-PCR results show that the expression of transcriptional regulation factors *lmo2* and *scl*, which associated with primitive hemotopoiesis were downregulated in 18h, 24h, 30h and 36h groups compared with control group. *c-myb* and *runx1* were also downregulated. **conclusion** These results revealed that *lmna* may have a relationship with hemotopoietic transcriptional regulation factors and have a negative effect on embryo development.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (NO. 31260284). We thank all members of the Department of Immunology and the Center of Stem Cell and Tissue Engineering Research for discussions and valuable comments.

Z6125B Establish an Tg(*zgata1:g6pd*-EGFP) zebrafish with a deficiency of 118-144 site on *g6pd*. *l. shu*, J. Song, Y. Tuo, L. Shang, Y. Zhou, X. Wu, Z. He. Guizhou Medical University, guiyang, guizhou, CN.

Li-Ping Shu *, Jin Song *, Yuan-Yuan Tuo,Lu-Jun Shang,Yan-Hua Zhou,Xi-Jun Wu, Zhi-Xu He[#] (* co-author [#] correspongding author((hzx@gmc.edu.cn)

Objective: To establish zebrafish model of G6PD dificiency.With dominant negative, expression of the mutant G6PD can impact the normal G6PD protein expression ,which result G6PD activity reducing.This modle can be used to study G6PD deficiency molecular mechanisms and provide an experimental basis for mass drug screening .**Methods**: To constrct a *gata1:g6pd*$^{M118\text{-}144}$-EGFP-PBSK-IsceI recombinant

plasmids.The plasmid were microinjected into tüebingen wild-type zebrafish embryos of single-cell stage.zebrafish embryos, which is injected into *gata1:g6pd*$^{M118\text{-}144}$-EGFP -PBSK-IsceI plasmid, express green fluorescent protein in the blood circulation that be treated as F0 generation G6PD transgenic zebrafish. Feeding F0 generation transgenic zebrafish until breeding period, each F0 generation transgenic zebrafish mate with wild-type zebrafish, the green fluorescent protein expression was observed in blood circulation in F1 generations transgenic zebrafish .Then using genomic PCR,whole mount in situ hybridization (WISH),Western blotting identify the Tg:*gata1:g6pd*$^{M118\text{-}144}$-EGFP F1 generation transgenic zebrafish on levels of DNA, mRNA, protein .**Results:** z*gata-1-g6pd*$^{M118\text{-}144}$*-EGFP*-pBSK-I-SceI recombinant plasmid is constructed and injected into wild-type zebrafish embryos.We screen zebrafish embryoes,which express green fluorescent protein as Founder 0 generation G6PD transgenic zebrafishs. we got five F0 generation G6PD transgenic zebrafishs. Expressing of the green fluorescent protein in F0 generation transgenic zebrafishs begins in ventral mesoderm at 11 hpf, and circulates with blood after 24 hpf ,and which can be passed on to the offspring. The *g6pd*$^{M118\text{-}144}$ is amplified from the genome of F1 generation transgenic zebrafish.With WISH,the mRNA of EGFP is detected at erythropoiesis site of F1 generation transgenic zebrafish. With Western blotting, expression of G6PD$^{M118\text{-}144}$-EGFP fusion protein is detected in F1 generation transgenic zebrafish.To sum up,we can identify that the *gata-1-g6pd*$^{M118\text{-}144}$*-EGFP* has integrated into the zebrafish genome and transcribed into mRNA, translated purposes fusion protein.**Conclusions** Identified the Tg:*zgata-1:g6pd*$^{M118\text{-}144}$-EGFP transgenic zebrafish on levels of DNA, mRNA, protein.Establishment the zebrafish disease models of G6PD deficiency can achieve in-depth study related mechanism in G6PD deficiency and high-throughput screening of antimalaric drugs to treat G6PD deficiency disease.

Acknowledgments This work was supported by the National Natural Science Foundation of China (NO.31360285).

Z6126C Integrin Alpha 6 Is Required for Neurovascular Development Of The Hindbrain In Danio Rerio. *Vinoth Sittaramane*, Louise Zehr, Kayla Smith. Georgia Southern University, Statesboro, GA.

Stroke is one of leading causes of death and disability worldwide (CDC, 2014). Hemorrhagic stroke could be due to defective neurovascular development. Even though the occurrence of stroke happens in older age groups, genetic studies have found mutations (SNPs) in human integrin alpha 6 (ITGA6) linked to the predisposition of stroke. As of now, the roles of ITGA6 in the development of the neurovascular system are unknown. This study created itga6 deficient embryos using antisense nucleotides to investigate neurovascular development in zebrafish embryos using appropriate vascular transgenic lines. Deficiency in itga6 leads to severe loss of central arteries (CtAs), increase in endothelial cell protrusions, and dilation of several hindbrain blood vessels such as the basal artery (BA) and primordial hindbrain channels (PHBC). Itga6 mRNA injections were able to rescue deficient phenotypes, displaying return in number of CtAs, decrease in protrusions, and decrease in dilation. Further, time-lapse analysis also supported these findings in the deficient embryos showing an increase in endothelial protrusions with inability to make connections as seen in the controls. The loss of itga6 in embryos also displayed hemorrhaging in the hindbrain by 48hpf. These results indicate that itga6 does play a critical role in neurovascular formations. Itga6 is a transmembrane receptor for the extracellular matrix protein laminin enabling matrix remodeling during morphogenetic processes. During cancer metastasis Itga6 has been shown to cleave extracellularly allowing for cell migration. This study investigated whether integrin alpha 6's ability to cleave is also required for neurovascular development. Our studies have found that this cleavage mechanism in integrin alpha 6 is necessary for the CtA formation in the hindbrain as well.

Z6127A Deciphering the mechanism of action of ApoB lipoproteins on endothelial cells. *H. Tempelhof*, I. Avraham-Davidi, Y. Ely, K. Yaniv. weizmann institute of science, Rehovot, IL.

Apolipoprotein B (ApoB) is the primary structural protein in low-density and very low-density lipoproteins, and in chylomicrons. In addition to its established role in lipid metabolism, we have suggested in the past that ApoB negatively regulates angiogenesis during embryonic development. In this work we have used Talen and crispr technologies to generate zebrafish carrying mutations in two ApoB genes- *apoBa* and *apoBb.1*. In addition to complete depletion of lipoprotein production and secretion, the double mutant embryos were characterized by excessive angiogenesis. In contrast, a complementary model of hyperlipidemia and increased ApoB secretion, generated through overexpression of microsomal triglyceride transfer protein (*mtp),* resulted in inhibition of intersegmental vessel formation. Surprisingly, we have been able to detect the presence of ApoB inside the nucleus of endothelial cells both in cultured HUVECs and in FACS sorted zebrafish endothelial cells. These findings suggest that in addition to its well-established role in cholesterol transport, ApoB may act as a regulator of gene expression, functioning according to the metabolic state of tissues.

Z6128B Reck is a novel component of the canonical Wnt signaling pathway required for the formation of the brain blood vasculature and its barriergenic differentiation. *J. Torres-Vazquez*[4], Florian Ulrich[4], Jorge Carretero-Ortega[4], Carlos Narvaez[4], Belinda Sun[4], Eva Lancaster[4], Valerie Pershad[4], Sean Trzaska[4], Evelyn Veliz[4], Makoto Kamei[1], Andrew Prendergast[2], Kameha Kidd[1], Kenna Shaw[1], Daniel Castranova[1], Van Pham[1], Brigid Lo[1], Benjamin Martin[3], David Raible[2], Brant Weinstein[1]. 1) NICHD, NIH. Bethesda, MD; 2) University of Washington, Seattle, WA; 3) Stony Brook University, Stony Brook, NY; 4) New York University / Skirball Institute, New York, NY.

The cerebral vasculature provides the massive blood supply that the brain needs to grow and survive. By acquiring distinctive cellular and molecular characteristics it becomes the blood-brain barrier (BBB), a selectively permeable and protective interface between the brain and the peripheral circulation that maintains the extracellular milieu permissive for neuronal activity. In a forward genetic screen we isolated a mutant that specifically lacks most of the intracerebral central arteries but not other brain blood vessels. We found that the cerebral vascularization deficit of these mutants is caused by an inactivating lesion in reck (reversion-inducing cysteine-rich protein with Kazal motifs reck; which encodes a membrane-anchored tumor suppressor glycoprotein). Our findings highlight Reck as a novel and pivotal modulator of the canonical Wnt signaling pathway that acts in endothelial cells to enable intracerebral vascularization and proper expression of molecular markers

associated with BBB formation. Additional studies with cultured endothelial cells suggest that, in other contexts, Reck impacts vascular biology via the vascular endothelial growth factor (VEGF) cascade. Together, our findings have broad implications for both vascular and cancer biology.

Z6129C The function of *prdxI* during vascular development in zebrafish. *Chang-Yi Wu*, Po-Chun Huang, Hai-Hong Syue. Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan.

The balance of reduction-oxidation (redox) has been shown acting an important role in vascular growth. However, most studies have done in adult animals under pathological conditions, and very limited information about oxidative stress and vascular development during embryogenesis. Here, we report a novel biological function of *prdx1* (*peroxiredoxin1*) that play critical roles in vascular formation during zebrafish development. Prdx1 belongs to a member of the peroxiredoxin family of antioxidant enzymes. We showed *prdx1* is expressed in developing vessels and knockdown of *prdx1* by morpholino injection impairs the growth of ISV (intersegmental vessel) and CVP (caudal vein plexus), suggesting the role of *prdx1* in promoting vessels growth. We further showed the reduction of ISV cells is due to a decrease of cell proliferation and migration, but not results from cell death in non-endothelial cells. The molecular evidence of vascular defects in *prdx1MO* is related to the decreased expression of vascular markers, *flt4, mrc1*, *stabilin* and *ephrinb2*. Loss of *prdx1* results in vascular defects suggest the antioxidant function is important, thus, we test if oxidative stress could cause vascular defects in H_2O_2-treated embryos. Our data showed H_2O_2 treatment impaired CVP formation and caused mild ISV defects. While H_2O_2-treated embryo combined with knockdown of *prdx1*, synergetic effects are observed. In addition, exogenous N-acetylcysteine (*NAC*) treatment rescues the vascular defects in *prdx1MO*. Those data suggested the oxidative stress indeed can disturb vascular development. We further showed the different regulation pattern of antioxidant genes *SOD1, SOD2* and *catalase* in *prdx1* morphants from H_2O_2-treated embryos. To confirm the role of *prdx1* in vasculature, we overexpressed *prdx1* in the embryos and found overexpression of *prdx1* promotes ISV and CVP growth. Meanwhile, overexpression of *prdx1* can rescue the loss of *prdx1*. Those data suggest *prdx1* functions in vascular development necessary and sufficient. Interestingly, we found the increased expression of blood markers (*gata1, globin* and *lmo2*) coincident with the decreased expression of endothelial markers *flk1* and *fli* in *prdx1* MO, suggested *prdx1* likely regulate hemangioblast fate decision at earlier developmental stage. We further demonstrated *prdx1* likely interacts with Notch to control cell fate switch. Together, we showed *prdx1* play novel and critical roles in vascular growth during zebrafish development.

Z6130A A 24-hour Buffet: Effects of Culturing Zebrafish Under Continuous Illumination from Fertilization to Adulthood. *Thomas A. Delomas*, Mackenzie Miller, Konrad Dabrowski. The Ohio State University, Columbus, OH.

Investigation into the effect of continuous light on zebrafish biology and life cycle is lacking. This study examines growth rate, survival, and generation time of zebrafish raised under continuous illumination (24:0 photoperiod) from the time of fertilization to adulthood. Based on previous experiments in our laboratory with other cyprinid fishes, faster growth rate and shorter generation time under continuous illumination were predicted.

Larviculture methods utilizing rotifers, *Brachionus plicatilis*, and brine shrimp, *Artemia salina*, nauplii, were modified for this study. Zebrafish larvae were stocked in aerated stagnant water tanks at 5 days post fertilization (dpf) at a density of 100 larvae/L, which is 10 times higher than previously described. Feeding began at 6 dpf with live rotifers. At 12 dpf, half of the population was transitioned to live brine shrimp nauplii while the other half remained on rotifers for the duration of the study (46-50 dpf).

Fish achieved an average length of 16.1±0.7mm and weight of 61.6±6.7mg at 20 dpf, 2-5 and 10-30 times greater, respectively, than the length and weight described in the literature. Daily growth rate (%) was 39.7% from 12 to 20 dpf. Survival at 12 dpf (7 days post initial feeding), when larvae are most sensitive, was 75.6±10.6%. Once fish reached a size where gender could be visually distinguished (42 dpf), spawning pairs were set up daily (this included dark:light regime, 10:14, for 1-2 days) until a successful spawning occurred. Generation time (from egg to egg) was determined as 44 dpf (when the first pair spawned), 5 days earlier than the shortest generation time reported thus far in the literature (Aoyama et al. 2015. Zebrafish 12:288). This demonstrates that zebrafish can be successfully grown under continuous illumination to produce higher than average growth rate and shorter generation time than that suggested under light/dark regime. Using this method, five consecutive generations have been produced in 228 days. This rearing protocol will drastically reduce the time required for multigenerational experiments and mutant strain development, thereby considerably decreasing the cost of zebrafish culture.

Z6132C Optimization of Larval Zebrafish Husbandry: Getting More with Less. *David Zitser*, Nastassja Carusetta, Anish Bhandari, Robert Nissen. California State University of Los Angeles, Tarzana, CA.

The zebrafish *Danio Rerio* is a well-established model organism used to study normal development as well as disease states. While the use of dry feeds in zebrafish husbandry has grown substantially over the past decade, further optimization of the care and feeding of zebrafish breeding colonies may save significant time and resources. Thus, we sought to optimize larval care towards maximizing survival rate while also minimizing the amount of hands-on care required to achieve a still acceptably high survival rate. Many factors potentially affect larval survival rate. We sought to optimize food amount, feeding frequency, and water change frequency during the first 21 days of life. First, we optimized the quantity of 100-micron Golden Pearl (GP100) to be fed per tank of 30 larval zebrafish in one liter of fish system water. Second, we optimized the frequency of feedings and found that less frequent feeding may outperform daily feedings while simultaneously reducing the need for frequent water changes. Together, these changes yielded an average 81% larval survival rate (at 21dpf) with only 3 feedings per week and only a single water change per week, at which point the animals entered the adult colony and adult feeding regimen. These results may yield substantial savings of time and resources for breeding colonies.

Z6133A A zebrafish model of acute kidney injury associated with systemic infection induced by intravascular bacteria injection. *L. Cui*[1,2], X. Wen[1,2], S. Morrisroe[1,2], L. Brilli[1,3], X. Chen[1,2], N. Hukriede[1,3], J. Kellum[1,2]. 1) Center for Critical Care Nephrology, Pittsburgh, PA; 2) Department Critical Care Medicine, Pittsburgh, PA; 3) Department of Development Biology, University of Pittsburgh, Pittsburgh, PA.

Sepsis associated Acute Kidney Injury is a serious complication in critical ill patients, contributes to high mortality. To date, the underlying mechanisms responsible for kidney injury in sepsis remain poorly understood and treatments are still limited to supportive methods. The zebrafish is an economically attractive multicellular organism, provides unique visual access to the microscopic structure with cell-cell interactions. Both the embryonic and adult zebrafish show conserved components in both immune responses and functional nephrons with mammals, which makes this model promising for studying mechanisms and screening for treatment options. An acute nephrotoxic kidney injury model has been developed using gentamycin, but inflammation-associated AKI is more clinical relevant and yet no model in the zebrafish has been reported. E. tadar is a lethal pathogen in zebrafish, characterized by striking cytokine excretion and high mortality. Here we compared inflammatory findings of both bacteria injections into 3 days post-fertilization (dpf) zebrafish embryos as well as infection mediated kidney damage. We show dose-dependent mortalities for both E. coli and E. tadar intravascular injections, with E. tadar significantly higher than E. coli treated animals. We found remarkably decreased fluorescent dextran uptake and loss of tubule brush boarder occurred as early as 6-24 hours post injection in E. tadar treated larvae compared to E. coli treated ones as well as normal controls. Nephron-specific kidney injury molecular-1 (KIM-1) functions as phagocyte receptor, mediates uptake of dead cells and tissue debris. Immunofluorescence staining of KIM-1 appears at late time points post injection in both groups compared to no expressions in control groups. Prolonged expression of KIM-1 in mammalian kidney predicts AKI and maladaptive repair. These analyses have provided new insights for further understanding the physiological and molecular mechanisms that lead to kidney injury in the setting of infection and system inflammation.

Z6134B Host-Directed Therapies for Tuberculosis: Discoveries from a Zebrafish Chemical Screen. *M. A. Matty*, D. M. Tobin. Duke University, Durham, NC.

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, kills over 1 million people annually and drug resistance to current therapies is rapidly increasing. We have explored drug treatments to potentiate host responses to mycobacterial infection using a novel screening technique in Mycobacterium marinum (Mm) infected zebrafish to discover FDA-approved drugs that ameliorate Mm infection through host-directed processes. From this screen, we uncovered 10 novel compounds that reduce Mm burden in a host-dependent manner. Using CRISPR/cas9, we have found the target of one small moledule that ultimately benefits the host to control Mm infection by potentiating host innate immune signalling pathways. We have shown that this drug induces cell death and may alter the innate immune response through inflammatory cues. Utilizing the validated zebrafish:Mm infection model, this work will assist in development of novel therapeutics for human:Mtb infections.

Z6135C Role of Developmental Signaling Pathways in Mycobacterial Pathogenesis. *Allison Rosenberg*, David Tobin. Duke University, Durham, NC.

Tuberculosis (TB) kills 1.5 million people annually, with 8-9 million new active cases each year. The causative agent, *Mycobacterium tuberculosis (Mtb)*, manipulates host macrophages to survive within them. These infected macrophages, along with uninfected macrophages and other immune cells, aggregate into characteristic structures called granulomas where almost all bacteria reside in human *Mtb* infections. We have paired *Mycobacterium marinum (Mm),* the closest relative of the *Mtb* complex and a natural pathogen of ectotherms, with the zebrafish to establish a validated surrogate for human TB. The tuberculous granulomas that form in zebrafish recapitulate important features of human granulomas (organization, hypoxia, caseation necrosis) that are absent from standard mouse models. Here, we use the zebrafish to investigate how the central structure of mycobacterial infection is assembled and remodeled. We have used this model to make new findings about the tuberculous granuloma and have validated these findings in human specimens. Here, based on these findings and preliminary data, we hypothesize that macrophages, the central immune reservoir for pathogenic mycobacteria, deploy canonical developmental pathways, not traditionally considered in the context of immunity, in the construction of the granuloma. Using the zebrafish- *Mycobacterium marinum* model we will determine the role of developmental signaling in mycobacterial pathogenesis and granuloma formation.

Z6136A Investigating interleukin-2 receptor family signaling in zebrafish. *Robert Sertori*, Clifford Liongue, Alister C. Ward. Deakin University, Geelong, Victoria, AU.

The interleukin 2 receptor (IL-2R) family of class I cytokine receptors plays important roles in lymphocyte development and function. The mammalian IL-2R family utilize the shared signaling component interleukin 2 receptor gamma common (IL-2Rγc). Mutations of this receptor chain in humans or mice leads to severe combined immunodeficiency (SCID).

The purpose of this project was to examine the IL-2R family in zebrafish and generate a SCID zebrafish model.

Methodologies used include transcription activator-like effector nucleases (TALEN) induced mutagenesis, high resolution melt (HRM) and restriction fragment polymorphism (RFLP) to identify mutants and whole-mount *in situ* hybridization (WISH) and RT and QRT-PCR for expression analysis. Morpholino mediated knock-down was also used to explore the interleukin 15 receptor (il-15r).

Zebrafish possess 2 paralogues of *IL-2Rγc* with *il-2rγc.a* shown to have a conserved role in embryonic T lymphopoiesis. Therefore TALENs were targeted to *il-2rγc.a* to generate a SCID zebrafish model. Homozygous mutants were immunocompromised and showed reduced T cells at 5 dpf which was also evident at 28 dpf. B cells were expressed at 28 dpf indicating a T-B+ SCID model.

The zebrafish heterotrimeric receptor was also investigated for a conserved role in lymphopoiesis. Zebrafish only possess *il-15rα* at the *il-2rα*/*il-15rα* gene locus resulting in a single ligand specific chain that would signal with interleukin 2 receptor beta (il-2rβ) and il-2rγc.a. The

possible complex was investigated by morpholino mediated knockdown of *il-15rα*, *il-2rβ* and a possible *il-2rβ* like and examined for changes in *rag1* expression at 5 dpf.

Z6137B DBP is essentially required for zebrafish embryogenesis. *Seong-Kyu Choe*, Yong-Il Kim, SeongAe Kwak, Sushil Bhnadari, In-Koo Nam. Wonkwang University School of Medicine, Iksan, Jeonbuk, KR.

D-bifunctional protein (DBP) is a highly conserved enzyme involved in peroxisomal beta-oxidation. We investigated the role of DBP during zebrafish embryogenesis. Zebrafish *dbp* is expressed early during development. Morpholino-mediated Dbp knockdown generates embryo with small head, pericardial edema and short tail. In agreement with its involvement in peroxisomal fatty acid oxidation, yolk lipid consumption is highly reduced in Dbp knockdown embryos. Endoderm-derived organs such as liver and pancreas are almost absent in morphants. Dbp knockdown also affects other aspects of animal development, such as neuronal maturation, cartilage formation, blood cell development and vasculogenesis. Consistent with morphological defects upon Dbp knockdown, significant changes in the expression of various genes involved in ether phospholipid synthesis, mitochondrial biogenesis, peroxisomal protein import and beta-oxidation pathway in peroxisome. Taken together, these results indicate that Dbp deficiency causes severe developmental abnormalities some of which has not been reported yet in human or mouse studies, and suggest that zebrafish can be used as an animal model to study organelle functions in vivo.

Z6138C Ketohexokinase, a fructose metabolic enzyme plays an important role in somatogenesis and angiogenesis during early embryonic development. *C. Chun*, C. Chen, H. Chun, M. Segal. University of Florida, Gainesville, FL.

Ketohexokinase (Khk), also known as fructokinase is the first enzyme in a specialized catabolic pathway metabolizing dietary fructose to the glycolytic intermediate glyceraldehyde-3-phosphate. While the metabolic mechanism of glucose is tightly regulated by feedback mechanism, the metabolic mechanism of fructose bypasses feedback inhibition. Studies on metabolism of fructose have recently garnered attention due to the association of high fructose diets with an increased risk of diabetes and other diseases. These adverse effects in both human and animal models were exhibited during adult stage. However, the impact of fructose diet and Khk on fetal development has been overlooked. We utilized zebrafish as an animal model to study Khk function during early embryonic development. We utilized zebrafish as an animal model to study Khk function during embryonic development. mRNA of Khk is detected as early as 1-cell stage and an alternative transcript is detected from 10 to 48 hours post fertilization (hpf). We designed two morpholinos to block either translation or splicing of khk and injected into newly fertilized embryo. Knockdown (KD) of khk results in fused somites and defective intersomatic vessel (ISV). Utilizing CRISPR/Cas9 system we edit khk genomic DNA and observe similar phenotype to morpholino-based KD of khk. Taken together, these data suggest a role for khk in early embryonic development, especially in somatogenesis and angiogenesis. .

Z6139A Circadian modulation of autophagy rhythms directly through the nuclear hormone receptor Rev-erbα and indirectly via C/ebpβ in zebrafish. *Guodong Huang*, Fanmiao Zhang, Qiang Ye, Han Wang. Soochow University, Suzhou, Jiangsu, China.

Autophagy is a highly conserved intracellular degradation system, and recently was shown to display circadian rhythms in mice. The mechanisms underlying circadian regulation of autophagy, however, are still unclear. Here, we observed that numbers of autophagosomes and autolysosomes exhibit daily rhythms in the zebrafish liver, and *c/ebpβ* and various autophagy genes are rhythmically expressed in zebrafish larvae but significantly upregulated in *per1b* and TALEN-generated *rev-erbα* mutant fish, indicating that both Per1b and Rev-erbα play critical roles in autophagy rhythms. Luciferase reporter and ChIP assays show that the circadian clock directly regulates autophagy genes through Rev-erbα, and also regulates transcription of *c/ebpβ* through Per1b. We also found that fasting leads to altered expression of both circadian clock genes and autophagy genes in zebrafish adult peripheral organs. Further, transcriptome analysis reveals multiple functions of Rev-erbα in zebrafish. Taken together, these findings provide evidence for how the circadian clock regulates autophagy, imply that nutritional signaling affects both circadian regulation and autophagy activities in peripheral organs, and shed light on how circadian gene mutations act through autophagy to contribute to common metabolic diseases such as obesity.

Z6140B Involvement of The p62-Nrf2 Pathway as A Protection Mechanism against Spns1 Deficiency in Zebrafish. *A. Khan*[1], S. Lian[1], T. Sasaki[1], M. Kobayashi[2], S. Kishi[1]. 1) The Scripps Research Institute, Jupiter, FL; 2) University of Tsukuba, Japan.

The protein Nrf2 is a transcription factor which regulates the expression of various antioxidant genes in cells. p62/SQSTM1 functions as a cargo receptor for autophagy and also in the non-canonical activation of Nrf2. Using transgenic zebrafish that express multiple reporters which allow simultaneous monitoring of Nrf2 activation and autophagy, we demonstrate that chemical and genetic inductions of stress and autophagic responses are reciprocally linked to developmental senescence. Deficiency of the lysosomal sympoter Spns1 was exacerbated by loss of either Nrf2 or p53 individually. However, acceleration of senescence due to the loss of Nrf2 in *spns1*-mutant embryos was partially counteracted by the p53 defect, suggesting that Nrf2 plays a protective role against autolysosomal deterioration induced by Spns1 deficiency through a different mechanism from p53. Intriguingly, a newly identified chemical potentiator of Nrf2 in our system, but not other authentic Nrf2 activators, ameliorates *spns1*-mutant phenotypes. We further found that chemically and genetically enhanced p62 aggregations are involved in the mechanism of Nrf2-induced protection against Spns1-deficent pathogenesis. Together, these data suggest that chemical genetic boosting of the p62-Nrf2 axis protects against the deteriorative impact induced by Spns1 deficiency, a condition in which autolysosomal clearance is disrupted.

Z6141C Genetic Interaction between Spns1 and v-ATPase and Their Counteractive Dual Defects in Premature Autolysosomal Fusion and Developmental Senescence. *S. Lian*[1], T. Sasaki[1], K. Alam[1], W. Chen[2], D. Klionsky[3], S. Kishi[1]. 1) The Scripps Research Institute, Jupiter, FL; 2) Vanderbilt University School of Medicine, Nashville, TN; 3) University of Michigan, Ann Arbor, MI.

Spns1 [Spinster homolog 1 (*Drosophila*)] in vertebrates, as well as Spin (Spinster) in *Drosophila*, is a hypothetical lysosomal H^+-carbohydrate symporter, which functions at a late stage of macroautophagy (hereafter autophagy). The Spin/Spns1 defect induces aberrant autolysosome formation that leads to developmental senescence in the embryonic stage and premature aging symptoms in adulthood. However, the molecular mechanism of the specific pathogenesis still awaits elucidation. Using chemical genetic and CRISPR/Cas9-mediated genome-editing approaches in zebrafish, we investigated a mechanism that ameliorates Spns1 loss-mediated embryonic senescence as well as autolysosomal impairment. Unexpectedly, we identified the vacuolar-type H^+-ATPase (v-ATPase) subunit gene, *atp6v0ca* (ATPase, H^+ transporting, lysosomal, V0 subunit ca) as a spatiotemporal suppressor for senescence induced by the loss of Spns1, while the sole loss of Atp6v0ca led to senescent embryos. Moreover, we discovered that the concurrent heritable defect of both *spns1* and *atp6v0ca* still induced premature autophagosome-endosome and autophagosome-lysosome fusion without sufficient acidity in the lysosome. Our data suggest that Spns1 and the v-ATPase orchestrate proper endo-lysosomal biogenesis with optimal acidification that is critically linked to developmental senescence and survival.

Z6142A A transgenic approach to visualize mitochondrial dynamics associated with renal function and disease. *Y. Sugano*[1,2], R. Tomar[1,2], E. Merkel[1,2], I. A. Drummond[1,2]. 1) Massachusetss General Hospital, Charlestown, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA.

The kidney demands a high metabolic energy supply for its proper function. Mitochondria are the major source of energy supply in aerobic cells. Accumulating amounts of evidence implicate that mitochondria play a central role in renal function. For instance, individuals with mutations in genes required for mitochondrial coenzyme Q10 (CoQ10) biosynthesis pathway, such as *ADCK4* and *COQ6*, develop steroid-resistant nephrotic syndrome characterized by edema and severe proteinuria. Despite the importance, however, little is known about mechanisms by which altered mitochondrial function leads to development of renal disease. In this study, taking advantage of the optical clarity of embryos, we aim to establish the zebrafish as an *in vivo* system to analyze mitochondrial properties in the kidney. A previous work of others has developed a transgenic zebrafish with mitochondria targeted CFP and membrane targeted YFP under the UAS effector element [Tg(UAS:memYFP, mitoCFP)]. Crossings of this UAS effector line into Gal4 driver lines with *podocin* and *cdh17* promoter [Tg(*podo*:Gal4) and Tg(*cdh17*:Gal4)] drove specific expression of the transgenes in glomerular podocytes and tubular epithelial cells, respectively, in the pronephros, providing an intravital imaging platform to study mitochondria. We are currently investigating spatiotemporal characteristics of mitochondria by live imaging in these transgenic zebrafish. Zebrafish mutants for *adck4* and *coq6* are also being generated by CRISPR/Cas9 system in order to present models for nephrotic syndrome caused by mitochondrial defects. Combinations of the reporter fish lines with these mutants would serve as a novel and easily accessible system to study relevance of mitochondria in human glomerular disease.

Z6143B Characterizing the craniofacial *Tft^{9N}/ddx10* zebrafish mutant. *K. Alharthi*[1], P. Yelick[1], W. Goessling[2]. 1) Tufts University of Dental Medicine , Boston, MA; 2) 2Harvard Medical School, Boston, MA.

Craniofacial abnormalities including craniosynostosis and cleft lip and/or palate are considered among the most common congenital defects, occurring in 1/700 live births. Genetic mutations are known to play important roles in these craniofacial defects, but we have yet to elucidate the complex molecular processes leading to normal and syndromic craniofacial development. Regulatory factors controlling development are highly conserved in vertebrates, and over the years the zebrafish has emerged as a powerful animal model for studying human development. In addition to structural similarities between zebrafish and mammalian craniofacial development, the expression and regulation of the major signaling transduction pathways are also highly conserved. These characteristics, combined with experimental advantages of the zebrafish model, make them very attractive model .Zebrafish are particularly well suited for forward genetic mutagenesis screens, which have been used to identify a variety of craniofacial mutants relevant to human development and disease. Similarly, we used this approach to identify a number of skeletal/craniofacial mutants, including the *Tft^{9n}* mutant. Next generation sequencing was then used to identify *ddx10* as a candidate gene. The human *ddx10* DEAD (Asp-Glu-Ala-Asp) box polypeptide 10 is an RNA helicase that has been implicated in diverse cellular processes involving alteration of RNA secondary structure such as translation initiation, nuclear and mitochondrial splicing, and ribosome and spliceosome assembly. RNA helicases also contain ATPase activity, which helps to unwind secondary structures of mRNA. Previous studies showed that *ddX10* plays complex roles in tumor development, mutations in ATP-dependent RNA helicases result in deregulated protein function. There are no known associations of *ddx10* to craniofacial disorders at this time.The goals of this study are to define phenotypic abnormalities of *Tft^{9n}* mutants and to validate the candidate gene, *ddx10*. Identified *Tft^{9n}* heterozygous adults are being used to generate developmentally staged embryos for Immunohistochemical (IHC) analysis of Ddx10 protein expression, and to characterize wild-type and mutant ddx10 mRNA expression. Single cell injection of ddx10 mRNA is being performed to demonstrate rescue of the mutant phenotype. Preliminary analyses show that Ddx10 is expressed in muscle, cartilage and the neurocranium, consistent with the affected tissues in these mutants. These studies demonstrate, for the first time, roles for ddx10 in craniofacial development. These studies were supported by NIH/NIDCR R01DE018043 (PCY). .

Z6145A The regenerating fin as a model to examine the skeletal defects of Roberts Syndrome. *Rajeswari Banerji*[1], Diane M. Eble[2], M. Kathryn Iovine[3], Robert V. Skibbens[4]. 1) Lehigh University, Bethlehem, PA; 2) Lehigh University, Bethlehem, PA; 3) Lehigh University, Bethlehem, PA; 4) Lehigh University, Bethlehem, PA.

Roberts syndrome (RBS) is a rare genetic disorder characterized by craniofacial abnormalities, limb malformation, and often severe mental

retardation. RBS arises from mutations in *ESCO2* that encodes an acetyltransferase that modifies the cohesin subunit SMC3. Cohesins are critical for both inter-molecular DNA-DNA tethering (such as between sister chromatids) and intra-molecular DNA tetherings (stabilizing the base of DNA loops). It remains unclear which activity is adversely affected, although current models suggest that RBS arise through mitotic failure and limited progenitor cell proliferation due to premature sister chromatid separation. However our findings using the zebrafish regenerating fin reveal that *Esco2* depletion results in significant defects in both tissue regeneration and bone segment growth in the absence of elevated levels of apoptosis. In pursuing evidence obtained from genomic studies in mice and zebrafish, we found that Esco2 is a critical regulator of *cx43/gja1* (encodes the gap junction connexin subunit required for cell–cell communication) expression. The link between Esco2 and Cx43 is of immense interest since *cx43* mutations cause oculodentodigital dysplasia (ODDD) in humans and the skeletal defects of the mutant *short fin* (*sof*^{b123}). ODDD patients exhibit developmental defects that overlap with those of both RBS and a related disorder CdLS, conceptually linking ODDD to cohesinopathies. Among additional evidence that Esco2 and Cx43 function in a common pathway, we demonstrated that miR-133-dependent *cx43* overexpression rescues *esco2*-dependent growth defects. Further, preliminary evidence suggests that the target of Esco2-dependent acetylation, Smc3, is similarly critical for *cx43* expression. These results provide strong evidence that ESCO2 plays a transcriptional role critical for human development and that RBS arises through dysregulation of developmental programs.

Z6146B Zebrafish mutants lacking kiaa0753, a regulator of centriole duplication, phenotypically mimic human ciliopathies. *K. S. Bishop*[1], C. Chen[1], A. Weech[1], B. Carrington[1], W. Pei[1], P. M. Zerfas[1], M. English[1], E. A. Burke[1], M. Gunny-Aygun[1], T. Vilboux[2], S. Burgess[1], W. Gahl[1], R. Sood[1], M. C. Malicdan[1]. 1) National Human Genome Institute/NIH, Bethesda, MD; 2) Division of Medical Genomics, Inova Translational Medicine Institute, Falls Church, Virginia.

Centrosomes are microtubule organizing centers that include a pair of centrioles, one of which functions as a basal body serving as a template for cilia formation. Abnormalities in centriole biogenesis, centrosome structure/function, or formation and maintenance of cilia result in a heterogeneous group of human diseases, including central nervous system disorders, cancer and ciliopathies. We and others have recently described the human ciliopathies oral-facial digital syndrome type VI and Joubert Syndrome due to loss-of-function mutations in *KIAA0753*, a gene involved in centriole duplication. Current knowledge on how these mutations in *KIAA0753* affect basal body function and ciliogenesis is limited. In this study, we generated two *kiaa0753* null mutants in zebrafish using the CRISPR/Cas9 system. We observed that the Kiaa0753 protein is critical in zebrafish survival because mutants died between days 5 to 15. We demonstrated that the *kiaa0753* null mutants exhibited a phenotype associated with cilia defects, including a curved body, edema, retinal abnormalities, renal cysts, and lack of a swim bladder. Further analysis of the neural masts revealed that the mutant fish had shorter and deformed cilia. Cultured cells from mutant embryos showed defects in cell proliferation, supporting the importance of kiaa0753 in cell division. Our novel zebrafish model shows the importance of *kiaa0753* in embryonic development, specifically in ciliogenesis, and can be a useful tool for understanding the biology of centrosomes.

Z6147C Zebrafish as a model for eye disease: congenital cataracts. *Lindy K. Brastrom*, C. Anthony Scott, Diane C. Slusarski. Department of Biology, University of Iowa, Iowa City, IA.

Eye disease and visual impairment coupled with age-related vision loss are a significant public health concern. Modern genomics has enabled the identification of an unprecedented number of genetic variants, many of which are extremely rare, associated with blinding disorders. Zebrafish pose many advantages as a human eye disease model. Key among these are their rapid generation time, small size, eye structure similar to humans, and ability to screen many at a time. By 5 days-post-fertilization (dpf) zebrafish have quantifiable behavioral responses to visual stimuli. Our lab has adapted vision testing in zebrafish to facilitate high-throughput in vivo screens: Visual Interrogation of Zebrafish maNipulations (VIZN) and OptoMotor Response (OMR). VIZN uses interruptions in constant light to garner a startle response. This vision startle assay looks at the ability of the fish to see in a general capacity. Fish that are visually compromised will not respond to the interruption of light. Automated data collection and software to handle the large spreadsheets have been developed to provide optimization in the number of screens we can accomplish. While the VIZN assay works for blinding disorders, there is a need to test for partial vision loss such as those exhibited in cataracts. We are currently adapting OMR to assay partial vision loss. This is done by stripes of white and black passing under a plate of fish. Fish that can see will respond by orienting their body perpendicular to the lines. Vision impaired fish will be unable to respond and thus not orient themselves to the lines. The goal is to combine both assays to evaluate the spectrum of visual impairments. We are currently assessing conditions to test for partial vision impairment. These tests will allow us to screen many genes related to loss of vision quickly and efficiently using zebrafish. .

Z6148A Comparison of locomotion and cerebellar morphology in CRISPR snx14, pink1 and pla2g6 F0 mutants. *E. Buglo*[1], A. Abrams[1], R. Kozol[2], A. Rebelo[1], J. Dallman[2], S. Zuchner[1]. 1) Dr. John T. Macdonald Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Department of Biology, University of Miami, Miami, FL.

Creating a process that would allow for high-throughput and fast correlation of phenotypes to genotypes in a vertebrate model system is a valuable tool for gene discovery and functional confirmation. Zebrafish has many advantages as a model for neurodegenerative disorders and genetic screens, such as easy and fast generation of embryos, ease of direct injection, availability of transgenic lines, as well as measurable locomotive behaviors. Genetic screens in CRISPR/Cas9 induced F0 generations are time and cost-effective, but producing a reliable phenotype has been a challenge.

The inherited movement disorders such as cerebellar ataxia have not been clearly established in a zebrafish model, despite over 50 known ataxia disease genes in humans. It also has not been assessed whether locomotive phenotypes can be measurably distinguished between different movement disorders.

In this study we produce mosaic genotypes in F0 generation in zebrafish using CRISPR/ Cas9 targeting a known ataxia gene snx14, a gene for Parkinsonism disease (pink1), and pla2g6, a gene involved in a complex phenotype with parkinsonism, ataxia and neuroaxonal dystrophy. We perform high-throughput movement analysis using a video tracking system Danio Vision (Noldus Ethovision software). We estimate the differences in locomotive manifestations between Parkinsonism and ataxia. We then evaluate the cerebellar morphology of these mutants in a transgenic line with a Purkinje cell fluorescent marker (Aldoca: GAP-Venus). We then perform next generation deep sequencing to measure the Cas9 cutting efficiency at >10,000 read depth in these mosaic animals. Finally, genotypes are correlated with movement as well as cerebellar morphology. This allows conclusions regarding the necessary levels of genome editing to produce observable and measurable phenotypes, also addressing the question whether genetic mutations linked to movement disorders with distinct clinical symptoms in people produce distinct motor deficits in zebrafish models.

Z6149B Understanding Fanconi anemia core complex and associated proteins by multiplexed CRISPR/Cas9-mediated knockout mutant generation. *Blake Carrington*, Gabrielle Robbins, Kevin Bishop, Gaurav Varshney, John McElderry, Marypat Jones, Settara Chandrasekharappa, Raman Sood. National Human Genome Research Institute/ NIH, Bethesda, MD.

Fanconi anemia (FA) is a rare, mostly recessive, DNA repair deficiency disorder. Chromosomal instability is a cellular phenotype of FA, particularly when cells are exposed to DNA crosslinking agents. FA patients display developmental abnormalities, bone marrow failure (BMF), and predisposition to cancer including acute myeloid leukemia. Genetically, FA is a heterogeneous disease with 19 genes identified so far, and the encoded proteins participate in the FA/BRCA pathway that orchestrates repair of DNA damage caused by interstrand crosslinks. Eight FA proteins form a core complex and, along with FA associated proteins (FAAP), provide a key function that results in ubiquitination of two other FA proteins, FANCD2 and FANCI, a critical step in the pathway. But for FANCL and FANCT/UBE2T, biochemical functions of other Core proteins are yet unknown. Only a fraction of FA gene homologs are present in any invertebrate model organism, and thus zebrafish offers an opportunity to explore the functions of FA genes, particularly in understanding BMF, as hematopoiesis in zebrafish is well studied. We can knockout multiple genes in zebrafish with ease, so this allows exploring the consequence of loss of two or more genes that encode proteins known to interact. Except *FANCS/BRCA1*, homologs of all FA genes have been identified in zebrafish. Therefore, we used the CRISPR/Cas9 mediated targeted mutagenesis to generate loss of function mutants in *fanca, fancb, fancc, fancd1/brca2, fancd2, fance, fancf, fancg, fanci, fancj/brip1, fancl, fancm, fancn/palb2, fanco/rad51c, fancp/slx4, fancq/ercc4*, *fanct/ube2t*, and two FA-associated genes (*faap100* and *faap24*). We designed two sgRNAs/gene and tested them for target-specific activity using our CRISPR-STAT method. We performed multiplexed mutagenesis by pooling the sgRNAs to FA genes based on their known interactions. We used our fluorescent PCR method to identify germline-transmitting founders with mutations in individual or multiple genes that were pooled together. We have begun the phenotypic characterization of all mutants. We will present data on the embryonic phenotypes for each of the genes when mutated individually and in combination with its interacting partners.

Z6150C Model of lymphedema and rescue by regulating MEK/ERK activity. *J. Chan*[1,2], A. Dasgupta[1], S. Hazy[1], J. Mably[1,2]. 1) Skin of Color Research Institute, Hampton University, Hampton, VA; 2) Department of Biological Sciences, Hampton University, Hampton, VA.

The lymphatic system transports fluids and facilitating the return of extravasated cells and macromolecules back into the blood circulation. Obstructions in lymphatic vessel function, known as lymphedema, can occur as a primary or a secondary disorder. Milroy's disease is an example of a genetic or primary lymphedema where mutations in one allele of the VEGFR3 gene leads to pervasive lymphatic malfunction resulting in the accumulation of fluids in the lower limbs. In zebrafish, this disorder can be modeled by mutations in this receptor or its ligand, VEGF-C. In secondary lymphedema, an injury to lymphatic vessels can occur after surgery, causing a blockage in the lymphatic system and fluid accumulation in affected tissues. In both cases, pressure and massage have been the only treatments over the last 200 years. To understand the signaling pathways that might be involved in inducing lymphedema, we investigated the ability of preclinical cancer drugs targeting the VEGFRs, MEPK/ERK or PI3K/mTOR to disrupt lymphangiogenesis. We found that MEK1/2 inhibition over a 6-hour period at 3 days post fertilization to provide a dramatic blockade lymphatic function that cannot be recovered by drug removal. To determine whether transgenic overactivation of these pathways may rescue this phenotype, we created 2 transgenic lines, using the fli1 promoter to drive endothelial expression. Treatment with the MEK1/2 inhibitor typically impeded the formation of the thoracic duct in greater than 80% of zebrafish larvae. In the transgenic fli1::MEK1DD line, this lymphatic vessel is effectively rescued. However, increased endothelial mTORC1 in a fli1::rhebS16H transgenic line had no effect. To provide further mechanistic insight into the signaling components required for proper lymphangiogenesis, we are currently examining the ability of a number of chemical compounds for their ability to rescue this phenotype.

Z6151A Larval phenotype of the zebrafish model of Smith-Lemli-Opitz syndrome. *C. V. M. CLUZEAU*[1], K. M. TABOR[2], K. BURKERT[1], J. PICACHE[1], C. A. WASSIF[1], D. S. ORY[3], B. FELDMAN[2], H. A. BURGESS[2], F. D. PORTER[1]. 1) PEMG, NICHD, NIH, Bethesda, MD; 2) DDB, NICHD, NIH, Bethesda, MD; 3) Washington University School of Medicine, St. Louis, MO.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder characterized by multiple malformations, cognitive impairment and abnormal behavior, including autistic traits. Mutations of the 7-dehydrocholesterol reductase (*DHCR7*) gene, encoding the last enzyme in the cholesterol biosynthetic pathway, result in decreased cholesterol and accumulation of 7-dehydrocholesterol (7DHC). To gain insights into the pathophysiological mechanisms and produce a model allowing cost-effective *in vivo* drug screening, we disrupted the zebrafish *dhcr7* gene using Transcription Activator-like Effector Nucleases. We showed that fish homozygous for a frameshift mutation present the characteristic accumulation of 7DHC and decreased cholesterol in both liver and brain from 2 weeks old. Homozygous mutants also display delayed growth from 3 weeks old, and the transition from larval to adult features (*i.e.* body pigmentation, squamation and fins) is also delayed. The yolk from

eggs produced by mutant females contains high levels of 7DHC. The mutant progeny of the mutant females (maternal zygotic, MZ fish) have high levels of 7DHC during their first week of life, whereas their control siblings (M heterozygous fish) are only exposed to 7DHC until 4 days post fertilization. Interestingly, only the MZ fish present an abnormal acoustic startle response at 1 week old compared to control fish, with decreased responsiveness and altered movement kinematics for non-Mauthner escapes. Treatment of M heterozygous individuals with AY9944, a dhcr7 inhibitor, prevents them from correcting the biochemical defect and leads to a behavioral defect similar to the one observed in MZ fish. Surprisingly, given their exposure to high 7DHC levels during development, MZ larvae are morphologically normal. 7DHC has been shown to alter protein content in lipid rafts and modify membrane fluidity, which could impact signaling between cells during development. Fish are known to adapt to shifts in environmental temperature and membrane fluidity by modifying membrane lipid composition. We hypothesized that mutant embryos use such a mechanism to maintain normal membrane fluidity and signaling. Our preliminary results indeed identified several fatty acid chains in the phosphatidylethanolamine series as upregulated in MZ fish compared to controls. Future work will focus on characterizing the brain structure and transcriptome of *dhcr7*-deficient fish to elucidate the causes of the mutant abnormal behavior, and on analyzing the lipid content in mutant larvae to better understand the compensatory mechanism that preserves membrane fluidity.

Z6152B Generating zebrafish models of human disease to facilitate drug discovery. *Ann E. Davidson*, Sarah A. Hutchinson, James J. Dowling. Hospital for Sick Children, Toronto, ON, CA.

Advances in next generation sequencing have greatly accelerated the identification of novel disease-associated gene mutations. However, a prevailing challenge continues to be validation of the function of these variants, particularly in rare diseases. We have established a Zebrafish Genetics and Disease Models Facility that combines shared expertise and infrastructure at the Hospital for Sick Children, creating a pipeline from gene identification to functional validation and drug discovery. With international accessibility, our facility aims to provide the services required to efficiently generate and analyze zebrafish models that accurately recapitulate human disease. We use a high throughput CRISPR-Cas9 mutagenesis system along with high resolution melt (HRM) analysis to generate mutations in zebrafish that are targeted to putative human disease loci. We also utilize transgenic techniques to create "humanized" models of disease, as well as CRISPR-Cas9 to develop knock-in models of human mutations at conserved zebrafish loci. Additionally, we offer phenotypic analysis and drug discovery services. We are currently developing models for a diverse set of diseases including inflammatory bowel disease, pediatric cancer, cardiac arrhythmia and childhood muscle disease. In less than 2 years since establishment, we have tested nearly 100 gRNAs with a 30% success rate. To date, we have worked with 11 individual labs to generate 24 targeted mutations in 19 genes. Currently, our screening has yielded 18 successfully targeted F0s and 8 successfully generated F1s. In this study, we will present the results of our large-scale mutation generation effort, as well as the preliminary characterization of our first successful mutant strains.

Z6153C Functional Characterization of Epilepsy Related Genes in Zebrafish. *T. D. Fuller*[1,2], T. A. Westfall[1], D. C. Slusarski[1]. 1) Department of Biology, University of Iowa, Iowa City, IA; 2) Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA.

Statement of Purpose: Epilepsy is a chronic condition of recurrent seizures which affects approximately one percent of the general population. Though several causative genes have already been identified, these account for only a small percentage of all the genetically caused cases of epilepsy. Further, even when genes are identified, we lack tractable animal models to rapidly translate these findings into mechanistic insights and ultimately new anti-epileptic therapies. For this reason, the zebrafish is increasingly being used as a model of epilepsy due to its high genetic and physiologic homology to humans and its seizure-like behavior in response to various pharmacological and genetic manipulations. Using High throughput sequencing, a significant number of gene variants are being identified, yet their role in the disease state remain unknown. My project utilizes the zebrafish and focuses on characterizing the functional role of 15 genes in the NIH Undiagnosed Diseases Program for which mutations have been associated with epilepsy, and for which zebrafish orthologues have been identified. **Methods:** I isolated the zebrafish orthologues and characterized gene expression patterns by RTPCR and whole mount in situ hybridization techniques. We previously demonstrated that knockdown of Prickle (PK), a gene associated with human epilepsy, sensitizes zebrafish to seizure-inducing drugs through the use of larval motility assays. To facilitate high-throughput in vivo screens, I adapted this approach and developed a code to rapidly and efficiently analyze the generated data sets. This allowed for the characterization of the 15 candidate genes in the context of seizure sensitization. **Results:** Of the fifteen candidates, I found five: syne1b, sms, ccdc89, wscd1, and nid2a, to result in seizure sensitization when knocked down in the zebrafish. I show that each of these genes is expressed in specific regions in the brain during critical times of neuronal development. Further, I find genes expressed in the retina result in axon defects when knocked down.

Z6154A Determining the roles of mab21l2 in vertebrate eye development. *N. N. Gath*[1,2,3], J. M. Gross[1,2]. 1) University of Pittsburgh Medical School, Department of Ophthalmology, Pittsburgh, PA; 2) Fox Center for Vision Restoration, Pittsburgh, PA; 3) University of Texas at Austin, Austin, TX.

The eye is a complex organ, formed by many coordinated developmental processes. When these processes go wrong, clinically significant eye disorders can result. One such case is modeled by a zebrafish mutant in *mab21l2*. This gene is highly conserved amongst vertebrates, but very little is known about its function. No structural motifs are predicted for the protein, and its biological activity is unknown. *mab21l2* mutants present with lens defects and coloboma. Humans with mutations in *MAB21L2* possess colobomas and associated eye defects, but the molecular and cellular underpinnings of these defects are unknown. The goal of this project is to discover the mechanisms by which *MAB21L2* mutations result in developmental abnormalities in the eye. Evidence suggests mab21l2 may be a transcriptional regulator required to maintain proliferation during lens development and choroid fissure closure. Preliminary evidence supports chromatin association of mab21l2, where it could indeed be involved in transcriptional regulation. Preliminary work has also begun to explore the phenotypes and progression of defects in

the mutant zebrafish eye. Some mutants appear to completely fail to begin lens morphogenesis, while others begin morphogenesis with a slight delay and slower rate of growth, leading to dramatic differences in lens size at later ages. Identifying the functions of mab21l2 and the pathways it is involved with will increase the understanding of this enigmatic protein as well as conserved mechanisms governing eye development in humans.

Z6155B Loss of type I collagen telopeptide lysyl hydroxylation causes musculoskeletal abnormalities in a zebrafish model of Bruck syndrome. *Charlotte Gistelinck*[1], Paul Eckhard Witten[1], Ann Huysseune[1], Pascal Simoens[1], Sofie Symoens[1], Fransiska Malfait[1], Amelie De Muynck[1], Anne De Paepe[1], Ronald Y. Kwon[2], MaryAnn Weis[2], David R. Eyre[2], Paul J. Coucke[1], Andy Willaert[1]. 1) Ghent University, Gent, Belgium; 2) University of Washington, Seattle, USA.

Bruck syndrome (BS) is a disorder characterized by joint flexion contractures and skeletal dysplasia that shows strong clinical overlap with the brittle bone disease Osteogenesis Imperfecta (OI). BS is caused by bi-allelic mutations in either the *FKBP10* or the *PLOD2* gene. *PLOD2* encodes the lysyl hydroxylase 2 (LH2) enzyme, which is responsible for the hydroxylation of lysine residues in fibrillar collagen telopeptides. This hydroxylation directs cross-linking of collagen fibrils in the extracellular matrix, which is necessary to provide stability and tensile integrity to the collagen fibrils. To further elucidate the function of LH2 in vertebrate skeletal development, we created a zebrafish model harboring a homozygous *plod2* nonsense mutation resulting in reduced telopeptide hydroxylation and cross-linking of bone type I collagen. Adult *plod2* mutants present with a shortened body axis and severe skeletal abnormalities with evidence of bone fragility and fractures. The vertebral column of *plod2* mutants is short and scoliotic with compressed vertebrae that show excessive bone formation at the vertebral end plates, and increased tissue mineral density in the vertebral centra. The muscle fibers of mutant zebrafish have a reduced diameter near the horizontal myoseptum. The endomysium, a layer of connective tissue ensheathing the individual muscle fibers, is enlarged. Transmission electron microscopy of mutant vertebral bone shows type I collagen fibrils that are more widely spaced and less organized. In conclusion, *plod2* mutant zebrafish show molecular and tissue abnormalities in the musculoskeletal system that are concordant with clinical findings in BS patients. Therefore, the *plod2* zebrafish mutant is a promising model for the elucidation of the underlying pathogenetic mechanisms leading to BS and the development of novel therapeutic avenues in this syndrome.

Z6156C Establishing a zebrafish model for giant axonal neuropathy. *S. Gurung*, J. Fajardo, R. Kennedy, E. Asante, E. Murray, D. Hummel, K. Jasmer, M. Hannink, A. Chandrasekhar. University of Missouri, Columbia, MO.

Giant Axonal Neuropathy (GAN) is a rare neurodegenerative disorder affecting axons in the peripheral and central nervous system, accompanied by axonal swellings and axon loss. Disease onset is around 3 years of age, and is characterized by muscle weakness, impaired sensation, gait disturbance, mental retardation, and seizures. While GAN is caused by presumptive loss-of-function mutations in the human *GAN* gene, the etiology of the disease is poorly understood. We aim to establish a zebrafish model of GAN to gain insight into the cellular and biochemical mechanisms underlying the disease phenotype. *GAN* encodes a 468 amino-acid protein, Gigaxonin, a member of the BTB-Kelch superfamily involved in the ubiquitin-proteasome pathway. In mice, *gan* is expressed extensively in multiple tissues, including the brain and spinal cord. Similarly, zebrafish *gan* is expressed from 18 hpf to 4 dpf, with high expression in the brain at 4 dpf.

To investigate roles of *gan* in disease, we generated loss-of-function mutations in *gan* using the CRISPR/Cas9 system. Using guide RNA targeted to the 2nd exon (AA 84-90 of Gigaxonin), we identified two alleles with frameshift mutations resulting in premature stop codons. Since sensory-motor functions are compromised in GAN patients, we examined two behaviors in zebrafish *gan* mutants involving sensorimotor circuits: touch-evoked escape response and food intake. Wild type and mutant larvae exhibited normal escape responses when touched in the trunk. In contrast, a significantly larger proportion of mutants responded when touched on the head, consistent with extensive *gan* expression in the hindbrain, where touch responses are processed. Feeding is a complex behavior requiring the coordination of sensory (vision, olfaction) and motor (jaw movement, locomotion) activities. At 7 dpf, mutant larvae exhibited a significant decrease in food intake compared to wild type siblings, consistent with *gan* expression in the hindbrain, where the motor neurons driving jaw movements are located. These data suggest that zebrafish *gan* mutants have defects in sensorimotor functions consistent with the defects seen in the mouse GAN model and in human patients, and may represent a good model for GAN in a simple vertebrate.

Z6157A Functional study of appetite regulation in the arcuate nucleus of hypothalamus by zebrafish orexigenic models. *G. M. Her*[1], Y.-W. Hsieh[1], S.-H. Lai[1], C.-Y. Lai[1], C.-Y. Lin[1], K.-Y. Yeh[2]. 1) Department of Bioscience and Biotechnology, Keelung, Taiwan, TW; 2) Chang-Chung Memorial Hospital, Keelung,Taiwan.

Energy homeostasis is maintained by balancing energy intake and expenditure. Many signals regulating energy intake are conserved between the human and zebrafish. NPY (neuropeptide Y) and AgRP (Agouti-related peptide) are neuropeptide and specifically produced from the AgRP/NPY neuron in arcuate nucleus. Both NPY and AgRP promote the growth of adipose tissue and increased appetite. AgRP/NPY neurons counteract another arcuate nucleus cell population. The POMC (proopiomelanocortin) neurons, which are considered anorexigenic. α-MSH (α-melanocyte-stimulating hormone) is a peptide hormone derived by POMC, to be in part responsible for the decreases in food intake and increases in energy expenditure. α-MSH exerts its effect by binding to the MC4R (melanocortin 4 receptors). We have established obsogenic zebrafish by overexpression orexigenic genes including Agrp, and NPY and four TALEN knock-out obsogenic genes, (Carboxypeptidase E (CPE) , nescient helix loop helix 2 (nhlh2), MC4R and POMC). We also generated zebrafish fat models by transgenic overexpression lipogenic (PPAR-γ, DGAT2, and sar1b) and adipocytic genes (ATF4 and Mdx3). We also generated partially deled POMC gene resulted α-MSH talen knockout mutant. As the insights into our research aims for studying lipid metabolism in those zebrafish models, it is very likely that establishment of compound superobesity fish (SOBF) models for studying the connection between obesity (lipogenesis) and metabolic diseases. To observe

lipogenesis gene expression and obesity in SOBFs, we performed feeding tests which SOBFs fed with HFD (high fat diet) and ND(normal diet) and then examined by Nile Red and Oil Red O staining. We demonstrated the application of SOBFs by a high-throughput fluorescence-based assay system. Those results demonstrate that these SOBFs are valuable models for analysis metabolic syndrome and Energy homeostasis.

Z6158B Establishment of stable zebrafish genetic models for studying myotonic dystrophy. *M. N. Hinman*[1], J. S. Eisen[1], J. A. Berglund[2], K. Guillemin[1]. 1) University of Oregon, Eugene, OR; 2) University of Florida, Gainesville, FL.

Myotonic dystrophy (DM) is a genetic disorder most known for causing muscle weakness and myotonia, but it affects many body systems including the gastrointestinal (GI) tract. DM patients often experience altered GI tract motility and small intestinal bacterial overgrowth, but the underlying mechanisms are poorly understood. DM is caused by expansion of a CUG repeat RNA that is encoded by the 3' UTR of the DMPK gene. The MBNL family of RNA-binding proteins are sequestered by the CUG repeats, preventing them from regulating alternative splicing.

We generated several zebrafish DM models by creating CRISPR mutants of each MBNL gene and by inserting transgenes that overexpress CUG repeats globally and in specific tissues. Preliminary studies indicate that DM model fish exhibit some of the same alternative splicing changes that are seen in humans.

We will use these models to study the digestive phenotypes of DM and how microbiota contribute to them. Specifically, we will compare gut motility between WT and DM model fish, and will investigate the specific cell types that contribute to any phenotypes. We will ask how bacteria are altered in DM models, and whether this leads to intestinal inflammation. In addition, we will use germ-free fish to ask whether altered microbiota are necessary or sufficient to cause DM-related digestive phenotypes. Finally, we will investigate how alternative splicing changes contribute to digestive phenotypes.

Overall, these studies will provide important insight into the mechanisms behind the digestive symptoms of DM and the role, if any, that microbes play in them.

Z6159C Novel genes critical for hypoxic preconditioning in zebrafish are regulators of insulin and glucose metabolism. *F. B. Imam*, D. Zhang, G. G. Haddad. UC San Diego, La Jolla, CA.

Fine metabolic regulation to adjust for changes in oxygen and energy availability is a conserved, ubiquitous survival strategy of cells and tissues to unpredictable environments. Severe oxygen deprivation can overwhelm these protective strategies and is a common cause of major brain, heart, and kidney injury in adults and newborns alike. Intriguingly, mild hypoxia can be preventative against a later, more severe hypoxia exposure via "hypoxic preconditioning"—a protective phenomenon that is not yet fully understood. We have therefore established and optimized an embryonic zebrafish model to study hypoxic preconditioning in detail using a functional genomic approach. Using this developmental zebrafish model, we validated five novel hypoxia-protective genes from hundreds of hypoxia-regulated genes we identified via differential expression microarray: *irs2a*, *crtc3*, and *camk2g2* have been previously implicated in insulin and glucose metabolism, while *btr01* and *ncam2* are previously uncharacterized. Furthermore, we have generated null mutants using CRISPR in three of these genes (*irs2a*, *crtc3*, *btr01*) and have begun to characterize hypoxia-induced cellular and transcriptional mutant phenotypes. These results extend our understanding of the mechanisms of hypoxic preconditioning and affirm the discovery potential of this novel vertebrate hypoxic stress model.

Z6160A Identifying Mechanisms of Gastrointestinal Distress in Zebrafish Based Autism Models. *D. James*, E. Storrs, R. Kozol, J. Dallman. University of Miami, Miami, FL.

More than 70% of individuals with Autism Spectrum Disorder (ASD) experience moderate to severe Gastrointestinal (GI) distress with unspecified cause. Multiple studies have shown that individuals with ASD experience GI distress at a statistically higher percentage when compared to typically developing individuals, and that the type of GI distress (vomiting, diarrhea, allergy/intolerance) is highly variable. Due to the heterogeneity of both the autism spectrum and associated GI distress, combined with the relative ambiguity associated with potential roles for the microbiota, no strategies currently exist to address GI distress on a physiological or systems level (Hsiao, 2014). As a first step towards developing these strategies, we are employing zebrafish ASD models to gain mechanistic insight into how genetic variants with high autism relatedness impact GI function. Here we focus on the high-confidence ASD gene *SHANK3*, deletions of which contribute to Phelan-McDermid Syndrome (a form of ASD). This syndrome has gastroesophageal reflux and other gastrointestinal issues reported in nearly 50% of cases.

Our prior work has shown that knockdown of *shank3a* in zebrafish cause delayed mid- and hindbrain development (Kozol et al. 2015). Such delayed hindbrain development could have significant implications for GI regulation. Additionally, gene families like those of SHANK3 arose evolutionarily well before the appearance of neurons, suggesting a potential role in cell-to-cell contact or epithelial function, which could influence GI function (Alie and Manuel 2013).

To begin to address these diverse hypotheses, our initial study has two goals; 1) to test GI function in *shank3a/b* mutants/morphants and 2) to produce a viable reporter line that labels tissues expressing Shank3. Using standard cloning techniques, we adapted a strategy published by (Jia Li *et al.* 2015) to make a plasmid that would enable us to engineer the last exon of *shank3a.* The plasmid tags the endogenous protein with an HA epitope linked by a cleavable P2A peptide to a GFP reporter. By targeting the upstream intron using CRISPR/Cas9, we plan to insert our modified last exon upstream of the endogenous last exon. To test the efficacy of our guide RNAs *in vitro*, we will use our donor plasmid, as the guides should cut both the last intron insertion point, and the cloned intronic segment in the plasmid. Engineering endogenous *shank3* in this way will allow us to localize endogenous *shank3a* using HA antibodies in GFP expressing cells, circumventing low expression issues seen with *in situ* hybridization (especially in diffuse tissues like the enteric nervous system). With shank3a as a test case, we hope to vet this strategy to gain insight into mechanisms underlying ASD-associated GI distress.

Z6161B Functional Analysis of *Parla* and *Parlb* Paralogs in Zebrafish. *M. Jung*, S. Noble, M. Ekker. Center for Advanced Research in Environmental Genomics (CAREG), Department of Biology, University of Ottawa, Ottawa, ON, CA.

Parkinson's disease is a highly prevalent multifactorial neurodegenerative disorder caused by a complex cascade of interactions between various genetic and epigenetic factors. Due to this, the majority of cases are termed idiopathic, however, about 10% of PD cases are due to defined genetic factors. Interestingly, both idiopathic and familial cases of PD share mitochondrial dysfunction as a central component in the pathology of the disease. The mitochondrial protease, presenilin-associated rhomboid-like (PARL), is a Parkinson's disease-linked gene, functioning in regulated intramembrane proteolysis in the inner mitochondrial membrane. PARL is associated with diverse processes including mitochondrial dynamics, and is seen to actively inhibit unnecessary apoptosis and mitophagy in *Drosophila* and yeast. Here, we investigated the role of the two zebrafish *parl* paralogs, *parla* and *parlb*, through transient morpholino mediated loss and stable CRISPR-Cas9 mediated mutagenesis. Our morpholino results show a transient loss of *parla* and/or *parlb* function resulted in defects in the dopaminergic neurons, including mild neurodegeneration and mispatterning in the ventral diencephalon. Morphants exhibited extensive cell death throughout the entire body, as well as increased larval mortality. Additionally, for the first time in vertebrates, our results show evidence that the *parl* genes may function upstream of *pink1*, in a conserved pathway between *Drosophila* and zebrafish. In order to study the effects of heritable loss of *parl* function, *parla* and/or *parlb* mutant lines were produced via the CRISPR-Cas9 system. Several mutant zebrafish lines have been identified and current efforts focus on characterizing these mutant lines for further phenotype assessments. This research will shed light on the specific roles played by each of the two *parl* genes in zebrafish, potentially leading to a better understanding of Parkinson's disease etiology.

Z6162C Characterization of DPP6 Neuronal Expression in Zebrafish (*Danio rerio*). *E. P. Kite*[1], L. Burghi[2], K. Hu[2], M. Geng[2], B. R. Bill[1]. 1) University of Texas at Tyler, Tyler, TX; 2) University of California Los Angeles, Los Angeles, CA.

Autism spectrum disorders (ASDs) are a heterogeneous set of developmental disorders with complex etiology. *Dipeptidyl-Pepitdase 6* (*DPP6*) is a proposed autism candidate gene. Mouse models have been informative for function of this potassium channel regulator; however, a zebrafish model could provide a vehicle for high-throughput pharmaceutical screens or gene-gene interaction studies. Therefore, our goal was to determine the suitability of the zebrafish homologs for these studies. In zebrafish, there exists two homologs of *DPP6*, *dpp6a* (63.4% identity to *DPP6*) and *dpp6b* (59% identity to *DPP6*). Utilizing qRT-PCR and *in situ* hybridization, we identified expression of *dpp6b* as early as 2 days post fertilization (dpf) continuing through adult. Specifically, we observed staining within the zebrafish thalamus, hypothalamus, periventricular Gray of the optic tectum, and torus longitudinalis utilizing *in situ* hybridization for *dpp6b* expression. A more extensive analysis was performed with immunohistochemistry utilizing a commercial antibody raised against the human *DPP6* synthetic peptide, expression was observed in brain areas similar to that of the *in situ;* however, we saw broader staining in the cerebellum, fasciculus retroflexus, and several sensory-associated neurons. Based on conservation, expression, and the ability of the human antibody to cross react with the zebrafish dpp6, we propose that the zebrafish homologs will be functionally similar; therefore, a good tool for future studies. Preliminary studies with morpholino oligonucleotides and TALEN knockouts are being developed to determine anatomical and behavioral neuronal phenotypes.

Z6163A Precision medicine for hearing loss: zebrafish based drug screen. *A. Koleilat*[1], A. Lambert[2], T. Wiggin[2], M. Masino[2], S. Ekker[1], L. Schimmenti[1]. 1) Mayo Clinic, Rochester, MN; 2) University of Minnesota, Twin Cities, MN.

Homozygous recessive mutations in *MYO7A* cause Usher Syndrome Type 1 (UST1), characterized by profound congenital deafness and retinopathy. The *mariner* mutant is a zebrafish model of UST1 caused by mutations in *myo7aa* and exhibits deafness and circular swimming. The objective of this study is to facilitate drug screening that rescue the mutant swimming phenotype. We assessed zebrafish swimming and evaluated the efficacy of drugs by measuring average global change in body orientation (AGC). The AGC for wildtype fish was 680 +/- 130 radians/s. In contrast, the mutant fish had an AGC of 1100 +/- 300 radians/s ($p<0.0001$). Using a L-type calcium channel agonist, (±) Bay K 8644 the AGC was 780 +/- 200 radians/s. The drug did not have any adverse reactions on the wildtype fish. The AGC of the mutant fish incubated in 5 µM of (±) Bay K 8644 differed from the control mutant fish ($p<0.0001$) and did not differ from wildtype control fish ($p=0.0451$). We tested a second L-type calcium channel agonist, FPL 64176. Values of the AGC did not present statistical significance between the *mariner* mutants in the compound compared to the mutant controls. However, the pattern of swimming for the *mariner* mutants incubated in 0.5 µM FPL 64176 more closely resembles wildtype swimming. These preliminary studies support that using L-type calcium channels shifts swimming behavior towards wildtype swimming. This represents a significant step towards discovering compounds to treat hearing loss caused by mutations in MYO7A.

Z6164B Validation of a zebrafish FOP model. *M. LaBonty*, N. Pray, P. C. Yelick. Tufts University, Boston, MA.

Introduction: The zebrafish Type I BMP/TGFβ family member receptor, Acvr1l, modulates BMP/TGFβ signaling to promote cartilage and bone formation. Activating mutations in the human ortholog of Acvr1l, ACVR1, are associated with Fibrodysplasia Ossificans Progressiva (FOP), a disease characterized by the gradual ossification of fibrous tissues, including skeletal muscle, tendons, and ligaments. The **objective** of this work is to validate and characterize a zebrafish model for FOP to elucidate the molecular mechanisms driving heterotopic bone formation in FOP and other human diseases.

Methods: Gateway cloning, transgenesis, automated heat shock system, fluorescence imaging, Alcian blue stain, Alizarin Red Stain, µCT, histology, IHC

Results: Gateway cloning was used to create a vector containing the Hsp70 heat shock (HS) promoter driving the expression of mCherry-tagged constitutively active (CA) Acvr1l. Constructs were injected into single cell stage BMP response element reporter (BRE-GFP) zebrafish to create stable transgenic hs-CA-Acvr1l-mCherry::BRE-GFP lines. Developmentally staged transgenic animals were subjected to daily one-hour heat

shock treatments (3 weeks to 8 months) to induce CA-Acvr1l expression. An Activin A injection injury model was developed for heat shocked CA-Acvr1l expressing animals to define the time course of HO progression in these animals. We are currently performing μCT and Alcian blue/Alizarin red staining on these animals to characterize mineralization defects and to identify sites of heterotopic ossification (HO) as observed in human FOP patients. In addition, we are performing paraffin embedding and sectioning on selected specimens to perform histological and immunohistochemical analyses. Preliminary results show that CA-Acvr1l-expressing zebrafish exhibit enhanced/accelerated mineralization, vertebral fusions, osteochondroma formation, and HO phenotypes, as compared to HS WT and non-HS CA-Acvr1l transgenic zebrafish controls. IHC analyses of activated BMP/TGFβ signaling (pSmad1/5, pSmad2/3), and chondrocyte (Collagen II, Sox9), endothelial cell (Tie2, vWF) and osteoblast differentiation markers (Runx2, Osteocalcin) are being used to characterize these defects on a cellular level. Conclusions: HS-inducible CA-Acvr1l transgenic zebrafish provide an informative model for HO development in human FOP.

Z6165C Zebrafish as a disease model for Epidermolysis Bullosa Simplex. *S. A. MacDonnell*, M. A. Akimenko, R. Lalonde, J. Zhang. University of Ottawa, Ottawa, Ontario, CA.

Epidermolysis Bullosa Simplex (EBS) is a rare human genetic skin disorder that results from mutations in the *keratin 14*, *keratin 5*, or *plectin* genes, and occurs in 1 out of 20 000 live births. In most cases, EBS is caused by dominant-negative missense mutations in the *cytokeratin 5* (*K5*) or *14* (*K14*) genes. The primary symptom of EBS is the formation of blisters on the hands and feet of affected individuals. This project will focus on creating a zebrafish model for the disease using a two point mutation of *keratin 5,* identified to be linked with EBS in humans through the I161S and E477K mutations. The I161S and E477K mutants have been linked with mild and severe forms of the disease, respectively. Both of these mutations are found in the central rod domain of the keratin, which is involved in keratin assembly. The zebrafish and human *keratin 5* proteins share an 83.2 percent similarity in this domain. Moreover, the isoleucine (I161) and glutamic acid (E477) are also conserved. *Keratin 5* is ubiquitously expressed in the surface epithelial tissue of zebrafish embryos, as in humans. Using site-directed mutagenesis, we inserted point mutations in the zebrafish *krt5* cDNA to mimic the I161S and E477K mutations observed in humans. Our lab has recently characterized a *cis*-acting regulatory element specific to the ectoderm of the median and pectoral fin fold of developing zebrafish. To ensure embryo survival, we have used these elements to ectopically express mutant *keratin 5* only in the fin fold ectoderm. Mutant *keratin 5* is linked to an eGFP reporter gene via the 2A peptide for screening purposes. Multiple transgenic lines for the I161S mutation, *Tg(epi+β-globin:ker5 I161S-2A-eGFP)*, have recently been established. Preliminary phenotypic analysis shows little to no blistering. However, trauma or friction may cause blisters to form, as is the case in many forms of EB in humans. In an attempt to induce blistering, the zebrafish are currently being observed under different environmental stressors. Simple microscopy will be used to detect phenotypic defects in the skin, such as the formation of blisters. Further phenotypical analysis will include the structural integrity of the fin and the potential formation of keratin aggregates. The integrity of the keratin filament network in affected fins compared to wild type fins will be determined using atomic force microscopy. We will examine the keratin network and the possibility of keratin aggregates using immunohistochemistry and electron microscopy on the mutant fish as well as wild type fish. Our goal is to create a disease model for EBS that will be used for high throughput drug screening to identify candidate drugs to cure or alleviate the symptoms of EBS.

Z6166A Ewsa inhibits TP53-mutation dependent tumorigenesis in zebrafish. *Justin Mehojah*[1], Richard Galbraith[2], Hyewon Park[1], Mizuki Azuma[1]. 1) University of Kansas, Lawrence, KS; 2) Lawrence Memorial Hospital, Lawrence, KS.

ABSTRACT

Ewing sarcoma is a pediatric cancer of bone and soft tissue. The common molecular abnormality identified in this disease is a chromosomal translocation, which results in the expression of a chimeric fusion protein containing *EWS (Ewing sarcoma breakpoint region 1)*-derived sequences in the amino terminus fused to the carboxyl-terminus of an ETS transcription factor (*FLI1*, *ERG, ETV1, ETV4*, or *FEV*). Despite that the loss of *EWS* allele is a common character observed in this sarcoma, whether it contributes to the pathogenesis of Ewing sarcomas is unknown. To address this question, we utilized a zebrafish mutant for *ewsa* (a homologue of human EWS). Both *ewsa/wt,* Zygotic *ewsa/ewsa* and Maternal-Zygotic (MZ) *ewsa/ewsa* did not develop tumors. The MZ *ewsa/ewsa* mutants displayed a higher percentage of cells with aneuploidy at 27 hpf, compared to wildtype zebrafish. These results suggest that Ewsa maintains chromosomal stability. Because Ewing sarcoma is often associated with an impaired P53 pathway, we further generated a double-mutant line by intercrossing *ewsa/wt* and *tp53(M214K)/wt* mutants. As a result, the incidence of tumorigenesis in the *tp53/wt;ewsa/ewsa* line and the *tp53/wt*;*ewsa/wt* line was higher than in the *tp53/wt*;*wt/wt* line. The result suggests that endogenous Ewsa inhibits tumorigenesis in *tp53/wt* zebrafish. Moreover, the tumor samples obtained from (*tp53/wt;ewsa/wt*) or (*tp53/wt;ewsa/ewsa*) zebrafish were converted to (*tp53/tp53;ewsa/wt*) or (*tp53/tp53;ewsa/ewsa*). The result suggests that zebrafish from the (*tp53/wt)* line developed tumors by undergoing LOH (Loss of Heterozygosity) to the (*tp53/tp53)*, and wildtype Ewsa plays a role in inhibiting LOH induction. Here, we propose that the loss of *EWS* allele as a result of formation of *EWS*-fusion gene may play a role in the pathogenesis of Ewing sarcoma development.

Z6167B Discovery of neuroprotective small molecules to treat Parkinson's disease. *H. Mo*[1,2], J. Kim[1], H. Liu[1], S. Chen[1], M. Arkin[1], M. Keiser[1], B. Huang[1], S. Guo[1]. 1) University of California,San Francisco, San Francisco, CA; 2) Tsinghua University,Beijing,China.

Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder. Characterized by the loss of dopamine (DA) neurons in the substantia nigra (SN), the majority of PD cases are idiopathic with a small percentage being genetically determined. Studies of both sporadic and inherited forms of PD point to mitochondrial (mito) dysfunction and oxidative stress as underlying pathophysiological mechanisms.

Objective: 1. Optimize primary screening assay employing the chemo-genetic larval zebrafish DA neuron degeneration model. 2. Carry out a primary screen of compounds library through organism-based high content imaging to find candidate hits.

Methods: we have developed a chemo-genetic DA neuron degeneration model in larval zebrafish. This model expresses the bacterial enzyme nitro-reductase (NTR) in DA neurons. Upon addition of the prodrug metronidazole (MTZ, a commonly used antibiotic), NTR converts MTZ into a toxic compound that renders DA neuron degeneration. For screening, we have established whole organism-based high content imaging platform from which we can directly visualize the integrity of these neurons

Results: We have figured out the best conditions of Mtz treatment for our model and tested Z' factor (Z'=0.38) for assay evaluation and validation. For screening, we have validated 40 pairs of candidate hit compounds selected from a primary screen of 3,000 compounds and found a potential compound that led to a significant increase in DA neuron fluorescent intensity compared to MTZ-treated negative controls.

Z6168C Braciole: a novel motile cilia mutation which exhibits neural randomization and scoliosis. *Nicholas Morante*, Shin-Yi Lin, Stuart Carter, Eunice Lee, Daniel Grimes, Rebecca Burdine. Princeton University, Princeton, NJ.

Primary ciliary dyskinesia (PCD) is a relatively rare genetic condition, affecting around 1 in 10,000 people, that is caused by defects in ciliary motility. PCD patients often exhibit infertility, left-right (L-R) axis randomization, otitis media, and bronchiectasis. The involvement of motile cilia in the development of in these defects has been the focus of much research on motile cilia. However, in addition to these disorders, recent findings have unearthed new potential associations between motile cilia and diseases, notably in the etiopathogenesis of adolescent idiopathic scoliosis (AIS), highlighting the need for a close re-examination of the consequences motile cilia defects in vertebrates. Here we describe the characterization of a novel motile cilia mutant, *braciole (brcl)*, which exhibits developmental defects associated with motile cilia abnormalities. We demonstrate that *brcl* encodes a novel *dyx1c1* mutation that results in a loss of ciliary outer dynein arms and complete cilia immotility. We further develop a technique to allow us to rescue embryonic defects and thereby characterize later roles for motile cilia in juveniles and adults. We find that *dyx1c1*is required to maintain spine straightness during growth and that mutation causes three-dimensional spinal curvatures that closely model idiopathic scoliosis.

Z6169A Exploring the roles of Cytoskeletal Protein Mutations in Amyotrophic Lateral Sclerosis. *Kim Nguyen*, Kathryn Sheldon, Lisa Schneper, Zhonghua Gao, Keith Cheng, Zachary Simmons, Sue Patrick, Kendall Dubois, Tiffany Bohr, Syndi Reed, James Broach. Penn State Hershey Coll of Medicine, Hershey, PA.

Amyotrophic Lateral Sclerosis (ALS) is a rapid progressive degenerative disease of motor neurons that control muscles of limbs, tongue, and lungs. Unfortunately, this destructive disease still remains untreatable largely due to a limited understanding of disease mechanisms and the absence of a suitable model organism for drug screening. Currently, known causative genetic mutations only account for ~6% of ALS cases despite the total ALS heritability being 50%. Not only is there a tremendous knowledge gap in the genetic basis of ALS, but also the associated clinical phenotypes are complicated and disconnected to the known genetics. Therefore, the goal of our study is to *identify and characterize the genetic factors underlying the disease and to specifically correlate these alterations to various groups of ALS clinical features*. To understand genetic mechanism underlying ALS, we have recruited familial ALS (fALS) patients, sporadic ALS (sALS) patients, and sALS trios consisting of ALS patient and their unaffected parents. Consented patient samples were subjected to exome-sequencing, whole-genome sequencing, and SNP array analysis. As a result, we identified, in addition to rare damaging variants in ALS causative genes such as SOD1 and TDP43, de-novo compound heterozygous mutations in cytoskeletal-related proteins such as DNAH2, DYNC2LI1, STARD9 and NRP2. To functionally validate candidate genes, CRISPR knockout technology was performed in mouse embryonic stem cells (mESC) and the zebrafish model system. Two CRISPR targets per gene were generated and used for the knockout to allow for easy validation tests with PCR and RT-PCR. At present, homozygous knockout mouse embryonic stem cells and heterozygous knockout F1 zebrafish are available. Crosses are underway to produce homozygous knockout F2 embryos. In parallel, *ex vivo* differentiations of mESC are in progress to compare neurite outgrowth of motor neurons between the knockout and the wildtype. Preliminary results of NRP2-CRISPR injected embryos demonstrate a bent-body phenotype, resembling the phenotype of ALS zebrafish. DNAH2-, DYNC2LI1-, and STARD9-CRISPR injected embryos also showed slight locomotive defects. Taken together, our results suggest that dysregulation of cytoskeletal-related proteins could play an important role in ALS disease mechanism. Contributing to the field, we have identified probable causing genes of ALS, and provided cellular and whole-organism ALS models that are pathway-specific for specialized ALS drug screening.

Z6170B Mechanism that links vesicular fusion defects and apoptosis in photoreceptors. *Y. Nishiwaki*, M. Suenaga, M. Araragi, I. Masai. Okinawa Institution of Science and Technology Graduate University, Okinawa, Japan.

Intracellular protein transport is mediated by budding and fusion of transport vesicles on intracellular membrane organelles and often linked to photoreceptor degenerations in human. However, it is unclarified how protein transport defects cause photoreceptor degenerations. We previously reported that mutations of β-SNAP/*napbb*, a vesicular fusion regulator, cause apoptosis in zebrafish photoreceptors, and found that this apoptosis depends on BNip1. BNip1 is a component of syntaxin18 (stx18) SNARE complex, which regulates retrograde transport from Golgi to ER. BNip1 contains the BH3 domain, which activates the Bax-dependent apoptosis. The absence of β-SNAP compromises the disassembly of stx18 cis-SNARE complex, which is generated by vesicular fusion. Accumulation of stx18 cis-SNARE complex activates Bax-dependent apoptosis through its BH3 domain. Thus, BNip1 induces apoptosis in response to vesicular fusion defects. To understand physiological roles of BNip1 for photoreceptor apoptosis, we examined the relationship between β-SNAP functions and BNip1-mediated apoptosis. In the β-SNAP mutant, photoreceptor degeneration largely occurs at 2-3 dpf in the stage when protein transport is highly activated. We found that over-expression of β-SNAP during 2-5 dpf is enough to suppress photoreceptor apoptosis in the β-SNAP mutant at least by 3 wpf, indicating that BNip1-dependent apoptosis correlates with an excessive activated vesicular transport state, suggesting that BNip1 may be one of safe guard that limits the upper threshold of vesicular transport.

Z6171C Development of a Novel Zebrafish Sepsis Model for High-throughput Drug Screens. *A. M. Philip*[1,2,3], Y. Wang[1,3], C. dosSantos[1,2], X. Y. Wen[1,2,3]. 1) St. Michael's Hospital, Toronto, Ontario, CA; 2) Institute of Medical Sciences, Univ. of Toronto, Toronto, Ontario, CA; 3) Zebrafish Centre for Advanced Drug Discovery, Toronto, Ontario, CA.

Sepsis is primarily a state of uncontrolled systemic inflammation. Despite decades of sepsis research involving mammalian models and over 100 clinical trials, sepsis remains a major healthcare burden. Severe sepsis strikes the young and old alike with an incidence of ~750,000 cases per year worldwide, and an estimated mortality rate of 50-80%. As an alternative to traditional animal models, zebrafish have recently emerged as a powerful vertebrate paradigm to study human pathologies and for phenotype-based high-throughput drug screening. Here we show that sepsis can be effectively modeled in the zebrafish. Lipopolysaccharide (LPS) was used to induce sepsis-like pathology in 3dpf zebrafish by water delivery. Through the use of several tissue-specific fluorescent-reporter mediated transgenic lines, fluorescent microangiography, histological assessment, and gene expression analyses, we strived to assess LPS-induced systemic inflammation. LPS delivery to zebrafish embryos at a concentration of 100µg/ml effectively models the dynamics of sepsis progression, leading to 90% mortality. Our zebrafish sepsis model exhibits the major hallmarks of human sepsis including widespread vascular leakage, edema and tissue damage, increased inflammatory cell infiltration and reactive oxygen species (ROS) production, reduced blood circulation and increased thrombocyte aggregation. We validated the suitability of the model for phenotype-based drug screening using fasudil, a drug known to rescue LPS induced vascular permeability in non-primate sepsis models. LPS induced mortality, tissue edema and ROS production were identified as fast and reliable read-outs for high-throughput drug screening. This novel zebrafish sepsis model is expected to provide unmatched potential to screen and validate large numbers of compounds that can modify sepsis pathology *in vivo*.

Z6172A A rapid and effective method for screening, sequencing and reporter verification of engineered frameshift mutations in zebrafish. *Sergey Prykhozhij*[1], Jason Berman[1,2,3]. 1) Department of Pediatrics, Dalhousie University, Halifax, NS, CANADA; 2) Department of Microbiology and Immunology, Dalhousie University, Halifax, NS, CANADA; 3) Department of Pathology, Dalhousie University, Halifax, NS, CANADA.

Clustered Regularly Interspaced Palindromic Repeats (CRISPR)/Cas9 adaptive immunity against pathogens in bacteria has been adapted for genome editing and applied in zebrafish (*Danio rerio*) to generate frameshift mutations in protein-coding genes. Although there are methods to detect, quantify and sequence CRISPR/Cas9-induced mutations, identifying mutations in F1 heterozygous fish remains challenging. Additionally, sequencing a mutation and assuming that it causes a frameshift does not prove causality because of possible alternative translation start sites and potential effects of mutations on splicing. This problem is compounded by the relatively few antibodies generated to zebrafish proteins limiting validation at the protein level. To address these issues, we developed a detailed protocol to screen F1 mutation carriers, and clone and sequence identified mutations. For verifying that mutations actually cause frameshifts, we created a fluorescent reporter system that can detect frameshift efficiency based on the cloning of wild-type and mutant cDNA fragments and their expression levels. As proof-of-principle, we applied this strategy to three mutations in *pycr1a*, *chd7* and *hace1* genes. In *pycr1a* gene involved in proline biosynthesis, we identified an insertion of 7 nucleotides that at the mRNA level caused exon skipping. The fluorescent reporter approach revealed effective frameshifting only for 2-nucleotide deletion in *chd7*, a chromatin remodelling factor gene, suggesting activity of alternative translation sites in the other two. Thus, in addition to providing a protocol for characterizing frameshift mutations in zebrafish, this approach highlights the importance of checking mutations at the mRNA level and verifying their effects on translation by fluorescent reporters when antibody detection of protein loss is not possible.

Z6173B *In vivo m*odeling of copy number variants in Marfan Syndrome and Autosomal Dominant Polycystic Kidney Disease-associated phenotypes. *D. Schepers*[1], C. Golzio[2], E. Davis[2], C. Claes[1], E. Reyniers[1], A. Raes[3], N. Katsanis[1], L. Van Laer[1], B. Loeys[1]. 1) Center of Medical Genetics, University of Antwerp and Antwerp University Hospital, Antwerp, Belgium; 2) Center for Human Disease Modeling, Duke University Medical Center, Durham, NC, USA; 3) Department of Pediatrics, Ghent University, Ghent, Belgium.

Thoracic aortic dissections are among the most life threatening forms of cardiovascular disease. Thoracic aortic aneurysm, preceding dissection, is a prominent clinical feature of several heritable connective tissue disorders, including Marfan syndrome (MFS). MFS is caused by mutations in *FBN1*, which encodes fibrillin-1, an important extracellular matrix protein. Mutations in *PKD1* or *PKD2*, two polycystin encoding genes, are responsible for autosomal dominant polycystic kidney disease (ADPKD). Aortic and arterial aneurysms also occur in ADPKD. *Vice versa*, kidney cysts have also been observed in MFS. This clinical overlap suggests a mechanistic link between ADPKD and MFS.

Here we describe a four generation family with nine affected individuals presenting with both thoracic aortic aneurysm and mild cystic kidneys. *FBN1*, *PKD1* and *PKD2* were excluded as disease causing genes by linkage analysis and/or sequencing. Subsequently, whole genome linkage analysis resulted in the delineation of a unique linked region on chromosome 16q21-q24.1. Exome sequencing was performed but no putative causal variants were found in the linked region. Copy number variation analysis identified two duplicated regions in the linkage interval, one (chr16: 86862531-870228808) gene-less and the other (chr16: 86357163-86725305) containing seven genes, including three genes encoding transcription factors of the FOX gene family (*FOXC2*, *FOXF1*, *FOXL1*), one gene encoding a methenyl tetrahydrofolate synthetase containing domain protein (*MTHFSD)* and three long non coding RNAs (*LOC732275*, *FENDRR, FLJ30679)*. The presence of the first duplication and its segregation in the family was confirmed using Multiplex Amplicon Quantification analysis. By overexpressing these genes separately and in combination with each other in zebrafish, we will explore the pathogenic mechanisms underlying not only MFS and ADPKD, but aneurysm and cyst formation in general. Preliminary results of these experiments, revealing a potential role for *FOXF1* in cyst formation, will be presented.

Z6174C The functional role of actin associated CORO2B in the pronephros of *Danio Rerio*. *A. M. Schwarz*, L. Ebarasi, E. Raschperger, J. Patrakka. Karolinska Institutet, ICMC, Stockholm, SE.

Diabetic nephropathy (DN) is worldwide the leading cause of end-stage renal disease (ESRD), which requires dialysis or a kidney transplant. Diabetes impacts negatively on all cells of the kidney. Podocyte cell injury has been shown to be a major driver in the initiation and progression of DN. The podocyte actin cytoskeleton plays an essential role in the proper organization and function of the glomerular filtration barrier. In this study, we describe a new interaction partner of podocyte actin organization, coronin, actin binding protein, 2B (CORO2B). CORO2B has already been associated to the connection of the plasma membrane with the actin cytoskeleton in neuronal cells. In human DN patients, CORO2B is downregulated. In fish, there are two coro2b genes, coro2ba and coro2bb. We initially employed a morpholino-based approach to elucidate the role of the coro2b genes in the zebrafish pronephros. The morphant larvae showed abnormal morphology of the pronephros, with fewer podocytes, as well as hydrocephalus. At a functional level, proteinuria was observed in collected fish water suggesting a functional defect in the glomerular filtration barrier. Mammalian podocyte cell culture experiments show that CORO2B is crucial for the organization and maintenance of the actin skeleton of podocytes and by extension, the glomerular filtration barrier. These data are currently being validated by a CRIPSR knockout strategy in zebrafish.

Z6175A Chaperones and chromatin remodelers: functional non-cilia roles for established ciliopathy proteins. *C. Anthony Scott*[1], Xitiz Chamling[2], Lisa M. Baye[1], Val C. Sheffield[3], Diane C. Slusarski[1]. 1) Department of Biology, University of Iowa, Iowa City, IA, USA; 2) Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA, USA; 3) Department of Pediatrics and Ophthalmology, Carver College of Medicine, University of Iowa, Iowa City, IA, USA.

Bardet-Biedl syndrome (BBS) is a genetic disease that affects cilia function and/or maintenance. Other cellular functions, however, have not been well investigated. In this study we provide evidence that implicates BBS proteins as modulators of cytoplasmic-nuclear transport in addition to their cilia roles. To date, 20 genes have been identified as causes of BBS in patients. While BBS genes are from diverse gene-families, many of the BBS proteins form two major complexes: the BBSome (BBS1, 2, 4, 5, 7-9, and BBIP10) and the BBS chaperonin complex (BBS6, 10, and 12). The BBSome, which localizes to the basal body, is the main functional complex and is involved in trafficking of cargo to the cilium. Correct assembly of the large BBSome is mediated by the BBS chaperonin complex. Our lab has established the zebrafish as an *in vivo* model for BBS, and we have previously identified cilia-dependent as well as cilia-independent phenotypes. This has led us to investigate functions of BBS proteins outside of their defined roles in the cilia. Because of the similarities between the cilia transition zone (CTZ) and nuclear pores we hypothesized that BBS proteins regulate nuclear-cytoplasmic transport in addition to ciliary transport. Using zebrafish in conjunction with human tissue culture we identified an interaction between BBS6 and the SWI/SNF chromatin remodeler SMARCC1 (BAF155). We determined that BBS6 is actively transported between the nucleus and the cytoplasm and that perturbations of BBS6, as well as other BBS chaperonin complex members, affects the sub-cellular localization of SMARCC1. We conclude that BBS6 has a functional role beyond being a scaffold for BBSome assembly. Our work demonstrates that BBS6 functions (likely with other BBS proteins) to modulate nuclear-cytoplasmic transport of nuclear proteins and disruption of this may manifest as disease. This highlights the importance of looking at disease causing genes and alleles from a broader perspective.

Z6176B Functional characterization of the disease-associated Bardet-Biedl Syndrome 1 (BBS1M390R) allele in zebrafish. *D. C. Slusarski*[1,4], C. A. Scott[1], L. M. Baye[2], Q. Zhang[3], T. Westfall[1], V. C. Sheffield[3,4]. 1) University of Iowa, Iowa City, IA; 2) Augustana University, Sioux Falls, SD; 3) Carver College of Medicine, University of Iowa, Iowa City, IA; 4) Wynn Institute for Vision Research, University of Iowa, Iowa City, IA.

Dysfunctional cilia underlie a number of human genetic conditions that affect multiple organs, causing blindness, heart disease, infertility, obesity and/or diabetes. One such ciliopathy is Bardet-Biedl Syndrome (BBS). There is considerable interest in understanding the molecular mechanisms involved in BBS as phenotypes associated with this disorder are commonly found within the general population. BBS1 is the most commonly mutated gene in BBS patients, and a single missense mutation causing a methionine to arginine substitution at position 390 (M390R) accounts for 80% of all BBS1 mutations. $BBS1^{M390R}$ is predicted to be highly disruptive due to the introduction of a positive charge into the hydrophobic core of the folded protein. We use the zebrafish to understand the functional properties of BBS1 and the disease mechanism of $BBS1^{M390R}$. Analysis of BBS1 and $BBS1^{M390R}$ subcellular localization shows that $BBS1^{M390R}$ fails to localize to the centrosome. We also find that $BBS1^{M390R}$ maintains interaction with some, but not all, components of the BBSome complex, thereby likely disrupting its cellular function in cilia transport. To evaluate functional properties, suppression of knockdown defects (reduced Kupffer's vesicle cilia and intracellular transport delays) was tested. We also examined the extent to which the interaction of BBS1 with BBS4, a component of the BBSome, influences stability and localization as well as describe recently generated BBS1 genetic lines.

Z6177C Establishing PXE disease model in zebrafish. *J. Sun*[1,2,3], Tao Zhong[1,2,3]. 1) School of Life Sciences, Fudan University, Shanghai, CN; 2) State Key Laboratory of Genetic Engineering,Fudan University, Shanghai, CN; 3) Collaborative Innovation Center of Genetics and Development, Fudan University, Shanghai, CN.

Abstract:Pseudoxanthoma elasticum (PXE) is an autosomal recessive multi-system disease, manifesting pathologic mineralization of connective tissues, including the skin, eyes and cardiovascular system. The prevalence of PXE in population is about 1/50000. PXE is mainly caused by mutations in *abcc6*/*mrp6*, which encodes a member of the sub-family C of ATP-binding cassette (ABC) transport proteins. However, the roles of *abcc6* for organogenesis and mechanisms remain largely unknown. We have constructed *abcc6a* mutant with the same conserved domain substitution of PXE disease (G1302R) using CRISPR/Cas9-mediated knock-in system in zebrafish. We observed similar symptoms of human PXE disease in *abcc6a* mutants. The establishment of PXE disease model in zebrafish provides insights into pathological mechanisms of

PXE disease and opens novel avenues for developing potential therapy.

Keywords: PXE, ABCC6, disease model, zebrafish.

Z6178A The down-regulation of pank2 gene in zebrafish as a model of Pantothenate Kinase Associated Neurodegeneration. *N. Tiso*[1], D. Zizioli[2], G. Busolin[1], D. Khatri[2], R. Giuliani[2], G. Borsani[2], E. Monti[2], F. Argenton[1], D. Finazzi[2]. 1) University of Padova, Padova, IT; 2) University of Brescia, Brescia, IT.

The increased iron deposition is a hallmark of many neurodegenerative diseases, but its pathogenic role is still unclear. A strong link between iron and neurodegeneration is evident in a set of heterogeneous neurological disorders, known as Neurodegeneration with Brain Iron Accumulation (NBIA). The most common form of inherited NBIA is associated with mutations in the human *PANK2* gene (PKAN disease). Pank2 is the rate-limiting enzyme in CoA biosynthesis and its down-regulation in mammalian cells leads to perturbation of cellular iron homeostasis. In our work we have explored the Pank2 biological function in zebrafish (*Danio rerio*), proposing this system as an important new tool for the study of PKAN disease.

The zebrafish Pank2 protein shows 65% identity with the human ortholog. By qRT-PCR analysis on total RNA from embryos and adult tissues we have found that the expression of *pank2* transcripts is detectable in embryos from early stages to 72 hours post-fertilization, and that the brain is the tissue with the highest expression level of *pank2*. The whole-mount *in situ* hybridization (WISH) technique confirms the qRT-PCR results, showing high *pank2* expression in different brain structures, in the main vessels and in the venous plexus. The microinjection of *pank2*-specific morpholino oligos results in a clear-cut phenotype, with perturbation of CNS structures and vascular system development, suggesting the relevance of *pank2* expression for the normal nervous and vascular developmental process in zebrafish. Both the co-injection of *pank2* mRNA and the addition of pantethine 30 mM at the gastrula developmental stage can restore the wild type phenotype with high efficiency. The effects induced in the CNS and vascular structures have been characterized by WISH with different neuronal and vascular markers and by injecting *pank2* morpholino oligos in different transgenic lines. The results indicate a clear effect on the development of the forebrain, where also the nuclei corresponding to the human globus pallidus are located. The vascular arborization is also drastically perturbed, with severe fenestration of the main vessels and reduced connections of the inter-somitic vessels. Altogether these data indicate that the transient down-regulation of *pank2* gene expression in zebrafish represents an interesting model of PKAN disease, potentially amenable for high-throughput screening of molecules with therapeutic potential.

Z6179B Real-time Quantitative Assessment of Oxidative Stress as a Marker for Differential Nanoparticle Toxicity. *K. N. Wallace*, Rifat Emrah Ozel, Xiaobo Liu, Silvana Andreescu. Clarkson University, Potsdam, NY.

Rapid progress of nanotechnology and advanced nanomaterials production over the past decade offer significant opportunities for a wide range of applications in many fields ranging from medical diagnostics, imaging and drug delivery to sensing, catalysis and environmental remediation. While many engineered nanomaterials are commonly used in commercial products, their interactions with biological systems, their transport, kinetic, toxicity and accumulation in living organisms as well as their environmental and health effects are largely unknown and their use has recently become of particular concern. Several studies have shown that there is a direct relationship between the nanomaterial's structure and physicochemical properties and their impact in biological systems. The effect of NPs on biological systems varies broadly, and some reported results are contradictory, even in the case of the same type of materials. One of the key nanotoxicity mechanisms is the potential for induction of oxidative stress by generating nitrogen (RNS) species. However, assessing the extent of NPs induced oxidative stress has been a challenge as most RNS are highly reactive and short lived and therefore difficult to detect. We have developed electrochemical sensors for detection by direct real-time assessment of RNS species at the NPs accumulation site in living zebrafish embryos.

Z6180C Transcriptional Disease Signatures of Zebrafish Models of Fanconi Anemia. *C. Wilson*[1], W. C. Warren[2], J. H. Postlethwait[1]. 1) Institute of Neuroscience, University of Oregon, Eugene OR, USA; 2) McDonnell Genome Institute, Washington University, St Louis, MO, USA.

Fanconi Anemia (FA) is a rare pediatric disease caused by biallelic mutations in any of 19 different genes acting in a common DNA-crosslink repair pathway. FA involves bone marrow failure, hypogonadism, and high risk of hematopoietic and squamous cell cancers. Patient cells exhibit increased chromosome breakage when challenged with a DNA-crosslinking agent such as diepoxybutane (DEB) and show depressed stem cell renewal even in the absence of exogenous DNA damage. To better understand the mechanisms of FA and to facilitate the search for therapeutics, we used insertional mutagenesis and TILLING to generate mutant lines for the FA core-complex genes *fancc* and *fancl*, and the downstream gene *fancd1*(*brca2*). Zebrafish mutants for all three *fanc* genes exhibited increased cell death in response to DEB, as well as female-to-male sex reversal and gonadal dysgenesis. While the sex-reversal phenotype is caused by the death of meiotic oocytes in juvenile gonads, little is known about transcriptional changes underlying FA phenotypes during development of whole organisms. Tissues relevant to FA, including hematopoietic tissue, gonads, and the adaptive immune system, all develop in zebrafish in the first month post-fertilization. We used RNA-seq to compare gene expression between FA mutants and wild-type siblings in larval and juvenile zebrafish. We generated strand-specific cDNA libraries from 3-5 replicates of mutant and wild-type zebrafish at 4, 10, 14, 18, 22, 26, and 30 days post-fertilization and sequenced them at an average depth of 20 million reads on an Illumina HiSeq. Differential expression was found in genes involved in mitochondrial function and oxidative stress. These results provide a fuller understanding of the mechanisms of FA disease and may help identify new target genes for human therapies.

Z6181A Myelination deficiencies and pharmacological treatments in a zebrafish model for psychomotor retardation. *D. Zada*, A. Tovin, L. Appelbaum. Bar-Ilan University, Ramat-Gan, IL.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

Allan Herndon Dudley syndrome (AHDS) is a severe psychomotor retardation disorder characterized by neurological impairments and abnormal thyroid hormone (TH) levels. THs regulate metabolism, embryonic development and neurogenesis. Mutations in the TH transporter, monocarboxylate transporter 8 (MCT8), are associated with AHDS; however, the function of this gene and the mechanism of the disease remain elusive. Similar to human patients, MCT8-knockout mice exhibit impaired TH levels; however, they lack neurological defects. We utilized the zebrafish model to study MCT8 function and psychomotor retardation. This transparent vertebrate has emerged as a promising model to study neurobiology and development because it combines the advantage of high-throughput genetics of invertebrates with mammalian-like conserved genome and brain structure. Importantly, in contrast to mice, MCT8 mutant (*mct8*-/-) zebrafish exhibit impaired neurogenesis and altered behavioural performances. Here, we studied the development of myelination in *mct8*-/- zebrafish. We used the *myelin basic protein* (*mbp*) as a marker for mature oligodendrocytes in the CNS. The levels of expression of *mbp* mRNA were reduced in *mct8*-/- larvae. Furthermore, time-lapse live imaging of *mbp:EGFP* transgenic fish showed a decrease in the number of oligodendrocytes in different brain regions of *mct8*-/- larvae during development. In order to test potential treatments, the effect of a few drugs on myelination was determined during several developmental stages in live *mct8*-/- larvae. These treatments resulted in partial to full recovery of myelination. These results suggest that, similar to humans, loss of MCT8 affects the development of myelin in zebrafish larvae. It also suggests a putative pharmacological treatment for AHDS patients. Altogether, the *mct8*-/- zebrafish provide a model to study the mechanism and treatment of psychomotor retardation, and may help to understand other myelination disorders such as multiple sclerosis.

Z6182B Gene miles-apart is required for formation of otic vesicle and hair cells in zebrafish. *Jing-pu Zhang*, Zhan-ying Hu, Qing-you Zhang, Wei Qin, Jun-wei Tong, Bo Chen. Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences. Beijing, China.

Hearing loss is a serious burden to physical and mental health worldwide. Aberrant development and damage of hearing organs are recognized as the causes of hearing loss, the molecular mechanisms underlining these pathological processes remain elusive. Investigation of new molecular mechanisms involved in proliferation, differentiation, migration and maintenance of neuromast primordium and hair cells, will contribute to better understanding of hearing loss pathology. This knowledge will enable development of protective agents and mechanism study of drug ototoxicity. In this study, we demonstrate that the zebrafish gene *miles-apart*, a homolog of sphingosine-1-phosphate receptor 2 (s1pr2) in mammals, plays an important role in the development of otic vesicle, neuromasts and survival of hair cells. Whole mount *in situ* hybridization of embryos showed that *miles-apart* expression occurred mainly in the encephalic region and the somites at 24 hpf, in the midbrain/hindbrain boundary, the brainstem and the pre-neuromast of lateral line at 48 hpf in a strict spatiotemporal regulation. Both up- and down-regulation of *miles-apart* led to abnormal otoliths and semicircular canals, excess or few hair cells and neuromasts, and their disarranged depositions in the lateral lines. Miles-apart dysregulation also caused abnormal expression of hearing associated genes, including *hmx2*, *fgf3*, *fgf8a*, *foxi1*, *otop1*, *pax2.1* and *tmieb* during zebrafish organogenesis. Moreover, in larvae *miles-apart* gene knockdown significantly up-regulated proapoptotic gene *zBax2* and down-regulated prosurvival gene *zMcl1b*; in contrast, the level of *zBax2* was decreased and of *zMcl1b* enhanced by *miles-apart* overexpression. Collectively, miles-apart activity is linked to organization and number decision of hair cells within a neuromast, also to deposition of neuromasts and formation of otic vesicle during zebrafish organegenesis. At larva stage miles-apart as an upstream regulator of bcl-2 gene family, plays a role in protection of hair cells against apoptosis by promoting expression of prosurvival gene *zMcl1b* and suppressing proapoptotic gene *zBax2*.

Z6183C Fer1L-6 is a calcium signaling membrane protein that plays a critical role in skeletal muscle and heart development. *C. P. Johnson*, Chelsea Holman, Trisha Chau, Robert Tanguay. Oregon State University, Corvallis, OR.

The ferlin protein family are group of membrane proteins composed of multiple C2 domains with emerging roles in calcium regulated membrane trafficking and vesicle fusion events. Ferlin mutations are associated with muscular dystrophy and deafness, as well as infertility in Caenorhabditis elegans and Drosophila. Ferlin genes appear early in eukaryotic evolution and members are found in all eukaryotic kingdoms. Despite their prevalence, several vertebrate ferlins remain completely uncharacterized.

We present results of studies conducted on Fer1L-6, one of the uncharacterized vertebrate ferlins, in zebrafish. Fer1L-6 expression occurred early in zebrafish development and peaked 48 hpf, with greatest expression levels in muscle and brain. Knockdown of Fer1L-6 expression resulted in defects in skeletal muscle patterning, as well as cardiac chamber development and heart valve formation. We conclude that Fer1L-6 plays a critical role in the development of skeletal muscle and the heart.

Z6184A A dynamic anesthesia system for long-term imaging in adult zebrafish. *R. Y. Kwon*, B. M. Wynd, K. Patil, C. J. Watson, G. E. Sanders. University of Washington, Seattle, WA.

Long-term in vivo imaging in adult zebrafish (i.e., 1-24 hours) has been limited by the fact that regimens for long-term anesthesia in embryos and larvae are ineffective in adults, with respiratory arrest occurring within ~10-50 mins using MS-222 or MS-222 cocktails [1]. Here, we report the potential for dynamic administration of benzocaine to enable long-term anesthesia of up to 24 hours in adult zebrafish. We developed a computer-controlled perfusion system comprised of a 3D printed imaging chamber coupled to programmable peristaltic pumps that enabled automatic exchange between anesthetic and system water. Continuous administration of benzocaine in adult zebrafish resulted in a mean time to respiratory arrest of 5.0 hours and 8-hour survival of 14.3%. We postulated that an intermittent regimen consisting of ~100% of the average time to sedation and ~50% of the average time to recovery would prolong time to respiratory arrest compared to continuous administration. We measured characteristic sedation and recovery times in response to benzocaine, and used them to devise an intermittent dosing regimen consisting of 14.5 min of benzocaine followed by 5.5 min of system water. Intermittent benzocaine administration in adult zebrafish resulted in a mean time to respiratory arrest of 7.6 hours and 8-hour survival of 71.4%. To examine the potential for this regimen to extend anesthesia

beyond an 8-hour period, we performed a single 24-hour trial and found that intermittent dosing maintained anesthesia in an adult zebrafish over the entire period, with respiratory rates comparable to those observed in fish that survived to 8 hours in intermittent benzocaine trials. In summary, our studies demonstrate the potential for dynamic administration of benzocaine to significantly extend anesthetic periods in adult zebrafish, expanding the potential for imaging in adult physiologies that unfold over 1-24 hours.

References

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Z6185B Identification of skeletal disruptor compounds through *in vivo* screening in zebrafish. *SAVINI THRIKAWALA*[1], Rachna Sachanandani[1], Sanat Upadhyay[2], Clifford Stephan[3], Nghi Nguyen[3], Mary Sobieski[3], Richard Judson[4], Jan-Åke Gustafsson[1], Maria Bondesson[5]. 1) Department of Biology and Biochemistry, Center for Nuclear Receptors and Cell Signaling, University of Houston, Houston, TX; 2) Department of Computer Science, University of Houston, Houston, TX; 3) Center for Translational Cancer Research, Texas A&M Health Science Center, Institute of Biosciences and Technology, Houston, TX; 4) National Center for Computational Toxicology, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC; 5) Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX.

Exposure to teratogens during embryonic development may lead to skeletal birth defects, including cranio-facial and limb malformations. We have used zebrafish as a model organism to identify skeletal disruptors. Transgenic zebrafish expressing fluorescent markers in bone or cartilage tissues were used to visually monitor the development of the cleithrum and craniofacial cartilage structures under normal and perturbed conditions. Next, transgenic skeletal embryos were used in a primary high-throughput screen of 309 compounds of the ToxCast phase I chemical inventory, primarily consisting of pesticides and antimicrobials. We found that exposure to 82 compounds perturbed normal development of the cleithrum. The compounds causing an effect in the primary screen were further analyzed across a wider dose range. From this we identified 38 skeletal disruptors; 29 of them caused malformations in the cleithrum and 9 caused a short cleithrum. By further analysis in cartilage transgenic fish, we found that exposure to some, but not all, of the compounds caused perturbed development of both skeleton and cartilage.

Univariate analysis was performed to identify ToxCast *in vitro* assays that significantly correlated with the identified *in vivo* zebrafish skeletal disruptors. Ten assays were identified and were used to create ToxPi profiles for the skeletal disruptors and rank them based on the AC_{50} values of the ToxCast assays. A number of the corresponding molecular pathways to the ToxCast assays were further confirmed to be altered by exposures to skeletal disruptors in zebrafish. Additionally, RT-PCR was used to show that the expression of skeletal marker genes, such as Sox9 and Runx2, were affected by exposure to several of the skeletal disruptors. In conclusion, our results show that several environmental pollutants have skeletal disrupting capacity and that the developing zebrafish embryo is an efficient *in vivo* model that can be used for identification of skeletal disruptors and the molecular pathways that are affected by these chemicals.

Z6186C Multi-Modal High-Content Imaging Reveals Relationships Between Cell Signaling and Mineralization in Zebrafish. *Claire Watson*, Edith Gardiner, Werner Kaminsky, Ronald Kwon. University of Washington, Seattle, WA.

The generation of new bone requires activation of specific signaling cascades in a temporally and spatially distinct manner. However, until recently, a direct comparison between the dynamics of these signaling events and the formation of mineralizing bone has been precluded by the lack of strategies to simultaneously visualize these relationships *in vivo*. The optical clarity of the zebrafish caudal fin allows for high-content imaging of cell signaling in real time using fluorescent reporter strains. Previously, we demonstrated the potential to measure crystalline mineral accrual during fin regeneration using quantitative birefringence imaging (Rotopol microscopy [1]). Building on these findings, in this study, we developed a high-content, multi-modal system for tandem imaging of fluorescent transgenic reporters and bone mineralization within the same tissue. The system consisted of a motorized rotating polarizer integrated into a fully motorized Zeiss Axio Imager.M2 high-content fluorescence microscope, with custom software enabling interfacing between systems. We applied this imaging strategy to directly examine the relationships between canonical Wnt signaling and *sp7* (osterix) expression (using the Tg(7xTCF-Xla.Siam:GFP)ia4 and Tg(sp7:EGFP) reporter fish, respectively) with changes in cell metabolism (indicated by NADH autofluorescence) and mineralization (via Rotopol acquired birefringence) during bone formation and maturation in the regenerating zebrafish fin.

A custom mapping approach was developed to register images from different imaging days onto one another by creating landmarks of fixed pixel distances along each ray. Mapped images permitted the analysis of signaling events at any location in the regenerate, even before bony tissue was detectable. During the initial outgrowth phase, we find that acute activation of NADH is followed by Wnt signaling and subsequent expression of *sp7*. Interestingly, step-wise increases in mineralization coincide with second and third peaks in NADH autofluorescence. Furthermore, we find that mineralization is not complete even at 26 dpa in some fish. Using Rotopol imaging in concert with high resolution fluorescent imaging for the first time, we demonstrate that this system can be used to examine relationships between core bone formation events and maturation into fully mineralized bone.

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Z6187A Myomesin-1 stabilises sarcomeric structure acting as a shock absorber in skeletal muscle. *M. ZHAO*[1], A. Costin[2], C. Williams[1], G. Ramm[2], R. Bryson-Richardson[1]. 1) Monash Universtiy, Melbourne, Victoria, Australia; 2) The Clive and Vera Ramaciotti Centre for Structural Cryo-Electron Microscopy, Monash University, Melbourne, Victoria, Australia.

Skeletal muscle consists of bundles of myofibrils that act in concert to generate movement. Each myofibril is composed of repeating

contractile units, known as sarcomeres, which produce force by the sliding of the myosin-rich thick filaments along the actin-rich thin filaments. Thin filaments are anchored to the Z-disks that flank the sarcomere, whereas thick filaments anchor to the M-band at the centre of the sarcomere. In contrast to the extensive research into Z-disks and the identification of skeletal myopathies that result from their disruption, little is known about the M-band.

Myomesins (MYOMs) are a family of M-band-restricted proteins that crosslink thick filaments with MYOM1, MYOM2, and MYOM3, each occupying distinct locations within the M-band. MYOMs contain repeats of highly elastic domains and is able to reversibly stretch to 2.5 times of its original length under force, and thus MYOMs are predicted to act as molecular springs at the M-band. To investigate the roles of Myoms and the M-band, we used mutant lines and morpholino injections in combination to examine myomesin function in the zebrafish. Electron microscopic analysis showed the absence of Myom1 leads to contraction-dependent loss of the M-band and thick filament misalignment, showing that Myom1 is essential for sarcomeric integrity. We also show that loss of Myom1 does not affect maximal force generation but significantly decreases swimming activity. Our results suggest the M-band act as a shock absorber at the centre of the sarcomere, and demonstrate for the first time, Myomesin-1 is crucial in maintaining structural stability of the muscle.

Z6188B Defective Migration of Facial Branchiomotor Neurons Affects Jaw Movements and Food Intake in Zebrafish. *E. Asante*[1], J. Allen[1,2], B. Almadi[1], A. Chandrasekhar[1]. 1) University of Missouri, Columbia, MO; 2) Washington University School of Medicine, St. Louis, MO.

Migration of neuronal cell bodies from their birthplace to their final destination is a crucial step in brain development. Proper organization and function of neurons circuits depends on developmental processes such as neuronal specification and migration, axon guidance and maturation of synapses. Improper neuronal migration may play a role in human diseases such as schizophrenia, mental retardation, and autism. Despite its importance, the consequences of abnormal neuronal migration on circuit organization and behavioral function are poorly understood. To investigate migration of neurons we study the facial branchiomotor (FBM) neurons in zebrafish as a model system. In the zebrafish hindbrain, FBM neurons migrate caudally in the hindbrain from rhombomere 4 to their final destination rhombomeres 6/7, and innervate jaw and gill muscles. In order to examine the behavioral consequences of defective migration in FBM neurons, we characterized the maturation and function of the neural circuits controlling jaw movement in zebrafish larvae between 3-7 days post fertilization (dpf) by time-lapse imaging and quantitative analysis. At 3 dpf larvae exhibited little or no jaw movement and minimal gape activity (opening and closing of the lower jaw). At 5 dpf, larvae exhibited rhythmic jaw movements such that gape increases in amplitude and frequency. By 7 dpf jaw movement are very robust and rapid, with a doubling of frequency over 5 dpf larvae. These data indicate that jaw movements develop and mature between 3- 7 dpf**,** 24-36 hours after FBM neurons have completed caudal migration and 72 hours after completed axon projection to the jaw muscle. To study the consequences of defective neuronal migration, we measured the gape activity of *off limits* (*fzd3a-/-*) mutant larvae where FBM neurons fail to migrate out of rhombomere 4. Mutant larvae exhibited a small increase in gape activity from 3-7 dpf, resulting in very low gape frequency at 5 and 7 dpf compared to wild type siblings. These data suggest that a failure of FBM neuron migration leads to defects in motor circuits, resulting in a failure of jaw movement to be established properly. Given the defects in jaw movements in *off limits* mutants, we wondered whether another functional output of the branchiomotor circuits, namely food intake, was also affected in mutants. As expected, *olt* mutants exhibited a significant decrease in food intake. These results demonstrate that misslocation of the FBM neurons can lead to deficits in the functional output of the branchiomotor circuits. Our studies establish a foundation for dissecting the neural circuits driving a motor behavior essential for survival.

Z6189C Do fish itch: identifying mechanisms of pruritigen transduction and behavior in *Danio rerio*. *L. Condon*[1], K. Esancy[1], J. Feng[2], C. Kimball[1], A. Curtright[1], H. Hu[2], A. Dhaka[1]. 1) University of Washington, Seattle, WA; 2) Washington University, St. Louis, MO.

Chronic itch is a debilitating condition that affects a large portion of the population. It is a symptom of many illnesses including cancer, kidney failure, liver cirrhosis, MS, and shingles. Finding treatments for chronic itch would greatly improve many peoples' lives. The first step in developing effective treatments is to determine the cellular and molecular mechanisms responsible for itch. Using the zebrafish (*Danio rerio*) model system, we are exploring whether zebrafish experience pruritus as a discrete sensation from nociception and whether the itch transduction pathways identified in mammals are present in zebrafish. We expect that the advantages of the zebrafish model system, including rapid genetic manipulation, in vivo calcium imaging, and high throughput behavioral screening, will allow us to expand our knowledge of the cellular and molecular mechanisms that allow for the separation of nociception and this novel sensation. The Dhaka Lab is interested in determining what forms of itch, if any, exist in zebrafish so that we may leverage the advantages of the zebrafish model system to characterize the properties of itch stimuli transduction, thus expanding our knowledge of itch pathways and potentially gaining insight into the evolutionary origin of itch in vertebrates. We have been exploring whether compounds that induce itch in mammals, such as agonists of histamine receptor 1, protease-activated receptor 2, and toll like receptor 7, evoke a behavioral and somatosensory response in zebrafish. Our behavioral data shows that imiquimod produces both locomotive behavior in larval zebrafish and a novel lip rubbing behavior in adult zebrafish. Additionally when fish were exposed to imiquimod we observed activity in neurons of the trigeminal ganglion, an exclusively somatosensory cluster of neurons, which indicates that this compound elicits a somatosensory response in zebrafish. We plan to continue characterizing these pathways to increase our understanding of itch, which may aid in the development of novel therapies to treat itch.

Z6190A Photoreceptor development and regeneration examined by automated analysis of behavior. *Robbert Creton*, Danielle Clift, Robert Thorn, Emily Passarelli, Ruth Colwill. Brown University, Providence, RI.

Visual impairments affect 285 million people worldwide: 39 million people are blind and 246 million people have low vision. Several lines of evidence suggest that it may be possible to restore vision by regenerating photoreceptors and neural connections. However, as novel

methodologies are developed, a critical question will need to be addressed: how do we monitor in vivo for functional success? One approach to monitor for functional success in animal model systems is the analysis of behavior, since behavioral analyses can reveal subtle functional defects, even if the visual system appears normal by morphological criteria. The current study is focused on the automated analysis of behavior in response to visual stimuli, using zebrafish larvae as a model system. Zebrafish larvae are ideally suited for such studies, since high-throughput analyses of behavior can be combined with genetics, high-resolution imaging and experimental manipulations. We developed a novel behavioral assay using colored visual stimuli and found that 5-7 day-old zebrafish larvae display distinct responses to the visual stimuli. These behavioral responses were inhibited by photoablation of photoreceptors, but recover over time as the eye regenerates. The developed tools may be used in future research to screen small molecule libraries for novel treatments of blindness and low vision.

Z6191B Evaluation of the circadian biology of the neurohypophyseal hormones and their relationship with aggressive behavior in a vertebral model: Zebrafish. *L. A. Diaz-Arias*[1], S. A. Valencia[1], T. Manrique[2], V. Akle[1]. 1) Laboratory of neuroscience and circadian rhythms, School of Medicine, Universidad de los Andes, Bogota, DC, Colombia; 2) Brain and Behavior Laboratory, Pshycology department, Universidad del Rosario, Bogota, DC, Colombia.

The circadian rhythm is a physiological period of about 24 hours that coordinates the activity of different biological processes such as reproduction cycles, tissue regeneration, epigenetic, metabolism and behavior. Despite the fact that many studies have shown that aggressive behaviors as well as the expression of "social neuropeptides" follow a circadian pattern in different species, the relationship between aggression and the expression of the neurohypophyseal peptides has not been studied systematically. The objective of this study is to evaluate the role of the circadian system in aggressive behaviors of zebrafish and its relationship with the hormones isotocin (IT) and vasotocine (VT). For this purpose, we used a battery of behavioral tests that consisted of: Open Field test, Mirror test and Opponent test to assess aggression in the fish at specific time points during the light cycle. In subsequent experiments, using RT-PCR and immunohistochemistry the levels of IT, VT and its receptors at different times of day will be evaluated. Also, the receptors will be blocked to establish a possible mechanism of action of these neuropeptides. Preliminary results revealed that the mirror and predator assays acquire different kind of information. While the mirror test is appropriate as a measure of aggression, the encounter with the natural predator evaluates exploratory behavior or "shyness" more effectively. In general, there is a high variability in the behavior of individual fish, probably as a result of their social hierarchy. The behavior of males and females is statistically different in the Open Field test, while that difference is not evident in the Mirror test and Opponent test. Finally, even though there is a tendency toward more aggressive behaviors during the morning hours, the differences did not reach statistical significance. However, further tests are needed to clarify whether there are circadian differences in the aggressiveness of the zebrafish and between genders. This study represents the first step in exploring the role of the endogenous clock in the manifestation of aggression and neurohypophyseal expression of hormones in the vertebrate model zebrafish.

Z6192C Zebrafish: Lead and Learning. *M. A. Haasch*, Richard Sear, Simon Ng, Max Ho, Josephine Brenner, Alec Lau, Jeremy Kaine. Wauwatosa West High School, Wauwatosa, WI.

Zebrafish: Lead and Learning

Authors: Josephine Brenner, Maxmilian Ho, Jeremy Kaine, Alec Lau, Simon Ng, Richard Sear **Adviser:** Mary Haasch, Wauwatosa High School

Purpose: Adult zebrafish exposed to lead as embryos will exhibit lower learning and memory performance than adult zebrafish which were not exposed to lead as embryos.

Experiment: In collaboration with the Children's Environmental Health Sciences Center (CEHSC) at the University of Wisconsin-Milwaukee, students have tested the learning and memory capabilities of adult zebrafish exposed to 10uM lead as embryos, and adult zebrafish not exposed to lead as embryos. Using a simple T-maze, the number of trials needed for fish to learn to turn right (max. of 20 trials) and then learn the reverse by turning left (max. of 20 trials), was measured on three consecutive days and then 1 week after the initial day. Learning was measured by the number of trials needed to achieve 5 correct turns out of 6 consecutive trials. Memory was measured at 1 week using the same criteria.

Results: Adult zebrafish exposed to 10uM lead as embryos needed more trials to learn the right turn behavior compared to adult zebrafish not exposed to lead as embryos. Memories of learning the right turn in adult zebrafish exposed to 10uM lead as embryos needed a couple more trials compared to the adult zebrafish not exposed to lead as embryos.

Adult zebrafish exposed to 10uM lead as embryos needed more trials to learn the left turn behavior compared to the adult zebrafish not exposed to lead as embryos. Memory of learning the left turn at 1 week needed more trials by the adult zebrafish exposed to 10uM lead as embryos compared to the adult zebrafish not exposed to lead as embryos.

Conclusion: Lead exposure of zebrafish as embryos negatively affects learning as adults. Based on observations, students would like to compare the learning abilities of long finned to short finned zebrafish. Students would also like to pursue research into the affect of varying lengths of adult lead exposure on learning.

Z6193A Zebrafishbrain.org: developing a community neuroanatomical resource. *Thomas A. Hawkins*[1], Kate Turner[1], Andy Symonds[2], Mónica Folgueira[3], Jon Clarke[2], Stephen W. Wilson[1]. 1) UCL, London, London, UK; 2) King's College London, London, UK; 3) Universidade A Coruña, A Coruña, Spain; 11) Universidade A Coruña.

This poster will present an update on the development of zebrafishbrain.org and is intended to invoke a discussion on the best way forward for neuroanatomical atlasing resources for the zebrafish. Our objective with zebrafishbrain.org has been to create a web-based repository to communicate – in a user-friendly manner – information about the zebrafish brain.

Zebrafishbrain.org consists of annotated 2D projections of confocal data from transgenic and immunohistochemically stained larval zebrafish specimens. Many of these annotated images have also been incorporated into image and video-enriched tutorials about particular structures in the brain. The annotations and tutorials follow the standard ontology provided by ZFIN.org.

Recently, several brain data-registration systems have been independently developed by other groups and most are directed towards mapping expression or activity data to common anatomical frameworks.

We believe it is timely for these various resources to be brought together using one or few standardised approaches to ease the analysis of future anatomical, neuronal activity, and gene expression datasets. We present here some ideas about how best to achieve this through strengthening links between these disparate resources.

In addition, we will also present ideas about how best to generate high-throughput neuronal morphology data for incorporation into a standardised framework in order to build circuit information.

Z6194B A Zebrafish Model for Identifying Common Biological Mechanisms and Pharmacological Pathways in Autism Spectrum Disorders. *S. Ijaz*[1], E. Hoffman[1], M. Ghosh[2], J. Rihel[2], S. Wilson[2], M. State[1,3], A. Giraldez[1]. 1) Yale University, New Haven, Co; 2) University College London, London, UK; 3) University of California, San Francisco, CA.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social interaction and communication and restrictive, repetitive behaviors. ASD is considered one of the most heritable of neuropsychiatric disorders. Whole-exome sequencing has led to a growing list of genes that are strongly associated with ASD through the identification of an increased rate of de novo loss of function mutations in these genes in affected individuals. However, we are in need of model systems to identify common neurodevelopmental mechanisms that are affected by risk gene disruption. To address this, we are capitalizing on the advantages of zebrafish as a model system to investigate how disrupting these risk genes affects brain development and simple behaviors. First, we generated loss of function mutations in the zebrafish orthologs of nine ASD risk genes using CRISPR/Cas9 technology. These genes include: CHD8, SCN2A, POGZ, TBR1, GRIN2B, DYRK1A, KATNAL2, CUL3, and ANK2. For genes that are duplicated in zebrafish, we generated double mutant lines. Next, we are utilizing high-throughput behavioral analysis and high-resolution brain imaging to assess how disruption of these genes affects simple behavioral phenotypes and the development of excitatory and inhibitory neuronal populations. We are beginning to identify unique pharmaco-behavioral phenotypes among mutant lines. Next, our goal is to compare the behavioral profiles across mutant lines to identify pharmacological suppressors of abnormal behavioral fingerprints in mutants. Together, these studies have the potential to identify pharmacological pathways with relevance to ASD.

Z6195C Targeted knockout of a chemokine-like gene increases anxiety and social cohesion. *Y. Jeong*, J. Park, K. Ariyasiri, K. Lee, T. Kim, D. Jeong, K. Hong, C. Kim. Chungnam National University, Daejeon, KR.

Emotional responses such as fear and anxiety are essential for decision-making and survival. The habenula (Hb) is a structure in the epithalamus highly conserved during vertebrate evolution. It mediates behavioral responses to stress, anxiety, and fear. Dysfunction of Hb is associated with depression, post-traumatic stress disorder, and schizophrenia in humans. Furthermore, the ablation of Hb has been shown to profoundly alter fear and anxiety responses in fish and mice. However, the molecular mechanisms underlying the emotional responses and functional regulation of Hb remain largely unknown. Here we report the critical function of a novel chemokine-like protein, *samdori-2* (*sam2*), in anxiety and fear-related behaviors. We found that *sam2* is predominantly expressed by neurons in the dorsal Hb of adult zebrafish. Targeted knockout (KO) of the *sam2* gene by zinc finger nucleases in zebrafish did not affect viability, fertility, or general morphology. Furthermore, *sam2* KO fish showed normal development of neural circuits including Hb to interpeduncular nucleus (IPN) pathways. However, *sam2* KO fish exhibited robustly elevated anxiety-like behaviors in the novel tank and scototaxis tests, and showed increased social cohesion. In addition, nicotine-induced *c-fos* expression in the dorsal Hb-IPN pathway was significantly inhibited in the *sam2* KO fish. Consistent with these findings, Sam2 was found to strongly attenuate inhibitory synaptic function in neurons. These results establish a crucial role of *sam2* in regulating anxiety and social cohesion, and delineate a potential function of novel chemokine-like proteins in controlling neuronal circuit activity and behavior.

Z6196A Using larval zebrafish as an *in vivo* model system to study otoferlin, a protein expressed in the sensory hair cells and essential for hearing. *Colin Johnson*, Paroma Chatterjee, Murugesh Padmanarayana, Robert Tanguay. Oregon State University, Corvallis, OR.

Sensory hair cells convert mechanical motion into chemical signals. Otoferlin, a six-C2 domain transmembrane protein linked to deafness in humans, is hypothesized to play a role in synaptic vesicle exocytosis at hair cell ribbon synapses. To date however, otoferlin has been studied almost exclusively in mouse models which are technically challenging due to the inaccessibility of their hair cells and reluctance to genetic manipulation, and no rescue experiments have been reported. To overcome these challenges, we have developed zebrafish as a model for studying otoferlin function, and report the first otoferlin rescue experiments. We found that larval zebrafish have two otoferlin genes, otoferlin a and otoferlin b that are expressed early in development and are restricted to sensory hair cells and the midbrain. Knockdown of the otoferlin genes resulted in hearing and balance defects, and a phenotype associated with a looping and circular swimming pattern. Otoferlin morphants also had uninflated swim bladders. Microscopy revealed that otoferlin localized to both the apical and synaptic basolateral side of the cell and that knockdown resulted in abnormal presynaptic structures; namely changes in synaptic ribbon distribution and synaptic vesicle VGlut3 localization. Fluorescent mCLING dye loading experiments suggest that the loss of otoferlin also results in defective synaptic vesicle exocytosis and recycling. RNA-seq studies we have conducted provide a “big picture” view of otoferlin’s impact on zebrafish, and implicate otoferlin-related signaling in a variety of physiological processes extending beyond hearing. Rescue experiments conducted with mouse otoferlin

completely restored hearing, balance, and inflation of the swim bladder. Remarkably, truncated forms of otoferlin retaining the C-terminal C2F domain also rescued the otoferlin knockdown phenotype, while the individual N-terminal C2A domain did not. We conclude that otoferlin plays an evolutionarily conserved role in vertebrate hearing and that truncated forms of mouse otoferlin can rescue hearing and balance.

Z6197B Epidermal growth factor signaling regulates normal levels of sleep in zebrafish. *D. A. Lee*, J. Liu, D. A. Prober. California Institute of Technology, Pasadena, CA.

Sleep is an essential phylogenetically conserved behavioral state, whose regulation remains poorly understood. Several lines of evidence suggest that epidermal growth factor (EGFR) signaling may play an evolutionarily conserved and important role in regulating sleep. Genetic studies in *Drosophila* and *C. elegans* have demonstrated that EGFR signaling is required for invertebrate sleep. However, vertebrate studies have been inconclusive. To address this, we use the diurnal zebrafish to test the hypothesis that EGFR signaling is required for vertebrate sleep. We reveal that heat shock-induced overexpression of the EGFR ligand transforming growth factor alpha (TGFa) decreases locomotor activity and increases sleep, and that this phenotype is abrogated by small molecule inhibitors of MAPK, an EGFR effector pathway. We further observe that zebrafish containing mutations in EGFR ligands exhibit a dose dependent decrease in sleep. Corroborating this, animals with pharmacological inhibition and genetic loss-of-function of EGFR, demonstrate increased locomotor activity and decreased sleep during the day and night. Moreover, site of action analysis demonstrate that TGFa-induced sleep is centrally mediated. Similar to rodents and humans, EGFR is expressed in cells lining the brain ventricle of zebrafish. Following TGFa overexpressing, *c-fos* coexpresses with these *egfra* cells, suggesting that neural activity in this cell population is critical in mediating TGFa/EGFR-induced sleep. Taken together, these results indicate that EGFR signaling is both necessary and sufficient for normal vertebrate sleep, suggesting it participates in an ancient and central aspect of sleep control.

Z6198C The Role of Neurotensin Neuronal Networks in Zebrafish. *T. Levitas-Djerbi*, L. Appelbaum. Bar-Ilan University, Ramat Gan, IL.

Neurotensin (NTS) is a 13 amino acid neuropeptide that is expressed in the hypothalamus. In mammals, NTS-producing neurons that express leptin receptor (LepRb) regulate the function of hypocretin/orexin (HCRT) neurons. Thus, the hypothalamic leptin/NTS/HCRT neuronal network orchestrates key homeostatic output, including sleep, feeding and reward. However, the intricate mechanisms of the circuitry and the unique role of NTS-expressing neurons remain unclear. We studied the NTS neuronal networks in zebrafish and cloned the genes encoding the NTS neuropeptide and receptor (NTSR). Similar to mammals, the ligand is expressed primarily in the hypothalamus, while the receptor is expressed widely throughout the brain. A portion of hypothalamic *nts*-expressing neurons are inhibitory and some co-express leptin receptor (LepR1). As in mammals, NTS and HCRT neurons are localized adjacently in the hypothalamus. In order to track the development and axonal projection of NTS neurons, a partial NTS promoter sequence was isolated. Transgenesis and double labeling of NTS and HCRT neurons showed that NTS axons project toward HCRT neurons, some of which express *ntsr*. To determine the genetic, neuroanatomical, and behavioral role of NTS, we generated a CRISPR-based mutant zebrafish line (*nts*-/-). Using a video-tracking behavioral system, we studied the neurological and behavioral phenotype of the *nts*-/- fish. These findings suggest structural and functional circuitry between leptin, NTS and hypocretinergic neurons, and establish the zebrafish as a model to study the role of NTS in the regulation of key behavioral states such as feeding, sleep and wake.

Z6199A Statistical morphometric analysis and annotation of brain microstructure defects in larval zebrafish. *G. D. Marquart*[1,2], S. Pajevic[3], A. Heffer[1], T. Mueller[4], I. B. Dawid[1], H. A. Burgess[1,2]. 1) NICHD, Bethesda, MD; 2) UMD, College Park, MD; 3) NIH, Bethesda, MD; 4) KSU, Manhattan, KS.

Transgenic lines provide excellent tools to target defined groups of neurons within the developing nervous system. However, whole mount visualization frequently lacks the context to adequately align and annotate transgene expression. To address this we previously adapted volumetric registration techniques to align patterns from 109 transgenic lines into a common reference space and generated software to survey expression patterns at any point within the brain. This alignment allows us to better characterize expression, predict overlap and identify lines with expression in target regions. Without detailed anatomical annotation, however, the value of the data remains limited. While manual labeling addresses this, it is laborious, highly subjective, and constrained by our understanding of neuroanatomy, especially early in development when many primordial structures lack clear nuclear organization. However, we reasoned that local correlations in transgene expression might delineate emerging structures and allow us to computationally derive neuroanatomical annotations. Using spatially constrained data clustering of 112 transgenic lines, we were able to extract regions corresponding strikingly with previously identified neuroanatomical structures. These data-based annotations are less subjective than those manually defined and may allow identification of regions that would otherwise be impossible to reliably segment. Brain alignment also provides a framework for voxel-based morphometric comparisons, a statistically rigorous method for analyzing brain microstructure. As a proof of principle for the use of voxel-based morphometry in identifying discrete neuromorphological differences, we tested for differences in 10 wildtype brains and their left-right flipped counterparts. We speculated that if the analysis was both sensitive and selective, we should only pull out brain asymmetries. All statistically significant pixels that were found were in fact found in the habenula, the only asymmetric brain structure identified in larval zebrafish. Next, to test whether this analysis could locate novel brain abnormalities in mutant zebrafish, we aligned brains of kctd15a/b knockouts and compared them to their wildtype cousins. While kctd15 has been linked to human obesity, knockout larvae show no gross behavioral or morphological abnormalities yet lag behind wildtypes in their growth into adulthood. However, based on voxel-wise analysis of aligned brains, we identified a midbrain tegmental nucleus, the torus lateralis, that was absent in mutants. This finding was confirmed in six-month old mutants zebrafish using conventional histology. Together these results show that brain alignment can provide a powerful tool to screen for subtle neuroanatomical defects in mutants as well as to segment the larval brain into well-defined anatomical regions.

Z6200B Evaluating Pitch Perception via Acoustic Startle Behavior. *G. Ordiway*, R. Jain. Haverford College, Haverford, PA.

Interpreting auditory stimuli and triggering the appropriate physical responses are key biological processes through which individuals navigate their environment. A wide variety of acoustic frequencies are presented by the environment, and the relationship between frequencies can characterize complex hearing, and in part, predict responses to specific types of auditory stimuli. Due to the physics of sound waves, sound vibrations occur not only at the fundamental frequency (n) of that sound, but also double that frequency, or the first harmonic (2n). Perception across a variety of frequencies, as well as this idea of harmonics, we can more effectively test nuanced aspects of zebrafish hearing and interpretation. Since zebrafish use auditory hair cells for audition, characterizing their capacity for complex hearing will better relate them to mammalian and other higher order auditory models. Using habituation to measure non-associative learning, and prepulse inhibition to measure acoustic precision and sensitivity, assays normally designed to observe startle response modulation can now be used to evaluate pitch perception and interpretation. This study measures the baseline responsiveness for a variety of frequencies, and we hypothesize that if harmonics are interpreted as more similar than unrelated tones, habituation to a fundamental frequency (n), will accelerate the rate of habituation to a harmonic frequency (2n) more than to a frequency unrelated to the fundamental. Using high-speed recording at millisecond resolution and FLOTE tracking software, we analyzed the responses of five dpf zebrafish larvae. Frequencies of 200-2000Hz were used to test baseline response levels, as well as harmonic relationships (ie 200Hz vs 400Hz). Future studies could use the acoustic environment of an organism to rationalize aspects of complex hearing, including sounds that might indicate danger or predators. In addition, this study suggests a characterization of complex hearing that can be applied across species and animal models.

Z6201C Deciphering the role of Tmie in the mechanotransduction in sensory hair cells. *I. V. Pacentine*, R. Maeda, T. Nicolson. Vollum, Portland, OR.

The **t**rans**m**embrane **i**nner **e**ar (TMIE) protein is required for the function of auditory and vestibular hair cells in the inner ear. Mutations in *TMIE* cause deafness in humans (DFNB6) and previous work in mice suggests a role for TMIE in mechanotransduction (i.e. the conversion of mechanical energy into electrical signals). While we know that TMIE is essential for the mechanotransduction process, it is not clear whether it is more of an accessory protein of the mechanotransduction complex or whether it plays a more central role in this process. To address questions about the function of Tmie, we characterized two zebrafish *tmie* mutants: a previously published null mutant (*ru1000*) and an uncharacterized mutant expressing a truncated version of Tmie (*jp060*). In addition we examined Tmie's relationship with other known components of the mechanotransduction complex in hair cells (Lhfpl5a, Pcdh15a, Tmc2b), and we examined the function of various motifs that are present in Tmie. Both *tmie*^{ru1000} and *tmie*^{jp060} mutants have normal hair-cell morphology and Lhfpl5a, Pcdh15a and Tmc2b are present in hair bundles where transduction takes place. Tmie itself localizes independently of other transduction proteins. However, our preliminary evidence using a yeast two-hybrid assay suggests that Tmie can interact directly with the tip-link protein Pcdh15a. In *jp060* mutants, the truncation of Tmie, which eliminates the C-terminus of Tmie, results in a hypomorphic phenotype, with scant yet residual activity in hair cells. Consistent with the requirement of this region for the normal function of Tmie, the absence of the C-terminus abolishes the interaction with Pcdh15a in the two-hybrid experiments. Aside from potential interactions with Pcdh15a, we suspect that the lysine-rich C-terminus also interacts with phospholipids in the plasma membrane. Further inspection of other motifs in Tmie revealed that the first transmembrane domain, which is not conserved between fish and mammals at the amino acid level, is non-essential. However, the highly conserved second transmembrane is critical for stability and localization of Tmie at the site of mechanotransduction. Future experiments will include additional structure-function analysis to identify regions and residues critical to function, and yeast two-hybrid experiments to confirm and identify interacting partners of Tmie.

Z6202A Isolation and molecular characterization of a spinal interneuron that modulates swimming behavior. *Andrew Prendergast*[1], Urs Böhm[1], Lydia Djenoune[1,2], Laura Desban[1], Pierre-Luc Baradet[1], Claire Wyart[1]. 1) Institut du Cerveau et de la Moelle épinière, Inserm U1127, CNRS Université Pierre et Marie Curie UMR 7225, Campus Hospitalier Universitaire Pitié-Salpêtrière, Paris, France; 2) Evolution des Régulations Endocriniennes, UMR 7221 CNRS, Muséum National d'Histoire Naturelle, Paris, France.

Vertebrate locomotion relies on precisely timed output of motor neurons. Spinal cord circuits are known to autonomously produce locomotor rhythms following sensory stimulation or descending commands from the brain. In a changing environment, it is necessary to modulate CPG activity with external and internal sensory information. CSF-contacting neurons (CSF-cNs) project a ciliary tuft into the central canal of the spinal cord and make projections throughout the motor column. Consequently, these cells have been hypothesized to act as an internal sensory cell detecting cues from the CSF that modulate locomotor activity. We previously showed that CSF-cNs modulate locomotion in zebrafish larva. Here, we demonstrate that CSF-cNs detect curvature of the spinal cord during active and passive locomotion. However, the sensory mechanisms underlying CSF-cN function remain elusive. We have developed a transgenic line to label differentiated CSF-cNs in zebrafish. In order to identify novel candidate receptors underlying their sensory function, we isolated CSF-cNs by FACS enabling us to proceed to a transcriptome analysis. After identifying candidate molecules, we will disrupt their function and assess defects on CSF-cN activity *in vivo* and on innate locomotion. All together, this project will characterize the role of sensory information carried by the CSF and relayed to spinal circuits. In the future, our effort in zebrafish will be ported to mammalian studies.

Z6203B Ntrk2b expression and function in developing brain of zebrafish. *M. P. Sahu*, Pertti Panula, Eero Castren. Neuroscience Center, University of Helsinki, Helsinki, FI.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, plays an important role in neuronal network plasticity by promoting differentiation and survival of neurons during development and in adult brain. BDNF signals by activating its cognate receptor

tyrosine kinase B, NTRK2 and has trophic effects on the serotonergic system. The exact role of NTRK2 in the behavioral effects of serotonin remains poorly understood. Alterations in the BDNF-NTRK2 signaling and/or in 5-HT neurons have been implicated in the pathophysiology of psychiatric or mood disorders. This study pursues to interrogate the role of NTRK2 in brain development and more specifically the effects on the serotonergic system. The aim of this study is to understand the developmental effects of TrkB in the zebrafish model. The TrkB receptor has two forms in zebrafish Ntrk2a and Ntrk2b. The Ntrk2a is not found in significant amounts therefore the focus has been mostly on Ntrk2b.

The mRNA expression of Ntrk2b was assessed by in-situ hybridization in the larval stages and adult brain of zebrafish. Wide distribution of the mRNA was observed in the CNS such as dorsal telencephalon, diencephalon, in the parvocellular pre-optic nucleus, hypothalamus, positive radial glial cells lining the mesencephalic ventricle, cerebellum and dispersed positive fibers in the medulla oblongata. The expression of mRNA for BDNF complemented with specific Ntrk2b expression pattern. The morpholino oligonucleotides against Ntrk2b was designed and validated by western blotting using Trk specific antibody. The Ntrk2b morphants had no major gross phenotype and the motor behavior did not change significantly as compared to the controls. There was a significant difference in the monoamines levels of dopamine and serotonin observed in the Ntrk2b morphants. Immunoreactivity using antibodies for serotonin, GABA1H and tyrosine hydroxylase was reduced in the morphants as compared to the controls. The expression level of serotonin transporter (*serta*), and tyrosine hydroxylase (*th1*) was reduced in the morphants. The transient loss could be rescued by over expression of full length Ntrk2b mRNA. No major change in cell death in the Ntrk2b morphants was observed by TUNEL staining method. These results reveal potential role of NTRK2 during development and its effect on the monoaminergic system. The results establish that zebrafish Ntrk2b has wide spread distribution throughout development and loss of Ntrk2b plays an important role in monoamines mainly the serotonergic and the dopaminergic system.

Z6204C Effects of neurostimulation of the habenula in serotonergic and dopaminergic systems in zebrafish. *L. M. Sanchez-Lasso*[1], S. A. Valencia[1], H. Oliva[1], N. Prieto[1], J. C. López[1], G. Monsalve[1,2], A. Ávila[1], V. Akle[1]. 1) Universidad de los Andes, Bogotá, CO; 2) Hospital universitario de la Fundación Santafe de Bogotá, Bogotá, CO.

Neurostimulation is a therapy based in the chemical or electrical stimulation of the brain nuclei, which can generate variations in the production of neurotransmitters. The habenula is part of a conserved brain circuit, which has been considered as a target for the treatment of severe depression. However, studies evaluating the effects of electrical stimulation in the habenula are scarce. The object of the present study is to assess the effect of electrical stimulation of the habenula at different frequencies on the dopaminergic and serotonergic systems in zebrafish. Adult zebrafish were anesthetized, paralyzed and intubated during the deep brain stimulation procedure with frequencies of 20Hz and 130Hz. The stimulation is made with one of two types of microelectrodes: carbon fiber with a diameter of 7±0.6 µm and a resistivity of 2±0.3 10^{-5}Ω m and 30 µm diameter gold wire with a tip of 7±2 µm and a resistivity of 1.6±0.1 10^{-7}Ω m. Gold microelectrodes were fabricated by an electrochemical process in 0.5 M NaCl in DI water and the resistivity was determined by 4-point probe. After the stimulation, the fish are evaluated with immunohistochemistry procedures to identify the changes in the expression of tyrosine hydroxylase and tryptophan hydroxylase, the rate limiting enzymes in the production of dopamine and serotonin, respectively. Also, the receptors zD1 and zSER will be studied across the entire brain. Preliminary results show that zebrafish can be paralyzed at a dose of 1.5µg/g of rocuronium and the effects last for about 4 hours. Fish are anesthetized, paralyzed, intubated using a custom-made system and received a craniotomy of 1mm of diameter above the habenular region. The maximum restrictive amplitude voltage in the zebrafish brain is 4V. This study tests the hypothesis that stimulation of the habenula results in changes in expression on the enzymes and receptors of the serotonergic and dopaminergic systems. Studying the mechanisms of the neurostimulation of the habenula is a step toward understanding the effect of neuromodulator that may be beneficial as an adjuvant therapy against neuropsychiatric disorders.

Z6205A From drug discovery to mechanism: comparison of nicotine-induced locomotor activity in freely swimming and embedded zebrafish larvae. *H. Schneider*, J. M. Abarr, K. Y. Chen, E. E. Clor, B. Edwards, R. Fantus, S. India-Aldana, B. F. Kopecky, R. Miller, C. A. O'Brien, S. Owiredu, M. Pogue, S. Ramayadan, N. J. Snyder. DePauw University, Greencastle, IN.

Potential new pharmacotherapeutics for smoking cessation therapy can be identified in simple neurobehavioral assays using zebrafish larvae. Recording the activity of neural networks or individual neurons in individual larvae could ultimately lead to a better understanding of underlying neuronal mechanisms of drug action thus augmenting drug discovery. Certain adjustments of nicotine dosage and application are required as behavioral experiments on freely swimming larvae are translated into experiments that record nicotine-induced changes of neuronal activity in agarose-embedded larvae. Embedding larvae does not seem to change the overall response to nicotine. However, an increase in locomotor activity occurs with a 10 to 15 min delay after addition of nicotine to embedded larvae compared to the immediate onset in freely swimming larvae. Higher dosage of nicotine used in short behavioral assays of freely swimming larvae, is not suitable for monitoring neural activity in embedded larvae for several hours as nicotine appears to cause irreversible changes in locomotor activity. Lower nicotine dosage allows repeated application of the drug that alternates with "rinse" phases during which locomotor activity can recover. Initial experiments demonstrate that chemicals that reduce the nicotine-induced locomotor activity in freely swimming larvae also reduce recorded muscle activity in embedded larvae. Focal application of chemicals to the head region or injection into the brain can provide further evidence of the specific action of a drug. Establishing nicotine-response assays for embedded zebrafish larvae represents the first step towards measuring nicotine-induced neuroadaptations of larval brain activity using methods such as calcium-imaging.

Z6206B Development of an assay to identify novel modulators of spinal motor activity from the venom of the fish-hunting cone snail *Conus catus*. *J. R. Schulz*, K. Medina, J. Sasaki, N. Leung. Occidental College, Los Angeles, CA.

The zebrafish model is rapidly being developed for use in neurophysiological studies. Here we describe the development of a novel small-

volume assay utilizing larval zebrafish to identify novel modulators of spinal motor activity in parallel. The assay is able to quantify the amount motility as well as distinguish the types of movements made in the absence of supraspinal inputs.

Using this assay we have identified novel modulators of spinal motor activity from the collected venom of the fish-hunting cone snail *Conus catus*. We have also utilized this approach to identify active synthetic neuroexcitatory peptides based on novel sequences identified through deep amplicon sequencing of products specific to the A-superfamily of *Conus* venom peptides. This assay, in conjunction with *in situ* and single cell recordings, extends the utility of the zebrafish model system for venom peptide research and pharmacology. We foresee that the approaches described here will be enhanced by the growing complement of genetic resources available to the zebrafish community.

Z6207C Molecular-genetic analysis of simple decision-making in larval zebrafish. *Hannah Shoenhard*[1], Roshan Jain[2], Marc Wolman[3], Kurt Marsden[1], Michael Granato[1]. 1) University of Pennsylvania; 2) Haverford College, PA; 3) University of Wisconsin-Madison.

Decision-making requires animals to integrate information from the environment with their experiences and their internal state in order to select appropriate behaviors. Although animals continuously make decisions critical for their survival, the genetic and molecular pathways underlying the assembly and function of decision-making circuits in vertebrates are not well understood. We recently developed an assay of larval zebrafish that measures in response to an acoustic stimulus the behavioral choice between a rapid escape behavior and a reorientation-like behavior (Jain et al., in preparation). We will present data demonstrating that this assay fulfills key criteria of decision-making, including dynamic biasing in response to stimulus quality, stimulus history, and internal state.

Using this assay, we conducted a forward genetic screen to identify genes required for this simple decision behavior. This screen yielded nine mutant lines in which decision-making is disrupted. While overall responsiveness is largely unaffected in these mutants, their response bias is shifted such that stimuli that preferentially result in an escape response in wild type instead preferentially elicit a reorientation-like behavior. We will present ongoing efforts to identify the causative mutations, as well as progress in applying whole-brain imaging (Randlett et al. Nature Methods 2015) to identify neurons selectively active during reorientation-like behaviors. Together, these approaches will yield new understanding of how the circuits involved in simple vertebrate decision-making develop and function.

Z6208A Role of Autism Susceptibility gene *Topoisomerase 3B* (*top3b*) in neural and behavioral development in Zebrafish larvae. *Vinoth Sittaramane*, Sydney Doolittle, Danielle Lott, Kori Williams, Paul Lascuni. Georgia Southern University, Statesboro, GA.

Autism Spectrum Disorder is a class of developmental disabilities characterized by social, communication, and behavioral impairments that has a wide range of severity between individuals. Studies have shown these defects stem from abnormal brain development during crucial developmental stages during early development. The underlying genetic cause of these impairments is not well understood but is believed to be a combination of a complex pairing of genetic and environmental factors. Several papers have indicated that topoisomerase 3B (top3b) plays a role in phenotypic defects in mice and fruit flies role indicating a defect in synaptic formation, a key component in developing verbal and social skills. These hindered neural circuits could potentially be the cause of the cognitive deficits characteristic in Autism. Our current research aims to better understand the specific roles of top3b in early neurological development though an *in vivo* study using zebrafish embryos as a vertebrate model system. We used a technique called RNA in situ hybridization and identified the regions of zebrafish nervous system where top3b is present during early neural development. We identified top3b is present in the forebrain, notochord, and visual neural pathways. Further, we used anti-sense nucleotides and created top3b deficiency in zebrafish embryos. Top3b deficient zebrafish embryos revealed defects in spinal motor nerves in the trunk and visual neural pathways in the head. We labeled the motor nerves and their muscle targets (Acetylcholine receptors) using molecular labeling techniques and identified that top3b deficient embryos lack connectivity of nerves and their target muscles. Further, when we re-introduce top3b RNA into deficient embryos, the defects were largely reduced and the embryos develop with normal spinal and visual neural pathways and are able to make connections to their muscle targets. Activation of Acetylcholine receptors have been shown to produce a variety of behavioral and physiological effects such as cognitive performance, vigilance, locomotor activity, respiration, and cortical blood flow. Location of top3b regions and the defect patterns seen in the deficient embryos implicate top3b plays a role in visual neural pathways and neuromuscular connectivity. Developing and analyzing the *top3b* deficient zebrafish model would enable us to use this model to identify potential drug targets against human neurological disorders such as autism.

Z6209B Developing an inducible gene regulation system with spatiotemporal precision at cellular resolution. *Mahendra Wagle*, Zhiqiang Dong, Annam Jayabhargav, Seema Niddapu, Su Guo. University of California San Francisco, San Francisco, CA.

The ability to regulate gene expression with saptiotemporal precision is highly desirable to study various biological phenomena. There has been considerable development in tools and methods available for controlling gene expression in zebrafish. Morpholino knockdown and CRISPR knockouts are most widely used methods for downregulating genes of interest, but have limitations with respect to controlling it to desired cells. RNAi mediated gene knockdown has been successfully demonstrated in zebrafish. We further develop this technique by combining GAL4-UAS and drug inducible TetON system to efficiently knockdown genes of interest. By using tissue specific promoters or available enhancer trap lines to drive Gal4 in specific cell types, it will be possible to restrict gene knockdown in desired cell types at various time points during the development. To achieve such regulation at single cell level, we are currently developing light inducible GAL4 system for gene expression and knockdown.

Z6210C The Role of Non-Neuronal SNAREs on Synaptic Transmission in Zebrafish Hair Cells. *Mike Waltman*[1], Suna Li[2], Katie Kindt[1]. 1) NIH/NIDCD, Bethesda, MD; 2) University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Mechanosensitive hair cells and their associated ribbon synapses are required for hearing and balance. Hair cells have a unique presynaptic

structure called a ribbon body which functions to tether glutamatergic vesicles near adjacent presynaptic L-type calcium channels. The ribbon body is believed to be important for encoding vestibular and auditory information by maintaining fast, sustained exocytosis. Despite their similarity to neuronal synapses, previous work suggests that hair cells do not require the classical neuronal soluble NSF attachment protein receptors (SNAREs) VAMP1 and VAMP2 for vesicle release (Nouvian et al., 2011). Therefore, the SNARE machinery functioning in hair cells is not known.

To determine the SNARE machinery required in hair cells we utilize the genetically tractable zebrafish model. The zebrafish has hair cells in their inner ear as well as in rosettes of hair cells that compose the lateral-line system. The zebrafish is an excellent model to investigate the molecular basis of ribbon synapse function as previous work has shown that ribbon synapses are structurally conserved amongst vertebrates (reviewed in Nicolson., 2015). Due to this level of conservation, combined with CRISPR-Cas9 advances in zebrafish, have enabled us to use the zebrafish to investigate what VAMP family member or members are required for hair-cell exocytosis.

We are currently examining CRISPR-Cas9 generated knockouts of the v-SNAREs (vesicle SNAREs/VAMPs). We are currently screening all VAMP family members. As of yet, no single VAMP has been directly linked to a complete loss of exocytosis or endocytosis in the hair cell. This suggests that no single VAMP subfamily plays a role in hair-cell synaptic transmission, but may require redundant VAMPS or an interaction with the complimentary subfamily of SNARES: t-SNARES (target SNAREs/SNAPs). We are exploring both possibilities now and continue to examine our current mutant *vamp* strains.

Lastly, in order to quantify alterations to synaptic vesicle release between wildtype and mutant strains, we are utilizing a transgenic line that expresses synaptophluorin in hair cells to measure vesicle release using swept-field confocal microscopy. In the future we plan on expanding our analysis to include electrophysiological studies in order to further examine alterations to neuronal function based on deficiencies in hair-cell release. An update of this research will be presented.

Z6211A Roles of *per1b*, *per2* and *rev-erbα* in zebrafish circadian behaviors. *H. Wang*[1]. 1) Center for Circadian Clocks, Soochow University, Suzhou, Jiangsu, China; 2) School of Biology & Basic Medical Sciences, Medical College, Soochow University, Suzhou 215123, Jiangsu, China.

Zebrafish are an important model for investigating vertebrate circadian clocks. However, scarce of zebrafish genetic circadian mutants has hindered further advancement of the zebrafish circadian field. We set out to dissect molecular genetic mechanisms underlying zebrafish circadian clocks. Using retroviral insertional mutagenesis, TALEN or CRISPR-Cas9, we have generated a library of zebrafish circadian mutants. Here we report characterization of zebrafish *per1b*, *per2* and *rev-erbα* mutants. Behavioral assays showed that under light/dark (LD) condition, *per1b* mutant larvae are approximately three times more active than their wild-type counterparts and the amplitude of *rev-erbα* mutants increases more than 40% during the whole duration of a 24-hour day and night, while *per2*mutant larvae have approximately 30% reduced activity amplitude in comparison with wild types; under constant darkness (DD) condition, locomotor activity of *per1b* mutant larvae is phase advanced by two hours, while locomotor activity of *per2* mutant larvae is phase delayed by two hours, and *rev-erbα* mutants display 0.5-hour phase delay and 0.25-hour shortened period. Under DD condition, *per1a*, *per2* and *per3* were all significantly up-regulated in *per1b* mutants, indicating that Per1b serves as a negative regulator in the zebrafish circadian system. Under LD condition, *bmal1a* and *annat2* were significantly up-regulated but *per1a*, *per1b* and *bmal1b* were significantly down-regulated in the *per2* mutant fish. *In Vitro* cell transfection assays showed that Per2 not only acts through E-boxes to repress E-Box-containing genes but also acts through RRE-boxes to activate RRE-box-containing genes. Under DD condition, both *bmal1b* and *bmal2* are significantly up-regulated in *rev-erbα* mutant larvae. We also found that zebrafish *per1b* mutants display hyperactive-, impulsive-, and attention deficit-like behaviors and low levels of dopamine, reminiscent of human Attention Deficit Hyperactivity Disorder (ADHD) patients. Rev-erbα modulates autophagy rhythms directly via controlling expression of autophagy and autophagy-related genes, and also plays regulatory roles in stress response, metabolism, signal transduction and posttranslational modification. Taken together, our findings demonstrate that Per1b, Per2 and Rev-erbα are essential for the zebrafish circadian clock and also play regulatory roles in numerous fundamental life processes, and highlight zebrafish as an attractive model for investigating vertebrate circadian rhythmicity.

Z6212B Retinal patterning and saccadic eye movements in Zebrafish require Down Syndrome Cell Adhesion Molecule-Like 1. *Tong Wang*[1], Manxiu Ma[1], Avirale Sharma[1], Christopher Kuang[1,7], Rachel Roberts[1,8], James A. Gagnon[2], Andrea Pauli[2], Steve Zimmerman[2], Shengdar Q. Tsai[3,4], Deepak Reyon[3,4], J. Keith Joung[3,4,5], Alexander F. Schier[2], Y. Albert Pan[1,6]. 1) Department of Neuroscience and Regenerative Medicine, Medical College of Georgia, Augusta Univ., Augusta, GA; 2) Department of Molecular and Cellular Biology, Harvard Stem Cell Institute, Center for Brain Science, Harvard Univ., Cambridge, MA; 3) Molecular Pathology Unit, Center for Computational and Integrative Biology, and Center for Cancer Research, Massachusetts General Hospital, Charlestown, MA; 4) Department of Pathology, Harvard Medical School, Boston, MA; 5) The Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA; 6) Department of Neurology, James & Jean Culver Vision Discovery Institute, Medical College of Georgia, Augusta Univ., Augusta, GA; 7) Medical Scholars Program; 8) Graduate Program in Neuroscience.

Down syndrome cell adhesion molecule (Dscam) family genes play diverse roles across many species. The *Drosophila dscam* is alternatively spliced and serves as intra- and intercellular recognition cues for repulsive interactions between axons and dendrites. In mice and chicken, *Dscam* and *Dscam-like 1* (*Dscaml1)* are involved in promoting cell death, maintain cellular spacing, and laminar targeting of neurites in the developing visual system. The functional and behavioral importance of *Dscam* and *Dscaml1*, however, are less clear. In this study, we generated a TALEN-mediated *dscaml1* mutant in Zebrafish (*Danio rerio*) and investigated how loss of *dscaml1* affects visual system development and visual behaviors. The small size, translucent brain, powerful genetics, and robust visual behaviors of zebrafish make it an ideal vertebrate model system. Consistent with *dscaml1*'s function in mammals, we found significant aggregation and misplacement of retinal cell types, particularly the serotonergic amacrine cells and muller glia. The mutant brain was narrower, with increased neuropil thickness and

reduced width. Despite these abnormalities, *dscaml1* have normal light-on and light-off induced locomotor response and can respond to moving vertical bars while performing the optokinetic response (OKR), suggesting that perception of visual stimulus is grossly normal, though light sensitivity is reduced. However, when challenged with a faster or more prolonged moving stimulus, the *dscaml1* mutants display severe deficits in saccadic (fast-resetting) eye movements and the eyes eventually become fixated at eccentric positions. These results suggest that *dscaml1* is required for the development or function of the motor systems downstream of saccade generation centers. Work is currently in progress to investigate the neural correlates of the saccade deficits and the temporal requirements of *dscaml1*.

Z6213C Comparative analysis of cart peptide expression and function. *Ian Woods*, Emily Conklin, KathyAnn Lee, Haley Coleman, Andrew Rodenhouse, Anastasia Koulopoulos. Ithaca College, Ithaca, NY.

Neuropeptidergic modulation of neuronal signaling facilitates context-dependent differences in neuronal circuit activity, and can thus generate profound differences in behavior. We study Cart (Cocaine- and Amphetamine-Regulated Transcript), a neuropeptide with proposed roles in the regulation of several behaviors, including arousal, anxiety, appetite, and reward. In mammals, a single Cart gene exhibits complex expression in many brain regions. In contrast, Cart is encoded in zebrafish by seven separate genes, each with a relatively simple expression pattern. The genetic tractability of zebrafish, along with its optical transparency and wide range of experimentally validated behavioral assays, provides an unparalleled system to link functions of Cart in specific brain regions with specific behaviors. First, we are pinpointing regions of Cart expression in zebrafish larvae via colocalization with markers of conserved neurotransmitter and peptidergic systems. Second, we are working to elucidate the development and targeting of Cart neurons using transgenic larvae that express GFP driven by cis-regulatory sequences of each Cart gene. Third, we are working to analyze the behavioral functions of each zebrafish Cart gene. Specifically, we have generated fish with loss-of-function mutations in four Cart genes, and transgenic lines that enable inducible overexpression of four Cart genes. Analogous reagents for the other Carts are in progress. We and others have established behavioral assays that are especially suited for analyzing behaviors relevant to Cart function, including arousal, sensory responsiveness, anxiety, and appetite. Hierarchical clustering of behavioral outcomes will elucidate the roles of each Cart gene in regulating specific behaviors. Finally, to explore the significant difference in Cart peptide family representation between species, we are using computational tools to model patterns of gene gain and loss in all neuropeptide families across a panel of vertebrate species.

Z6214A Origin and Functional Heterogeneity of Zebrafish Lateral-line Hair Cells. *Q. Zhang*, C. Wong, J. He, K. Kindt. National Institutes of Health, NIDCD, Bethesda, MD.

Hair cells are the sensory receptors required for proper hearing and balance. In hair cells, function is shaped by summation of local calcium signals that ultimately trigger vesicle release in order to encode a sensory stimulus. We studied calcium signals and vesicle release in neuromast hair cells of the lateral-line system. We observed that calcium signals were extremely heterogeneous within a neuromast cluster, with a subset showing a more robust and persistent calcium influx. It was only in this later subset of hair cells that we observed vesicle release. The purpose of this study is to understand the basis for this functional heterogeneity and to understand why a sensory organ would be organized to function in this manner.

We have developed several transgenic lines that express calcium indicators (GCaMP6s, GCaMP3, RGECO or GGECO) that allow us to stimulate and monitor calcium signals in hair cells or postsynaptic afferents. In addition, we use SypHy, an indicator to monitor hair-cell vesicle release. Overall, this is a very powerful system, where we can continuously record larvae over multiple days to understand how activity of a neuromast or a single hair cell changes over time or during development. This long-term functional imaging, combined with mutant analysis and pharmacology have allowed us to better understand the basis of hair-cell functional heterogeneity.

Our long term imaging experiments indicate that hair cells located around the periphery of the neuromast are most likely to have robust calcium signals and release vesicles. The periphery of the neuromast is where new hair cells are generated. Our data shows that as hair cells mature at the periphery, they have robust calcium signals. Whether they continue to release vesicles ultimately depends on location within the neuromast and whether additional cells are added to the neuromast. Damage to hair cells with robust calcium signal enables other hair cells to release vesicles, indicating all hair cells are primed and competent to encode stimuli. Using genetics, we have found that the functional heterogeneity of hair cells does not result from afferent or efferent modulation. Lastly, our pharmacological results suggest that instead, connections between hair cells or supporting cells may facilitate functional heterogeneity. From these results we hypothesize that not all hair cells may be required to encode sensory signals. In addition, the heterogeneity within the neuromast may play an important role in development and maintaining a healthy balance for encoding sensory information.

Z6215B Pharmacological reprogramming of lateral line neuromast support cells to a migratory progenitor state. *Paige Brooks*, Anastasiya Yandulskaya, Hannah Akre, Jason Meyers. Colgate University, Hamilton, NY.

The lateral line is a collection of small sensory organs called neuromasts that run along the body of fish and amphibians, and consists of sensory hair cells homologous to the hair cells within the inner ear. Each neuromast consists of sensory hair cells surrounded by support cells with a ring of mantle cells at the outer edge of the neuromast. Between 24-48 hours of development, protoneuromasts are deposited by a migratory primordium with Wnt positive mesenchymal-like cells proliferating at the leading edge and FGF positive cells forming into epithelial rosettes at the depositing end. Although the role of Wnt and FGF signaling has been well studied in the migratory primordium, the on-going roles of those signals in the deposited neuromasts is less clear. Given this, and the interaction between Wnt and FGF signaling in initial patterning of the primordium and protoneuromast deposition, we have examined the effects of Wnt and FGF manipulation to neuromasts after the migratory primordium has completed neuromast deposition. When we pharmacologically block FGF signaling, the neuromasts become

elongated as mantle cells begin to stream away from the neuromasts anteriorly and posteriorly. During FGF inhibition, the mantle cells begin expressing Wnt reporter genes, suggesting that one of the functions of FGF might be to limit Wnt signaling in deposited neuromasts. The upregulation of Wnt signaling in the mantle cells paired with the migration of these mantle cells along the horizontal midline suggests that Wnt signaling in the absence of FGF signaling promotes a migratory state similar to that in the leading edge of the migratory primordium. Consistent with this, when we couple the inhibition of FGF signaling with a pharmacological activation of Wnt signaling, driving Wnt reporter expression in all cells of the deposited neuromasts, all of the supporting cells adopt a migratory mesenchymal-like fate, leading to dissolution of the neuromasts and streaming of the cells both anteriorly and posteriorly along the lateral midline. This reversion of the epithelial cells to a migratory state is coupled with loss of cadherin expression and flattening of the cells. Remarkably, following washout of the drugs, the migratory cells re-epithelialize and reconstitute neuromasts. Many more neuromasts reform after washout than were present initially, and each reconstituted neuromast reforms sensory hair cells. We also show that these signals are utilized during neuromast regeneration following tail amputation to promote migration and reformation of neuromasts. Together, these data suggest that Wnt signaling may promote a reversion of supporting and mantle cells to a migratory state capable of reconstituting full neuromasts, but that this is normally repressed by FGF signaling working to maintain the cells in an epithelial state.

Z6216C Regulation of neural stem cell division modes in the developing zebrafish brain. *R. Choi*, X. Zhao, Z. Dong, S. Guo. University of California San Francisco, San Francisco, CA.
Zhao X[1,2] Choi R[1,2] Dong Z[1], Guo S[1*]
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Asymmetric cell division (ACD) is a conserved and fundamental process in neurogenesis for generating cellular diversity across both invertebrates and vertebrates. As the principal neural stem cells (NSCs), radial glia progenitors (RGP) undergo ACD to generate self-renewing and differentiating daughter cells in the developing central nervous system. Until now, the cellular and molecular mechanisms of ACD and subsequent daughter fate choice is not well understood in vertebrates.
By using zebrafish, we have identified that the cortical polarity regulator Partitioning defective protein-3 (Par-3) plays a crucial role in the establishment of ACD through localizing the ubiquitin E3 ligase Mindbomb (Mib), which activates Notch by ubiquitinating the Notch ligand unequally in the apical daughter. Using immunocytochemistry, in vivo imaging, and other molecular genetic and biochemical methods, we are elucidating the nature of Mib asymmetry and the underlying mechanisms that orchestrate such asymmetry.

Z6217A Hereditary cerebellar ataxia and the role of CAMTA1, a zebrafish study. *c. cianciolo cosentino*[1], p. ender[1], j. loffing[2], s. neuhauss[1]. 1) Institute of Molecular Life Sciences, University of Zurich, Zurich, CH; 2) Institute of Anatomy, University of Zurich, Zurich CH.
Hereditary cerebellar ataxias are a wide and heterogeneous group of neurological disorders characterized by motor incoordination, instability and developmental delay, with cerebellar neuron degeneration as a common hallmark. The genetic cause and the underlying pathophysiological mechanisms for many forms of ataxias are still unclear and, currently, there are no effective therapies. Recent studies have shown that loss-of-function mutations in the calmodulin-binding transcription activator 1 (CAMTA1), a calcium responsive transcription factor, are associated with childhood cerebellar ataxia and intellectual deficiency in humans. Zebrafish have been successfully used to model a range of neurological and behavioral abnormalities. In an attempt to establish a zebrafish model of hereditary cerebellar ataxia, we characterized the zebrafish CAMTA1 orthologue *camta1a* and its transcription pattern, and by making use of morpholino-mediated gene inactivation and the CRISPR/Cas9 genome editing tools, we analyzed the phenotype induced following zebrafish *camta1a* depletion. The amino acid sequence of *camta1a* display a 73% overall similarity with the human protein, and, like in mice and humans, expression analyses showed brain-enriched expression, with highest levels of transcript in the cerebellar granule cells and Purkinje cells. The injection of *camta1a* antisense morpholino oligonucleotides recapitulated salient features of ataxia. In particular, *camta1a* morphants presented abnormal movement pattern, with increased spontaneous contractions and shorter swimming path. Moreover, *camta1a* MO larvae showed a loss of Purkinje neurons. To confirm the phenotype obtained with *camta1a* knock-down experiments, we have generated *camta1a* genetic mutants by CRISPR/Cas9 genome editing, targeting the DNA binding domain of the protein. The analysis of *camta1a* homozygous mutant zebrafish (camta1a $^{-/-}$) is under way, together with the identification and characterization of genes transcriptionally regulated by *camta1a* in the cerebellum. Our results so far validate zebrafish as a valuable model organism in which to investigate the molecular mechanism leading to the cerebellar neuron degeneration responsible for the ataxic phenotype in humans with CAMTA1 mutation.

Z6218B Screening for genetic interactions in the blood-brain barrier *in vivo* in the zebrafish, *Danio rerio*. *Thomas Clements*[1], Shinya Hirota[2], Sam Kwiatkowski[2], Cecilia Guerra[1], Aryeh Warmflash[1], Joseph McCarty[2], Daniel Wagner[1]. 1) Rice University, Houston, TX; 2) University of Texas M. D. Anderson Cancer Center, Houston, TX.
In the blood-brain barrier (BBB), glial cells interact with vascular endothelial cells (ECs) to control blood vessel development and formation.

However, the molecular mechanisms of these interactions remain mostly uncharacterized. In order to screen for genetic interactions amongst genes of interest, we used MOs to repress the translation of the zebrafish homologs of these genes *in vivo.* Morpholinos (MOs) are an established reverse genetic technique that prevents splicing of pre-RNA or translation of mRNA by binding to complementary regions of RNA *in vivo*. Using MOs allows us to titrate the single doses down to the lowest effective levels and then screen combinations for more drastic effects, which cannot be done using traditional gene knockout methods. The zebrafish is ideal for studying genetic interactions in the blood-brain barrier because fertilization is external and the embryo is transparent, thus allowing for easy visualization of neural development.

It is hypothesized that an uncharacterized signaling network controls gene expression to regulate cerebral blood vessel development mediated by *αVβ8* integrin-activated (*Igtβ8, IgtαV)* TGF-*β*s. Thus, it is our goal is to confirm crosstalk and coordinated regulation of specific genes in this pathway using conditional systems in mice (done by our collaborator), MO as well as CRISPR-Cas knockouts in zebrafish, and the use of endothelial specific Smad-4 GFP transgenic line. Here we report a synergistic relationship between *Igtβ8* and Neuropilin1a (Nrp1a) *in vivo* as ascertained through the use of MOs, which was done by quantifying loss of transcript through observable brain hemorrhages and RT PCR.

Z6219C Molecular mechanisms of Schwann cell development and function in the peripheral nerve system. *M. R. D'Rozario*[1], S. D. Ackerman[1], A. Mogha[1], S. Giera[2], R. Luo[2], X. Piao[2], K. R. Monk[1]. 1) Washington University School of Medicine, St. Louis, MO; 2) Boston Children's Hospital and Harvard Medical School, Boston, MA.

Schwann cells (SCs) are the myelinating glia of the peripheral nervous system (PNS) that associate with and wrap around axons to form the insulating myelin sheath. During development, immature SCs select appropriately sized axons (a process termed radial sorting) and repeatedly wrap their membranes around their selected axon. The importance of SC myelin is best underscored in diseases in which SC dysfunction leads to peripheral neuropathies such as Charcot-Marie-Tooth Disease; importantly, defects in radial sorting are frequently observed in human PNS disease. Advances in identifying growth factors and signaling molecules expressed by SCs reveal the myriad roles of glial cells in the nervous system and their interaction with each other, neurons, and the surrounding extracellular matrix (ECM). We have previously identified the adhesion G protein-coupled receptor (aGPCR) GPR56/ADGRG1 as a critical regulator of PNS myelination. Loss of *Gpr56* function in zebrafish and mouse mutants results in delayed radial sorting. Consistent with the ability of aGPCRs to transduce extracellular stimuli into intracellular signals, we demonstrate that GPR56 signals through RhoA to promote SC radial sorting prior to myelination. Despite delays during early development, radial sorting in *Gpr56* mutants recovers with time. However, the mature myelin ultrastructure is disorganized and we observe increased outfoldings with age. In spite of these key functions, it is unclear what developmental cues activate GPR56 in the PNS. In other contexts, GPR56 has two known binding partners, the ECM proteins collagen III and tissue transglutaminase 2, and our efforts to define the roles of these potential GPR56 interactors in the PNS will be discussed. Interestingly, however, an unbiased proteomics screen has identified additional candidate binding partners for GPR56 in the PNS. Currently, we are using CRISPR/Cas9-mediated genome editing to generate zebrafish mutants for these candidate genes in order to define their role in radial sorting as well as perform genetic interaction studies to test whether these mutants enhance *gpr56*$^{stl13/stl13}$ phenotype. Together, our studies define the role of GPR56 PNS development, and our work has important implications for human neuropathies and myelin diseases.

Z6220A Genetic regulation of photoreceptor specification in zebrafish as a model for understading photoreceptor variation in diurnal species. *J. M. Fadool*, M. Sotolongo-Lopez. Florida State University, Tallahassee, FL.

The visual system is highly adapted to convey detailed ecological and behavioral information essential for survival of a species. The retinas of the majority of vertebrates are dominated by 4 cone subtypes sensitive to UV, blue, green and red wavelengths of light. However, much of our understanding of photoreceptor development is based on findings from mammalian models which are highly adapted to dim light conditions with rods vastly outnumbering the sparse and less diverse cone subtypes and thus fail to explain the tremendous diversity of cone subtypes and variation of rod and cone numbers in many species. The spatial patterning of zebrafish photoreceptors combined with classical genetics and emerging gene-targeting technologies, offer unprecedented opportunities to investigate photoreceptor biology. In the larval zebrafish retina the four cone subtypes grossly outnumber the far fewer, assymetrically distributed rods. Previously, we showed that loss-of-function and hypomorphic alleles of *tbx2b* result in an increase in the number of rods across the retina and a decrease in the number of UV-sensitive cones. Subsequently we showed that knockdown of *six7* results in a nearly identical rod phenotype, and knockout alleles result in the additional loss of the green-sensitive cone subtype. We report that hypomorphic alleles of *six7* and *tbx2b* are additive in their effects upon the number of rods. In wildtype larvae, cones outnumber rods 20:1, but in double mutants, the rods account for approximately 40% of the total number of photoreceptors. Furthermore, spatial pattern analysis indicates that the rods are uniformly distributed across the mutant retinas. Genome sequence analysis of the hypomorphic allele identified the deletion of a putative *cis*-regulatory module that may underlie the photoreceptor alterations. Lastly, additional gene knockdown approaches show the potential of epistatic interactions to drive further changes in rod and cone ratios. These data demonstrate the effects of subtle alteration of gene expression upon photoreceptor patterning, and the potentail for alterations in the gene-regulatory network as a driving forces underlying variation in rod and cone numbers across species.

Z6221B A Novel Developmental Requirement for NMDA Receptors in Axon Guidance is Disrupted by Hypoxic Injury. *J. Gao*, T. Stevenson, J. Bonkowsky. Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT.

Hypoxic injury in the developing human brain is a major cause of chronic neurodevelopmental impairments in part through loss of normal connectivity although the mechanisms are poorly understood. Here we found that hypoxic injury down-regulates N-methyl-D-aspartate receptors (NMDAR) expression in the developing brain. NMDARs are glutamate-gated heteromeric ion channels widely expressed in the CNS

that play key roles in excitatory synaptic transmission in the adult brain and in synapse stabilization. We found that commissural pathfinding is disrupted by pharmacological inhibition of NMDARs. We demonstrate that the NMDAR NR1 subunit is expressed in commissural axons, and that *vglut1*, the biosynthesis enzyme for glutamate, is expressed in neurons adjacent to the commissural axons. We further show that NMDAR agonist can rescue the hypoxic-induced commissural neuron pathfinding defects. In summary, we report an unexpected developmental role for NMDARs in axon pathfinding, and show that disruption of normal NMDAR function by hypoxia contributes to connectivity disruption.

Z6222C Zebrafish Rfx4 is required for neural tube morphogenesis. *Y. Grinblat*, I. Sedykh, A. Keller. Univ Wisconsin, Madison, WI.

Rfx family of wing-helix transcription factors are key regulators of the core ciliogenesis program in vertebrates. Recently, Rfx genes have been shown to function independently of ciliogenesis, e.g. during brain ventricle morphogenesis. To better understand the mechanism of Rfx gene function during neural tube development, we have generated frame-shift alleles in the zebrafish *rfx4* locus using CRISPR/Cas9. Rfx4 mutant embryos develop with a curvature characteristic of disrupted ciliogenesis, as do Rfx4 morphants. However, transcriptome analysis of Rfx4 mutant vs sibling embryos suggests that the primary role of Rfx4 is to promote expression of neural tube roof plate markers, e.g. *zic2a*, and floor plate markers, e.g. *fox2a*. Further, transcriptome analysis suggests that zebrafish Rfx4 is essential for Hh signaling but does not play a major role in ciliary gene transcription during embryogenesis. These findings are consistent with a recent mouse study that identified a cilia-independent role for Rfx4 as a transcriptional regulator of *foxa2 i*n the floor plate and *zic2* in the roof plate. Notably, curvature reminiscent of the *rfx4* phenotype has been reported in *foxa2/monorail* mutant zebrafish. Collectively, these data shed light on an important, yet incompletely understood conserved node in the gene regulatory network that controls patterning and morphogenesis of the neural tube.

Z6223A Distinct roles for the adhesion molecule Contactin2 in the development and function of neural circuits in zebrafish. *S. Gurung*, E. Asante, A. Chandrasekhar. University of Missouri, Columbia, MO.

During development, newborn neurons frequently migrate before assembling into neural networks that control complex motor and cognitive functions. In addition, neuronal growth cones must mediate precise axon pathfinding to generate functional neural circuits. Cell adhesion molecules play important roles during development, including in neuronal migration and axon guidance. Contactin2 (Cntn2)/Transiently-expressed Glycoprotein 1 (Tag1), a glycosylphosphatidylinositol anchored cell adhesion molecule of the immunoglobulin superfamily, is expressed in specific neuronal types during vertebrate nervous system development. In zebrafish, the facial branchiomotor (FBM) neurons, a subset of the hindbrain motor neurons, express *cntn2* while migrating caudally from rhombomere 4 to rhombomeres 6 and 7. Our previous studies using morpholinos suggested that *cntn2* is necessary for FBM neuron migration, and that it genetically interacts with the planar cell polarity gene *vangl2* during FBM neuron migration. To validate these data, we generated loss-of-function mutations in *cntn2* using CRISPR/Cas9, and identified three alleles including two frameshift mutations generating premature stop codons (*zou20* and *zou22*) that exhibited identical phenotypes. *cntn2* expression was reduced in $cntn2^{zou20}$ *and* $cntn2^{zou22}$ homozygotes, and Cntn2 protein was undetectable in both cases, suggesting these identified alleles are null. In contrast to the morphant phenotype, zygotic and maternal-zygotic (MZ) $cntn2^{-/-}$ mutants exhibited normal FBM neuron migration, suggesting either that the morphant phenotype may be non-specific or that there is genetic compensation from related genes in MZ mutants. Consistent with the latter, FBM neurons failed to migrate caudally in a significant fraction of $cntn2^{+/-}$*; vangl2*$^{+/-}$ embryos obtained from $cntn2^{-/-}$ mothers, supporting the genetic interaction observed using morpholinos.

Previous studies showed that morpholino-mediated *cntn2* knockdown generated defasiculation defects in midbrain nucMLF and spinal cord Rohon-Beard (RB) axons. However, MZ $cntn2^{-/-}$ mutants exhibited no nucMLF defects. To assay for defects in the sensorimotor circuits of RB neurons in *cntn2* mutants, touch-evoked escape responses was evaluated. Wild type and mutants larvae exhibited normal escape responses when touched on the head. In contrast, mutants were significantly less responsive when touched in the trunk consistent with putative defects in RB circuits. Together, these data support distinct roles for Cntn2 in the development and function of neural circuits in zebrafish.

Z6224B Before Neural Circuit Formation: A Role for Semaphorins on Retinal Progenitor Cells. *R. Halabi*[1,2,4], S. McFarlane[1,2,3,4]. 1) Hotchkiss Brain Institute; 2) Department of Neuroscience; 3) Department of Cell Biology and Anatomy; 4) University of Calgary, Calgary AB Canada.

Coloboma is a congenital ocular malformation caused by the failure of the choroid fissure to close during development. Although mutations in a number of genes have been identified, the cellular and molecular mechanisms in the pathogenesis of the disease remain poorly understood. Here we identify the secreted guidance molecule, Semaphorin3f (Sema3f), as a novel factor involved in retinal development. We used embryonic transgenic (Tg) zebrafish injected with antisense morpholino or CRISPR interference RNA to block Sema3fa production, and analyzed embryos throughout retinal development. Histological sections, in situ hybridization for retinal polarity markers and analysis of the retinal ganglion cells (RGCs) using the Tg(isl2b::GFP) reporter line were used to characterize the Sema3fa deficient embryos. We find that *sema3fa* is expressed around the choroid fissure and temporal retina prior to fissure closure and RGC axonal exit in wildtype embryos. Sema3fa deficient embryos present with coloboma of the eye at 48 hours post fertilization, defective patterns in the expression of retina polarity markers, and proliferation deficits in the temporal retina. During RGC axonogenesis, embryos present with aberrant axonal sprouts into the neural retina from the ganglion cell layer and optic nerve. Overall, these data suggest Sema3f as a novel factor involved in eye and choroid fissure morphogenesis, progenitor behaviour and neural retina development.

Z6225C *actr10* is a regulator of myelinating glial cell development. *A. L. Herbert*[1], K. Drerup[2], B. L. Harty[1], S. D. Ackerman[1], R. S. Gray[3], T. O'Reilly-Pol[1], S. L. Johnson[1], A. Nechiporuk[2], K. R. Monk[1]. 1) Washington University in St. Louis, St. Louis, MI; 2) Oregon Health and Science University, Portland, OR; 3) University of Texas at Austin, Austin, TX.

Myelin is the lipid rich sheath that surrounds axons and promotes rapid action potential propagation in the vertebrate nervous system.

Myelin is made by specialized glial cells called oligodendrocytes (OLs) in the central nervous system (CNS) and Schwann Cells (SCs) in the peripheral nervous system (PNS). Loss of or disruption to the myelin sheath can result in debilitating human conditions including multiple sclerosis and Charcot-Marie-Tooth disease, and understanding glial cell development is critical for development of therapies to treat myelin diseases. Our lab therefore performed a large-scale forward genetic screen in zebrafish to look for novel regulators of myelination and identified a mutant, *stl83*, that exhibits both axon and myelin defects. Using whole genome sequencing, it was determined that the *stl83* phenotype results from a missense mutation in the gene *actr10,* which encodes the protein Arp11. This was confirmed by a complementation cross using a previously identified mutant *actr10* allele, *llg22*, which results in a point mutation in the start site. Arp11 is a component of the dynactin complex, which is necessary for retrograde transport by the molecular motor dynein. *In-situ* hybridization (ISH) for *myelin basic protein* (*mbp*) mRNA to mark mature glial cells showed that both $actr10^{stl83/stl83}$ and $actr10^{llg22/llg22}$ mutants have drastically reduced *mbp* expression in the CNS and PNS at 5 dpf. Transmission electron microscopy analysis revealed that many fewer axons are myelinated in the CNS and PNS of both mutants. In addition, those axons that are myelinated have a thinner myelin sheath. We are currently teasing out the mechanism resulting in reduced *mbp* expression and transport by marker analysis for different molecular makers of oligodendrocyte and Schwann cell development, cell death and cell proliferation assays, live imaging using fluorescent transgenic reporter lines, and chimera analysis to determine autonomy of the myelin defects.

Z6226A *ZC4H2*, an XLID gene, is required for the generation of GABAergic interneurons. *K. Hwang*, T. Choi, M. Thiruvarangan, S. Lee, S. Hyung, H. Hwang, K. Lee, C. Kim. Chungnam National University, Daejeon, KR.

X-linked intellectual disability (XLID) is a well-known form of intellectual disability which is specifically associated with X-linked recessive inheritance. To investigate the function of XLID gene, *ZC4H2*, we generated zebrafish mutant for *ZC4H2* homologue gene using TALEN methodology. Zebrafish *zc4h2* homozygous mutant larvae exhibited abnormal swimming, stationary eye movement, and hyperactivity of jaw and pectoral fin. Because of the behavioral defects were consistent with hyperactivity, we focused on underlying neuronal defects. Through various molecular markers, we observed a striking reduction in GABAergic interneurons. Analysis of cell type specific markers showed a specific loss of V2 interneurons in the midbrain tegmentum and spinal cord. Also, we found that loss of Zc4h2 function affects in misexpression of *dbx2*/*nkx6.1* boundary. To restore the Zc4h2 function in the mutant, we microinjected human and zebrafish mRNAs in *zc4h2* mutant embryos, and examined *gad1b*-positive neurons. As a result, human and zebrafish mRNAs rescued the mutant phenotype. However, mutant zebrafish microinjected with human p.L66H or p.R213W mRNA failed to be rescued, while the p.R18K mRNA was able to rescue the interneuron defect. In this studies clearly support *ZC4H2* as a novel XLID gene with a required function in V2 interneuron development. Loss of function of ZC4H2 thus likely results in altered connectivity of many brain and spinal circuits.

Z6227B Goosecoid regulates a Spemann organizer-like function for neurogenesis in the inner ear. *H. Kantarci*, A. Gerberding, B. Riley. Texas A&M University, College Station, TX.

In all vertebrates hearing and balance related information is transmitted from the ear to the brain by the neurons of the Statoacoustic Ganglion (SAG). SAG neuroblasts originate in the inner ear epithelium, undergo epithelial-mesenchymal transition, then leave and migrate to a region between the ear and the hindbrain to subsequently differentiate into mature neurons. Locally expressed Fgf sources initiate cell fate specification of SAG neuroblasts by inducing expression of Ngn1 in the ear epithelium. However, there are no proposed mechanisms to explain how transition to mesenchymal state is induced during SAG development. Here, we report Goosecoid (Gsc) as a key regulator of this process, a gene famous for inducing the formation of the Spemann organizer and initiating the migratory movements that define the future embryonic axes. In zebrafish, Fgf signaling co-regulates expression of *gsc* and *ngn1* in partially overlapping domains. Loss of Gsc function impairs the ability of SAG neuroblasts to leave the ear and results in a significant loss of mature SAG neurons, whereas overexpression of Gsc increases the number of mesenchymal cells. Our analysis also reveals that Gsc induces transition to mesenchymal state without affecting the size of the neurogenic domain in the inner ear. Thus, Gsc and Ngn1 regulate epithelial-mesenchymal transition and neuronal specification as two distinct outputs of Fgf signaling. Furthermore, we discovered that medially expressed transcription factor Pax2a completely blocks Gsc function in the non-neurogenic regions of the developing inner ear, spatially restricting epithelial-mesenchymal transition to ventrolateral regions. The role of Gsc in SAG neurogenesis, together with its regulation of cell movement in the Spemann organizer and promotion of tumor metastasis indicates that Gsc might have a more widespread role in epithelial-mesenchymal dynamics than previously thought.

Z6228C A zebrafish model of vanishing white matter disease. *Matthew D. Keefe*. Department of Neurobiology, University of Utah, Salt Lake City, UT.

Vanishing white matter disease (VWMD) is an inherited leukodystrophy, causing CNS demyelination and high rates of morbidity and mortality. VWMD is autosomal recessive, and is caused by mutations in the five subunits of the eukaryotic translation initiation factor 2B (eIF2B) complex. eIF2B is the guanine nucleotide exchange factor for eIF2, and together they govern the rate of global protein synthesis. VWMD is characterized by reduced numbers of oligodendrocytes and astrocytes, as well as the progressive loss of myelinated axons. How mutations in eIF2B, a complex universally required for protein synthesis, disproportionately affects oligodendrocytes and astrocytes is not known. In yeast, null mutations in *eIF2B(2-5)* are non-viable which may indicate why in humans, mutations that prevent the expression of full-length *EIF2B* subunits have only been reported in the compound heterozygous state with a missense mutation as the second mutation. Because null mutations are most likely lethal in humans, this will limit the severity of phenotypes observed in individuals that survive to birth, making it difficult to determine the effects of *EIF2B* mutations in development. Importantly, mutations that severely compromise the function of eIF2B have not been studied in a vertebrate model system. The goal of our study is to develop a model of VWMD in zebrafish that

recapitulates the clinical features of the disease, and to discover developmental defects associated with impaired function of eIF2B.

We have generated mutant alleles in the zebrafish *eif2b* subunits 2 and 5. Our characterization of these mutants has revealed a diversity of phenotypes that range in phenotypic severity from mild growth defects to early lethality. The *eif2b5* alleles alter somatic growth rates in development, while only some alleles impact survival. To date, a point mutation in *eif2b2* is the most severe allele characterized. *eif2b2* mutants appear to develop normally, but by 5dpf are smaller in size compared to their siblings and display increased apoptosis in the fore-and-mid-brain, followed by early lethality by 10dpf. Our current study represents an essential step in the study of eIF2B related leukodystrophies, specifically by testing the function of several *eif2b* alleles in a vertebrate model organism. The genetic and developmental toolbox in the zebrafish field makes it a good candidate to develop disease models for the study of how developmental defects impact disease progression.

Z6229A Development of a Novel Pharmacological Model of Okadaic Acid-induced Alzheimer's Disease in Zebrafish. *D. Koehler*, F. Williams, Z. Shah. University of Toledo, Toledo, OH.

The present study aimed at establishing a new pharmacologically-induced Alzheimer's (AD) model in zebrafish by administering okadaic acid (OKA). This model involves most of the pathophysiological conditions predominant in AD while also cutting down time constraints and cost which hinder previously established AD models. Zebrafish were divided into 5 groups containing 5-6 fish per group. Each group was exposed to a respective concentration of OKA of 10nM, 100nM, 500nM, and 1µM along with a control group. Each exposure lasted for 9 days with a learning and memory test performed at the end. Fish were tested individually in a spatial alternation task in order to assess learning and memory capabilities. Fish were placed in a 10 L aquarium that was divided into two equal sections by a white divider that allowed for adequate room for the fish to swim from one side of the aquarium to the other. Each trial was initiated with a light tap at the center of each aquarium. After the light tap, there is a 5 second delay followed by food presentation on one side of the tank. In 20 minutes intervals, food presentation continued on alternating sides for a total of 28 trials (14 on each side). A response was considered correct if the fish was present on the side of food presentation during or shortly after food presentation. Each trial ended 5 seconds after the food was presented. Zebrafish are deemed to have learned the task when 75% or more of the responses are correct. After completing the learning and memory tests, fish were euthanized and their brains were further analyzed by conducting immunohistochemistry analysis for phospho-glycogen synthase-3 α/β (p-GSK-3 α/β), GSK-3 α/β, Aβ, p-tau, and tau. All parameters of expression of p-GSK-3α/β, GSK-3α/β, Aβ, p-tau, and tau were analyzed using a student's t-test. A value of $p<0.05$ was considered to be significant. It was observed that brain hemorrhages and higher mortality rates were caused in fish with increasing concentration of OKA. The 500nM and 1µM exposure groups observed the highest mortality rates of 60%-70%. The control group demonstrated rapid learning with maximal learning shown to be between 75%-80%. The exposed groups never demonstrated learning with their probabilities of correctness continuously staying around 50%. OKA exposure resulted in the increased phosphorylation of GSK-3 α/β, increased phosphorylation of tau, Aβ deposition, and the formation of Aβ plaques. These experiments were able to establish an AD model that incorporates most of the hallmarks of AD pathophysiology. This model can now be used to study drug discovery for AD and the molecular mechanisms involved in AD without the time and cost constraints that other AD models entail.

Z6230B Regulation of the cell cycle and cell fate by TGFβ signaling in larval and adult zebrafish. *J. R. Lenkowski*, M. Downey, K. Brandt, D. Meir-Levi. Goucher College, Baltimore, MD.

Several studies indicate that the TGFβ signaling pathway plays an important role in regulating proliferation of progenitors and cell differentiation during mammalian retinal development and zebrafish retinal regeneration. We are using chemical genetics and genetic approaches in zebrafish to manipulate TGFβ signaling in order to study how the signaling pathway regulates the cell cycle and cell differentiation during retinal development, which has not yet been described, and regeneration of the adult retina. Specifically, TGFβ signaling has been shown to promote Müller glial cell fate, inhibit proliferation, and increase differentiation into photoreceptors during rodent retinal development, and in the regenerating adult zebrafish retina, proliferation and regeneration are impaired when canonical TGFβ signaling is mis-regulated. Our preliminary studies of retinal development in zebrafish using fluorescence immunohistochemistry suggest that the CMZ may be expanded when TGFβ signaling is inhibited. In the adult damaged retina, the initial damage response by Müller glia is extremely similar between mammals and zebrafish, but the zebrafish is able to achieve robust regeneration of destroyed neurons while mammals are limited. In the zebrafish retina, Müller glial cells respond to damage by dedifferentiating and generating a neurogenic progenitor that goes on to regenerate lost neurons. Ongoing experiments are clarifying whether manipulating TGFβ signaling in the adult zebrafish retina promotes a more mammalian-like response to acute retinal injury.

Z6231C Making Functional Neuronal Circuitry: Interneuron specification in the spinal cord. *K. E. Lewis*. Syracuse University, Syracuse, NY.

CNS damage caused by abnormal development, injury or disease profoundly impacts quality of life. For example, spinal cord injuries are relatively common, but strategies to repair or regenerate damaged sites remain elusive. To develop more effective treatments it is essential that we understand the molecular mechanisms that produce functional spinal cord circuits. In addition, if we are to understand the genetic basis of neuro-developmental and behavioral disorders we need to understand how specific genes regulate the development of neuronal circuitry and hence enable particular behavioral repertoires. A particularly tractable model system for this research is spinal cord circuitry. This is partly because the development and functions of this circuitry can be studied in simpler vertebrates such as zebrafish and partly because the spinal cord is a much simpler structure than the brain. Specification of distinct types of neurons with particular functional properties is a crucial step in circuit formation. Interneurons constitute most of the neurons in the CNS and they function in almost all neuronal circuits. However, there are significant gaps in our knowledge of how spinal interneurons with specific functional characteristics develop and form appropriate neuronal circuitry. All of the evidence so far, suggests that the properties of distinct interneurons are determined by the transcription factors

that these cells express as they stop dividing and start to differentiate. However, in many cases, it is still unclear which transcription factors specify particular properties, at least partly because we do not yet know the full complement of transcription factors expressed by any population of spinal interneuron.

To address these critical gaps in knowledge we have expression-profiled different spinal interneurons using transgenic zebrafish lines that specifically label these cells. Using these data, we have identified transcription factors that are candidates for specifying particular interneuron properties and we are currently testing the functions of these using mutations. Notably we have already identified several families of transcription factors that are required to specify and/or maintain the neurotransmitter properties of particular spinal interneurons. For example, we have shown that Pax2 & Pax8 are required for the inhibitory fates of many distinct spinal neurons and Evx1 & Evx2 are required for the excitatory fates of V0v neurons. We have also identified additional transcription factors that are required either for the inhibitory fates of distinct ventral neurons or the excitatory fates of specific dorsal neurons or to maintain the glutamatergic fates of some excitatory neurons.

Z6232A Telomere-Dependent and -Independent Functional Roles of A Telomeric Factor TRF2 in Early Vertebrate Development and Neurogenesis. *S. Lian*[1], A. Khan[1], T. Sasaki[1], M. Rebagliati[2], L. Bally-Cuif[3], H. Sirotkin[4], S. Kishi[1]. 1) The Scripps Research Institute, Jupiter, FL; 2) Institute of Genetics and Molecular and Cellular Biology, France; 3) Institute of Neurobiology Alfred Fessard, France; 4) Stony Brook University, Stony Brook, NY.

Telomere repeat-binding factor 2 (TRF2) is critical for telomere integrity in dividing stem and somatic cells, and its role is essential for vertebrate early development because animals carrying a null *terf2* mutation have early embryonic lethality due to an unknown molecular mechanism. Besides telomeric homeostasis by protecting telomere ends, it has been shown that TRF2 can interact with the neuronal gene-silencer repressor element 1-silencing transcription factor (REST), whose instability via TRF2 affects neuronal cell differentiation *in vitro*. However, the mechanism that couples TRF2-mediated features to cellular differentiation programs has yet to be elucidated *in vivo*. Loss of TRF2 in zebrafish embryos recapitulates key aspects of telomere attrition, including the DNA-damage response and cell-cycle arrest, as well as neurodegeneration. Intriguingly, TRF2-deficient animals develop similar but more severe neuromuscular defects observed in spinal muscular atrophy, a genetic disorder characterized by a loss of alpha motoneurons in the spinal cord. Using *terf2* zebrafish embryos that carry the concurrent triple- or quadruple-defects of the *rest, tert, tp53 and/or terf1* genes with the expression of the various transgenic reporter genes including the zebrabow system, we show motor axon-specific pathfinding/guidance abnormalities that could be linked to aberrant Wnt and Hedgehog pathways in parallel with telomere fusion/attrition. Our results show for the first time, *in vivo*, that TRF2 functions in motor axon development and suggest that *terf2* mutation leads to early developmental senescence and motoneuron defects in both telomere-dependent and -independent manners.

Z6233B The Role of the CoREST Family in Early Neurodevelopment. *C. M. Monestime*, Andrew Taibi, C. R. Milano, Neal Bhattacharji, Esha Chebolu, Ibrahim Elmaghrbi, H. I. Sirotkin. Stony Brook University, Stony Brook, NY.

Neurogenesis is a highly regulated process that results in the emergence of diverse neural cell types from progenitor and stem cell pools. The RE 1-silencing transcription factor (REST) has been proposed to play a role in regulating neurogenesis, fine-tuning expression of neural genes, and suppressing expression of target genes in non-neural tissues. REST is a part of a neuronal macro-repressor complex. REST acts by binding to a highly conserved DNA element, the RE1 site; once bound, it recruits co-repressors Sin3 to its N-terminal domain and the CoREST family to its C-terminal domain, which subsequently recruit chromatin modifiers to form the repressor complex. Initially, CoREST was identified in association to the REST complex, but it is now recognized that CoREST also works independent of REST. In vertebrates, the CoREST family contains three members, RCOR1, RCOR2, and RCOR3; distinct activities and roles have yet to be established for each of these proteins. To tease apart the REST-dependent and REST-independent roles of the CoREST family in neural development, we used transcription activator-like effector nucleases (TALENS) to disrupt all three CoREST genes in zebrafish. The three single mutants and all three double mutant combinations are viable. qPCR analysis revealed that expression levels of RE1- containing genes differs between the CoREST mutants, suggesting unique roles for each protein. In addition, analysis of larval locomotor behavior of each of the mutants revealed distinct behaviors were produced in the absence of each CoREST gene. These results show that members of the CoREST family differentially mediate gene expression and larval behavior and suggest both redundant and unique functions for zebrafish CoREST family members during development.

Z6234C Dissecting the Endocannabinoid System using the zebrafish model. *F. Oltrabella*[1], B. Nguyen[2], S. Guo[1]. 1) UCSF, San Francisco, CA; 2) UC Berkeley, Berkeley, CA.

Recent trends in marijuana legislation have been a topic of significant public attention. Legalization of the drug in many US and overseas countries will increase its daily usage, which in turn will necessitate the need to understand the fundamental mechanisms underlying marijuana's effect *in vivo*, as it remains rudimentary. Δ9-tetrahydrocannabinol (THC), the main psychoactive ingredient in marijuana interferes with the endogenous cannabinoid system (eCBs), which includes cannabinoid receptors, endogenous ligands, and enzymes involved in the synthesis and metabolism of these ligands. The eCBs modulate various cognitive and physiological processes associated with addiction including reward, stress responsiveness and appetite stimulation. Limited functional studies of eCBs have been carried out in zebrafish, which have relied on the use of morpholinos and THC treatment but little is known regarding the role of eCBs on brain development. Through genomic analysis we identified components of eCBs in the zebrafish genome, which include:

- orthologous receptors (Cb1 and Cb2)
- synthesis enzymes (Abdh4, Daglα and Daglβ)
- catabolic enzymes (Faah and Mgll)

• cannabinoid receptor 1 interacting proteins (Cnrip1A and Cnrip1B)

Here we show expression analysis of eCBs genes in the zebrafish in different embryo time points and within tissue types in adults. To further characterize the molecular mechanisms by which endocannabinoid system works we targeted and disrupted eCBs associated genes using CRISPR/Cas9 technology. We successfully generated F1 hets with indels for all the eCB genes and obtained F2 for the majority of them. Here I am presenting the preliminary characterization of these mutants revealing new insights into the mechanism of action of eCBs on neurodevelopment, physiology and behaviour.

Z6235A Gene Expression Changes during Brain Regeneration in Adult Zebrafish. *Kanagaraj Palsamy*, Jack Parent. Department of Neurology, BSRB, University Of Michigan, Ann Arbor, MI, USA.

In the mammalian brain, neural stem cells and neurogenesis persist into adulthood mainly in the subependymal zone-olfactory bulb pathway and hippocampal formation. Adult neurogenesis is proposed to play an important role in learning and memory, but it also offers the potential for replacing injured or diseased brain tissues through regeneration by neural stem cells. Despite the persistence of neurogenesis, mammals have limited brain regenerative capacity. Zebrafish, however, display robust regeneration in many different brain regions and can make mature, functional neurons after injury. We use adult zebrafish to explore the molecular networks induced by traumatic brain injury and quinolinic acid (QA)-induced neurotoxicity. We performed RNA sequencing on telencephalic tissue collected 24 or 48 hours after brain injury, with or without QA lesioning, and identified many interesting candidate genes involved in various biological processes. Our gene ontology and pathway analysis study confirms upregulation of immune response, tissue regeneration, wound healing, cell migration, angiogenesis processes, and Jak-Stat, interleukins, inflammatory-induced chemokines and cytokines, notch, and B cell and T cell activation pathways respectively. Based upon expression level changes and their known roles in neuroprotection or stem cell function, we selected 8 candidate genes to explore their potential involvement in different cellular and molecular pathways necessary for brain regeneration. We first confirmed the RNAseq expression changes of the candidates after injury using real-time RT-PCR. Work is ongoing to examine cellular expression changes by in situ hybridization, and to generate loss-of-function models to examine how alterations in the candidate genes influence brain injury and the regenerative process. We are also using the QA excitotoxicity model in mouse striatal lesioning with the goal of comparing gene expression changes with the zebrafish model. Our eventual aim is to shed light on mechanisms underlying the limited regenerative capacity of the mouse brain with the goal of improving brain repair.

Z6236B A Zebrafish Screening Platform for *In Vivo* Pro-Myelinating Drug Discovery. *M. A. Preston*, L. Finseth, W. B. Macklin. University of Colorado Anshutz Medical Campus, Aurora, CO.

Myelin is produced by oligodendrocyte lineage cells in the central nervous system. Numerous extracellular and intracellular molecules influence myelination, but how these diverse cues are integrated at the level of transcription to control myelination remains poorly understood. Nuclear Receptors (NRs) are transcription factors that bind a wide variety of lipophilic molecules and activate or repress transcriptional programs that control differentiation and maturation of cells. One NR signaling cascade important for myelination is mediated by Retinoid X Receptors (RXRs), which dimerize with other NR family members, potentially influencing multiple NR signaling cascades. Signaling interactions between RXRs and other NRs have been tested with *in vitro* cell assays, which fail to replicate the complex intercellular relationships and signaling interactions that occur *in vivo*. The zebrafish embryo is an excellent model to study myelination *in vivo* as embryos develop *ex utero,* myelination of the spinal cord occurs during the first week of life and each pair of zebrafish produce hundreds of embryos, allowing multiple drugs or combinations of drugs to be tested simultaneously for their impact on myelination. As such, we have developed a novel zebrafish model which quantifies the promoter response of a critical myelin gene, myelin protein zero (*mpz*), to RXR and NR ligands using a fluorescent or nanoLuciferase-based reporter system.

The *mpz* reporter embryo responds to known positive and negative regulators of myelination, consistent with changes in myelin mRNAs as quantified by qPCR. Initial studies of known RXR agonists and antagonists indicate that endogenous ligands such as 9-cis retinoic acid (9cRA) or Docosahexaenoic acid (*DHA*) have no significant effect on myelination *in vivo*, while synthetic molecules such as Fluorobexarotene, SR11237, UVI3003, PA 452, HX 630 and HX 531 can have significant dose-dependent effects on *mpz* promoter activity. These studies suggest that RXR-mediated signaling *in vivo* is more complicated than previously predicted and that investigating RXRs in an *in vivo* context will be essential for understanding the underlying mechanisms of action. Future experiments will use the *mpz* reporter to identify RXR and NR-mediated signaling interactions using an *in vivo* screening approach with the goal of identifing other pro-myelinating signaling interactions.

Z6237C N-cadherin is required cell-autonomously for the collective migration of facial branchiomotor neurons. *J. K. Rebman*, G. S. Walsh. Virginia Commonwealth University, VA.

Neuronal migration is a key mechanism during neural development, as it allows cells to reach their final destination, establishing the basis for the subsequent wiring of neural circuitry. Collective cell migration, the coordinated migration of a cell population through cell–cell contact, is a recognized mode of migration for a variety of cell types, but its role in neuronal migration has been poorly characterized. We have recently published that facial branchiomotor (FBM) neurons, that migrate from rhombomere (r)4 to r6 in the developing hindbrain, use a collective mode of migration in which one FBM neuron can influence the migration of neighboring FBM neurons presumably through cell-cell contact. Here, we examined whether N-cadherin is a candidate cell adhesion molecule mediating the collective migration of FBM neurons. To accomplish this, we generated stable transgenic fish expressing a dominant-negative N-cadherin, lacking the extracellular domain (*ncadhDEC*), specifically in cranial branchiomotor neurons using the *islet1* promoter. We find that caudal migration of FBM neurons is blocked in *Tg(isl1: ncadhDEC)* fish, as they fail to exit r4. Timelapse imaging revealed that loss of N-cadherin function does not block cell motility as FBM neurons

migrate randomly and dorsally within r4. To determine whether N-cadherin is required for collective migration, we generated mosaic embryos in which some FBM neurons expressed *ncadhDEC* adjacent to wild-type FBM neurons. We found that few, if any, *ncadhDEC*-expressing FBM neurons could be rescued via collective migration by neighboring wild-type neurons. Taken together, our data suggest that N-cadherin plays an essential cell-autonomous role in mediating the collective migration of FBM neurons.

Z6238A Paclitaxel-induced epithelial damage and ectopic MMP-13 expression promotes neurotoxicity in zebrafish. *S. Rieger*[1], T. Lisse[1,2], L. Middleton[1], A. Pelegrini[1], P. Martin[1], E. Spaulding[1], O. Lopes[1], E. Brochu[1], E. Carter[1], A. Waldron[1]. 1) MDI Biological Laboratory, Salisbury Cove, Maine; 2) The Jackson Laboratory, Bar Harbor, Maine.

Paclitaxel is a microtubule-stabilizing chemotherapeutic agent that is widely used in cancer treatment and in a number of curative and palliative regimens. Despite its beneficial effects on cancer, paclitaxel also damages healthy tissues, most prominently the peripheral sensory nervous system. The mechanisms leading to paclitaxel-induced peripheral neuropathy remain elusive and therapies that prevent or alleviate this condition are not available. We established a zebrafish *in vivo* model to study the underlying mechanisms and to identify pharmacological agents that may be developed into therapeutics. Both adult and larval zebrafish displayed signs of paclitaxel neurotoxicity, including sensory axon degeneration and the loss of touch response in the distal caudal fin. Our studies in zebrafish larvae further showed that paclitaxel promotes epithelial damage and decreased mechanical stress resistance of the skin prior to induction of axon degeneration. Epithelial damage correlates with rapid accumulation of fluorescein-conjugated paclitaxel in epidermal basal keratinocytes, but not axons, and upregulation of the collagenase matrix-metalloproteinase 13 (MMP-13, collagenase 3) in basal keratinocytes. Intriguingly, pharmacological inhibition of MMP-13 largely rescued paclitaxel-induced epithelial damage and neurotoxicity. Thus our studies provide evidence that the epidermis plays a critical role paclitaxel neurotoxicty, and we provide a new candidate for therapeutic interventions.

Z6239B Integration of multiple signaling pathways in habenular development. *S. Roberson*[1,2], M. E. Halpern[1,2]. 1) Carnegie Institution for Science, Baltimore, MD; 2) Johns Hopkins University, Baltimore, MD.

The habenulae are paired nuclei of the epithalamus, which have been implicated in a number of behaviors, such as sleep, fear/anxiety and reward, as well as in neurological diseases such as bipolar and major depressive disorder. Despite the importance of the habenulae, relatively little is known about their development. Wnt and Fgf signaling pathways have been implicated in the formation of the dorsal habenular (dHb) nuclei, but their interaction during dHb development has not been explored. Both pathways are necessary for the generation of habenular progenitors, which are recognized by their expression of the homeodomain transcription factor *dbx1b* (Dean et al., 2014, Vue et al., 2007). This progenitor population is dramatically reduced in *fgf8* and *wntless* mutants. Using lineage tracing, we confirmed that *dbx1b*-expressing progenitors contribute to the entire dHb of larval and adult zebrafish. The *dbx1b*-expressing progenitors were also found to persist past 12 days post fertilization. It was previously suggested that the chemokine receptor Cxcr4b was also produced in habenular progenitors or early habenular neurons. Our data indicate that *cxcr4b* is not transcribed in habenular progenitors; rather, *cxcr4b* expression increases as *dbx1b* expression decreases, supporting the hypothesis that *cxcr4b* demarcates newly born habenular neurons. Wnt and Fgf signaling both regulate the spatial domain of expression of chemokine pathway components in the dorsal diencephalon, with Wnt acting upstream of Fgf8. We will present new findings on both the function of the chemokine signaling pathway in habenular development and the role of Wnt and Fgf signaling in this pathway.

Z6240C Bsx in Neuroendocrine and Pineal Complex Development. *Theresa Schredelseker*, Wolfgang Driever. Developmental Biology, Biology I, University of Freiburg, DE.

By releasing secreted factors to the blood upon receiving neuronal input neuroendocrine cells represent the main integrators connecting nervous and endocrine systems. The pineal gland is a small neuroendocrine structure in the dorsal diencephalon crucial for the regulation of circadian rhythm, partially via secretion of melatonin. Several nuclei at the base of the diencephalon form the hypothalamus, a master gland from which various factors controlling body temperature, hunger, thirst, fatigue and reproduction are released.

The highly conserved homeodomain factor *brain specific homeobox* (*bsx*) is expressed in both the developing pineal complex and hypothalamus. Using TALEN-mediated targeted mutagenesis in zebrafish, we generated the *bsx*^{m1376} mutant allele, in which a truncated Bsx protein lacking a functional DNA-binding domain is translated. In the rodent hypothalamus the orexigenic neuropeptide AGRP has previously been described as being significantly downregulated upon loss of functional Bsx. In *bsx*$^{m1376/m1376}$ larvae we observed drastically reduced *agrp* expression in the hypothalamus, suggesting a similar Bsx function in zebrafish.

Very little is known about the role of Bsx in the pineal complex. *In situ* hybridization revealed pineal gland expression of *tph2,* a key enzyme in melatonin synthesis, to be missing in *bsx*$^{m1376/m1376}$. Loss of Tph2 suggests absence of melatonin in *bsx*$^{m1376/m1376}$ and thus disturbed activity regulation during dark cycles. Reduction of *irbp* and *gngT1* expression further indicates disturbed photoreception via the pineal complex in *bsx*$^{m1376/m1376}$.

The teleost pineal complex more recently has also been implicated in background adaptation as well as the establishment of both anatomical and functional asymmetries in the epithalamus. We analyzed expression of *agrp2*, a neuropeptide crucial for melanocyte contraction, which we found to be significantly reduced in *bsx*$^{m1376/m1376}$. Furthermore, bsx$^{m1376/m1376}$ larvae display abnormalities in epithalamus asymmetry as revealed by absence of parapineal *otx5* expression as well as symmetrical expression of *lov* in the habenula. Through epistasis analysis including *floating head* and *one-eyed pinhead* mutants as well as injections of *otx5* and *crx* morpholinos, we seek to integrate Bsx into a comprehensive model of pineal complex development. Detailed knowledge about the mechanisms by which Bsx regulates neuroendocrine

development or differentiation in not only one but two neuroendocrine brain areas could contribute to a better understanding of numerous diseases associated with the neuroendocrine system.

Z6241A Pard3c, an unconventional zebrafish Par-3 ortholog for organogenesis: important for cell survival and proliferation but not for apicobasal polarity. *Z. Shi*, W. Fang, C. Guo, X. Wei. University of Pittsburgh, Pittsburgh, PA.

Vertebrate homologs of *Caenorhabditis elegans* Par-3 have versatile biological functions, including the prototypical role in cell polarization; such functional versatility may be partly explained by the variety of vertebrate Par-3 homologs. Thus, revealing the similarities and differences among individual Par-3 homologs is a prerequisite for a thorough understanding of their biology in vertebrates. Here, we identified and characterized Pard3c, one of the four zebrafish Par-3 orthologs. The Pard3c sequence is most closely related to that of Pard3, a zebrafish Par-3 ortholog that has been studied extensively. Surprisingly, unlike Pard3, Pard3c does not restrictively localize apically nor is it required for epithelial apicobasal polarity; instead, Pard3c localizes broadly to the cell membranes and is required for proper levels of cell survival and proliferation during the morphogenesis of the retina, lens, midbrain-hindbrain boundary, pharynx, and pectoral fin. Thus, despite the loss of the hallmark function of regulating apicobasal polarity as other Par-3 homologs do, Pard3c yet plays indispensable roles in zebrafish organogenesis.

Z6242B The role of microglia in neurogenesis and repair following telencephalic lesion in adult zebrafish. *Kaia Skaggs*. University of Findlay, Findlay, OH.

Zebrafish represent an attractive system for the study of neurogenesis following injury because, unlike mammals, they regenerate damaged neurons. After stab lesioning, initial cell death is followed by a robust inflammatory response 1-2 days post-injury and a marked increase in proliferation of radial glia-like progenitors that peaks 3-4 days post-injury. In order to study the role of the early inflammatory response on neurogenesis, we ablated microglia that responded to injury though concurrent injection of liposomes containing Clodronate at the injury site. Phagocytosis and subsequent degradation of the liposomal membrane releases Clodronate and causes microglial apoptosis. In the absence of microglia, proliferation and neurogenesis were markedly reduced. These effects persisted over time, resulting in incomplete repair in Clodronate-treated, lesioned brains compared to control liposome-treated, lesioned brains. These results indicate that the early inflammatory response following telencephalic lesioning is an important signaling event that stimulates neurogenesis and repair of adult zebrafish brain injury. A significant limitation of Clodronate treatment is that the critical time period during which microglia crucially affect regeneration cannot be determined. Microglia are dependent on colony-stimulating factor receptor 1 (CSFR1) for viability in other organisms. We used a small molecule inhibitor of CSFR1 to determine the effects of non-invasive microglial ablation on regeneration following brain injury and to further determine with increased precision the time period during which microglia are required to stimulate the neurogenic response following injury.

Z6243C Characterization of two protein repair enzymes in Zebrafish and their influence on the motor system. *Remon F. Soliman*, M. L. Cordero Maldonado, S. Perathoner, A. Crawford, C. Linster. LCSB, Belvaux, LU.

Most neurodegenerative diseases are associated with protein aggregation which in turn could be due to protein damage. One kind of protein damage results from the spontaneous isomerization of aspartyl and asparaginyl residues leading to structural alterations and potentially loss of function of the protein. A protein repair enzyme, namely Protein L-Isoaspartyl Methyltransferase (PIMT), specifically recognizes isoaspartyl residues and repairs them, restoring the original structure of damaged proteins. PIMT knock-out mice die at early stages due to fatal epileptic seizures. Better understanding the physiological role of PIMT may therefore enable us to modulate the progress of epilepsy. Due to limitations in using mouse models to investigate the molecular mechanisms involved in epilepsy, zebrafish (Danio rerio) emerges as a good alternative model organism to pursue this aim. There are two homologues of human PIMT in zebrafish: Pcmt and Pcmt-like. We found that both of them are ubiquitously expressed in many tissues, with the highest expression levels in the brain. We were able to show that both enzymes catalyse the same biochemical reaction as human PIMT. Both genes could successfully be knocked down using morpholino technology. Morphants showed a developmental delay and accumulated isoaspartyl-containing proteins. Preliminary results indicate that the morphants are prone to developing seizures and display disturbed calcium signalling, as observed in the transgenic zebrafish line GCaMP6. As calmodulin was previously found to be a major substrate of PIMT, our results suggest that PIMT is important to sustain normal brain activity, at least partially by supporting calmodulin-mediated calcium signalling.

Z6244A Wnt signaling and mediator 12 control development of the hypothalamus and pituitary. *E. D. Spikol*, E. Glasgow. Georgetown University, Washington, DC.

The brain is a complex structure composed of several distinct tissue types, the patterning of which requires the confluence of multiple signaling networks. The neuroendocrine system represents a vital connection between the brain and body that regulates homeostasis, metabolism, mood and sexual behaviors. Disruptions in the development and regulation of this system are associated with congenital abnormalities and neuropsychiatric disorders. Here, the role of Wnt signaling in the differentiation of neuroendocrine cells in the hypothalamic-pituitary axis is investigated. We examine the effect of the *apc* and *med12* mutations, and treatment with a Wnt-inhibitor compound on zebrafish embryos. Wnt signaling is upregulated in the *apc* mutant and putatively downregulated in the *med12* mutant. We analyzed morphology and used *in-situ* hybridization to detect neuroendocrine gene expression in the pre-optic area, anterior hypothalamus, and pituitary. Our morphology data are consistent with the hypothesis that *med12* acts downstream of *apc* to modulate transcription of Wnt-responsive genes, but suggest that this requirement is not absolute in all tissues. Our studies of brain development indicate that Med12 is

required and Wnt-signaling must be suppressed for normal development of hypothalamic and pituitary cells. However, it appears that Med12 acts independently of Wnt to control the development of the cell populations investigated. Mapping the impact of Wnt signaling and the role of Med12 contributes to a better understanding of the development of neuroendocrine cells, and in what ways alterations in this pathway harm the developing organism.

Z6245B The Retinoic Acid signaling pathway temporally influences enteric neural crest cell migration and differentiation during early phases of enteric nervous system formation in vivo. *R. A. Uribe*, S. Hong, M. E. Bronner. Caltech, Pasadena, CA.

The enteric nervous system (ENS), also known as the "gut brain", is an autonomous network of thousands of interconnected ganglia located within the walls of the digestive tract that regulates peristalsis, gut secretion and water balance. During development, the ENS is derived from "vagal" neural crest cells that emigrate from the hindbrain and, in response to environmental cues, migrate ventrally toward and caudally along the primitive gut. Once reaching their final destinations along the gut, they differentiate into distinct types of enteric neuron or glia. Whereas the migration of neural crest cells along the gut has been extensively studied, little is known about vagal neural crest migration toward the gut and the extrinsic signaling factors regulating their gut entry. Using FACS sorting, transcriptome and perturbation analyses, we have discovered that Retinoic Acid (RA) signaling pathway components are expressed in the zebrafish gut endoderm concomitant with invasion of the gut. Exogenous treatment with RA prior to and during vagal neural crest gut entry enhanced migratory progress, increased the number of gut resident neural crest cells and later of differentiated enteric neurons. Conversely, temporal attenuation of the RA pathway using Tg(*hsp70*:dnzRAR) heat-shocked embryos led to delayed gut entry by neural crest cells and live imaging revealed altered neural crest migratory behavior and progress along the gut. RA treatment was sufficient to expand *meis3* and *shha* expression domains, while its attenuation led to diminished *meis3* and *hoxb5b* expression. Together, these results suggest that the RA signaling pathway plays a key temporal role during the early phases of ENS development, thus enhancing our understanding of the genesis of the ENS *in vivo*.

Z6246C Role of local neurogenesis in functional recovery post spinal cord injury . *Deeptha Vasudevan*, Lisa Briona, Richard Dorsky. University of Utah, Salt Lake City, UT.

Successful treatment for human spinal cord injury (SCI) is currently an insurmountable goal. The biggest obstacle is the inability of severed axons and neurons to regenerate. Adult mammals have a conspicuous inability to regenerate post SCI, but zebrafish show full functional recovery throughout life due to axon regrowth, rewiring of existing circuits and/ or local neurogenesis. Our aim is to understand the role of local neurogenesis in functional recovery. Studies from our lab have shown that resident radial glia undergo a change in morphology, and actively make neurons post injury. To test the hypothesis that neurons born after SCI integrate into existing circuits and are required for recovery of sensorimotor behavior, we are using two approaches. In the first approach, we are genetically ablating neurons that arise from a radial glial lineage post injury to evaluate their specific requirement in swimming behavior. The second approach is to express a calcium reporter specifically in neurons arising from radial glia after injury, to determine whether they exhibit physiological activity during a defined escape behavior. We are also tracing the lineage of radial glia to test whether specific cell identities with known physiological properties are restored.

Z6247A Planar cell polarity components control anterior-posterior guidance of spinal commissural axons. *G. S. Walsh*, J. P. Miano, A. M. Purdy. Virginia Commonwealth University, Richmond, VA.

Spinal commissural neurons are an attractive model to investigate how multiple guidance cues control growth cone navigation along the dorso-ventral (D-V) and anterior-posterior (A-P) axes by both pre- and post-midline crossing axons. Commissural axons extend toward the floorplate, cross the midline, and turn anteriorly toward the brain. In the zebrafish, the earliest born spinal commissural neuron to navigate the midline and turn rostrally is termed CoPA (**C**ommissural **P**rimary **A**scending). We show an evolutionarily conserved role for planar cell polarity (PCP) components as essential for the anterior turning of CoPA axons in the zebrafish spinal cord. Specifically, we show that CoPA axons make randomized turns in *fzd3a* and *vangl2* mutants, with half of CoPA axons executing the correct rostral turn and the other half projecting posteriorly inappropriately. All other guidance decisions along the dorsal-ventral axis are unaltered in PCP mutants. We extend these findings to reveal a role for additional PCP components, *celsr3*, *scribble* (*scrib,*) and *protein tyrosine kinase 7 (ptk7),* in the anterior guidance of commissural axons. Specifically, we find randomized A-P guidance of CoPA axons in *celsr3* morphants, zygotic *scrib* mutants and maternal-zygotic *ptk7* mutants. We observe CoPA axons extend for long distances even after turning the wrong direction in PCP mutants, suggesting that PCP proteins are not required for axon growth *per se,* but appear to establish a "compass" within the growth cone from which directional pathfinding can be executed. Finally, we show that c-jun-N-terminal kinase (JNK) is an effector of PCP signaling in axon guidance. Embryos treated with the JNK inhibitor, SP600125, from 16-24 hpf, display a similar defect in A-P guidance of CoPA axons as seen in PCP mutants. Taken together, our data establish CoPA commissural axons as a model system to elucidate how planar cell polarity controls axon guidance decisions along the anterior-posterior axis. .

Z6248B A genomic approach to investigate the interactions between somatosensory neurons and skin. *Fang Wang*[1], Shawn Cokus[2], Maricruz De La Torre[1], Stacy Nguyen[1], Jueun Kim[1], Alvaro Sagasti[2]. 1) California State University, Dominguez Hills, Carson, CA; 2) University of California, Los Angeles, CA.

Somatosensory neurons detect mechanical, thermal, and chemical stimuli, which is crucial for animals to sense their environment and respond appropriately. Many studies have suggested that the interactions between somatosensory neurons and skin cells not only play an important role during development but also may be critical for neuron function. Thus, we have undertaken a genomic approach to investigate

the interactions between touch-sensing neurons and skin cells.

Peripheral axons of somatosensory neurons first arborize between the two epithelial layers (periderm and basal cells) that compose larval zebrafish skin, starting at the ~18 somite stage. Electron microscopy has revealed that the free endings of somatosensory axons become ensheathed by basal cells between 54 and 78 hours post fertilization (hpf). To identify genes that may be involved in axon/skin interactions, we examined the gene expression profiles of skin cells in zebrafish at three different stages: 20 hpf, 52 hpf, and 72 hpf. To accomplish this goal, periderm cells, basal cells, and non-skin cells were purified with fluorescent activated cell sorting of krt4:DsRed krt5:GFP transgenic fish that express DsRed in both epithelial layers, but only express GFP in periderm cells. mRNAs isolated from these cells were used for RNA-Seq. This method provides comprehensive and quantitative gene expression data. RNA-Seq was also performed on skin cells in wildtype fish as well as fish lacking somatosensory neurons to gain a genomic view of transcriptional changes in skin cells as they respond to axon innervation.

Currently, we are using molecular and cellular methods to verify the expression pattern of genes that are enriched in a specific skin layer and/or at a specific developmental stage. Loss-of-function studies will be conducted to unveil the functions of these genes.

Z6249C Lineage Tracing of Neuronal Progenitor Cells Expressing *dlx* Genes in the Zebrafish Brain. *H. C. Weinschutz Mendes*, C. M. Solek, S. Feng, E. Mahoney, M. Ekker. Department of Biology, University of Ottawa, Ottawa, ON, CA.

During development, the proper specification and migration of GABAergic interneurons is essential to the establishment of appropriate synaptic connections. Abnormal establishment of these neuronal types are often related to neurological and behavioral disorders. The *Dlx* homeobox gene family and the transcription factors they encode are involved in the proper specification of GABAergic interneurons in vertebrates. In the forebrain, the expression patterns of the *dlx1a/dlx2a* and of the *dlx5a/dlx6a* bigene clusters presents extensive overlaps and the functional specificity of the four *dlx* paralogs still remains elusive. The purpose of this research is to trace the lineage of *dlx*-expressing cells in the zebrafish brain, from early development to adulthood, and to compare the fate of cells expressing genes from the *dlx1a/dlx2a* and *dlx5a/dlx6a* clusters. We have produced a transgenic line that express the $CreER^{T2}$ recombinase under the control of *dlx1a/2a* or *dlx5a/6a* regulatory sequences and bred them with zebrafish that express a floxed GFP gene followed by the mCherry gene under the control of an ubiquitin promoter. These double transgenic lines allow us to permanently label *dlx*-expressing cells by introducing tamoxifen in the embryo medium and to observe the tracing of these cells on a time-related manner at different time points during early stages of development. We followed the migration and differentiation of these cells throughout the life of the zebrafish. Our findings indicate that, as predicted, the majority of labeled cells give rise to GABAergic neurons, although a small number of cells in the ventral telencephalic area are not immunoreactive for GABA or neuronal markers. The *dlx1a/dlx2a*-expressing cells labeled at 24 hours post-fertilization (hpf) seem to give rise to a relatively larger number of mCherry-positive cells when examined in older animals, compared to similar labeling of *dlx5a/dlx6a* fish. Furthermore, the *dlx1a/dlx2a*-labeled cells give a higher proportion of cells that remain close to the neurogenic zones, compared to *dlx5a/dlx6a*-labeled cells. Interestingly, a number of *dlx5a/6a*-expressing cells, labeled at 5 dpf, give rise to a large number of cells that populate the dorsal telencephalon, the periventricular grey zone and the hypothalamus, something that is not seen with *dlx1a/2a*-labeled cells nor when induction of *dx5a/6a*-driven Cre is performed earlier. This lineage tracing system can also be used to characterize the development of new GABAergic neurons in adult zebrafish both in conditions of homeostasis and during regeneration following injury. Supported by the Canadian Institutes of Health Research.

Z6250A Zebrafish Models for Parkinson's Disease. *J. Wint*, A. DellaPenna, E. Okoye, H. Sirotkin. Stony Brook University, Stony Brook, NY.

Parkinson's disease is the second most common neurodegenerative disorder. The majority of cases are idiopathic, having no identifiable cause. Approximately 5-10% of cases are due to inherited genetic mutations that provide a gateway to understand the mechanistic causes of this disease. The overt symptoms of Parkinson's disease are movement related and include shaking, rigidity, slowness of movement and difficulty with walking. These symptoms arise due to the death of dopaminergic neurons in the substantia nigra. About 70% of human protein-coding genes have homologs in zebrafish, thus allowing this model organism to provide a platform to fully elucidate and understand the mechanisms of these mutations. We are employing the CRISPR/Cas9 system to develop zebrafish models for the disease by altering zebrafish homologues of genes linked to the disease including LRRK2 (Leucine Rich Repeat Kinase 2) and PARK2. LRRK2 is a large multidomain protein with GTPase and kinase activity. The GTPase regulates kinase activity. We created mutations in the GTPase domain of LRRK2 as well as a single nucleotide change in the kinase domain. The single nucleotide change corresponds to the most commonly reported mutation of LRRK2, G2019S, which enhances the kinase activity of the protein. PARK2 is an E3 ubiquitin ligase and we have created a deletion mutation that is predicted to impair its activity. We are employing behavioral studies to screen through the various mutants and evaluate their locomotor defects. We will also analyze changes to dopaminergic neurons through staining with tyrosine hydroxylase. Zebrafish models would not only empower mechanistic and gene interaction studies, but enable behaviorally based small molecule screens to identify compounds that alleviate Parkinson's related phenotypes.

Z6251B Functional Genomics of Somatosensory Neuron Signaling And Morphology. *Victoria Wright*, Ian Woods. Ithaca College, Ithaca, NY.

The somatosensory system detects mechanical, thermal and chemical stimuli; abnormalities in somatosensory signaling can lead to migraine and chronic pain. Neurons within the somatosensory system are specialized to detect different types of sensory stimuli, via expression of various cell surface receptors, development of diverse branching morphologies, and appropriate targeting to the central nervous system. To uncover the molecular mechanisms that generate these differences, we employed genome-wide transcriptional profiling in purified subpopulations of somatosensory neurons. From our list of differentially-expressed candidate genes, we are focusing on secreted peptides, transmembrane proteins, cytoskeletal regulators, and transcription factors for follow-up studies, as these gene families likely

regulate development, morphogenesis, and function of somatosensory neurons. We are working to confirm enrichment of these genes in sensory neurons via fluorescence expression analyses, and to identify the neuronal subtypes in which these genes are expressed. Potential functions for the most promising candidates are being identified with genetic gain- and loss-of-function approaches. Specifically, neuronal outgrowth and branching morphology are quantified in embryos in which each candidate gene is overexpressed simultaneously with a fluorescent marker, driven by subtype-specific enhancer sequences. These embryos are also tested for changes in sensory responsiveness via high-throughput videotracking analysis. Similarly, CRISPR-generated knockouts will be analyzed for mutant phenotypes using assays of neuronal morphology and behavioral response to sensory stimuli of various modalities..

Z6252C Eyes shut homolog is localized near connecting cilia/transition zone and is required for cone photoreceptor survival in zebrafish. *Miao Yu*, Yu Liu, Jing Li, Brianna Natale, Jeffrey Amack, Huaiyu Hu. Upstate Medical University 750 E. Adams Street Syracuse, NY 13210.

Mutations in the extracellular matrix protein eyes shut homolog (EYS), also known as spacemaker or spam, make up a large number of autosomal recessive cases of retinitis pigmentosa (retinitis pigmentosa 25, RP25). Drosophila EYS protein is located in and required for the formation of the inter-rhabdomeral space. Vertebrate EYS is thought to be located in the analogous extracellular space of the outer segment layer. Because the mouse genome lacks an EYS locus, we have chosen zebrafish to study the roles of EYS in photoreceptor survival. Our results showed that EYS protein was not located at the outer segment layer. EYS immunostaining exhibited a punctate pattern on the basal end of the outer segment reminiscent of connecting cilia/transition zone (CC/TZ). Double immunostaining revealed that EYS protein was located on the basal end of acetylated a-tubulin but apical to g-tubulin, indicating that EYS was localized near CC/TZ. Using CRISPR/Cas9 technology, we generated several zebrafish EYS mutant lines. Although EYS deficiency did not cause any apparent retinal defect up to 40 days post-fertilization (dpf), progressive loss of cones became apparent at 4-, 6-, and 8-months post-fertilization (mpf). Guanine nucleotide binding protein (G protein) alpha transducin activity polypeptide (GNAT) 2 in the mutant retina, as well as the numbers of red-, green-, blue-, and UV-opsin positive cones progressively decreased. TUNEL-positive nuclei were also observed in the outer nuclear layer of cone photoreceptors. Contrary to the cones, rod markers Zpr3 and GNAT1 immunostaining and GNAT1 real-time RT-PCR did not reveal any significant changes in rod photoreceptor survival. Our results indicate that EYS is located near CC/TZ and is required for cone photoreceptor survival in zebrafish.

Z6253A Regulation of neural stem cell division modes in the developing zebrafish brain. *X. Zhao*, R. Choi, Z. Dong, S. Guo. Department of Bioengineering and Therapeutic Science, University of California, San Francisco, San Francisco, CA.

Asymmetric cell division (ACD) is a conserved and fundamental process in neurogenesis for generating cellular diversity across both invertebrates and vertebrates. As the principal neural stem cells (NSCs), radial glia progenitors (RGP) undergo ACD to generate self-renewing and differentiating daughter cells in the developing central nervous system. Until now, the cellular and molecular mechanisms of ACD and subsequent daughter fate choice is not well understood in vertebrates.

By using zebrafish, we have identified that the cortical polarity regulator Partitioning defective protein-3 (Par-3) plays a crucial role in the establishment of ACD through localizing the ubiquitin E3 ligase Mindbomb (Mib), which activates Notch by ubiquitinating the Notch ligand unequally in the apical daughter. Using immunocytochemistry, in vivo imaging, and other molecular genetic and biochemical methods, we are elucidating the nature of Mib asymmetry and the underlying mechanisms that orchestrate such asymmetry.

Z6254B Committed stem cells derived from the somites supply the osteoblasts during adult bone homeostasis and regeneration. *K. Ando*[1], E. Shibata[1], A. Kudo[1], G. Abe[2], K. Kawakami[2], S. Hans[3], M. Brand[3], A. kawakami[1]. 1) Tokyo Institute of Technology, Yokohama, JP; 2) National Institute of Genetics, Mishima, JP; 3) DFG-Center for Regenerative Therapies Dresden, Germany.

Calcified bone is a characteristic feature of vertebrates that is indispensable for maintaining their terrestrial lives. Since the bone matrix needs to be actively renewed and repaired throughout the animal life, a constant supply of osteoblasts is essential. It is thought that the progenitor cells of osteoblast, the osteoprogenitor cells (OPCs), which are derived from the mesenchymal stem cells (MSCs) in the bone marrow in the case of mammals, produce the osteoblasts in post-developmental stages; however, the identity, function and developmental origin of OPCs in adult vertebrates are unclear.

Here, from the analysis of transgenic (Tg) zebrafish of *matrix metalloproteinase 9* (*mmp9*), we identified a stem cell that produces the osteoblasts during adult zebrafish fin regeneration. In the *mmp9* BAC Tg, the EGFP localization was associated with bony tissues, and in particular a strong fluorescence was seen in the joints of adult fin. The Cre-loxP-mediated cell lineage tracing showed that *mmp9*-positive cells migrate from the nearby joints after fin amputation and produce the osteoblasts and new *mmp9*-positive joint cells as well, indicating that *mmp9*-positive cells are the committed stem cells, osteoblast stem cells (OSCs). The genetic ablation of *mmp9*-positive cells by nitroredutase and prodrug exhibited a significant decrease of the number of regenerating osteoblasts and the regenerating bone, indicating that the OSCs indeed play a role in bone regeneration, although they are not an only source of osteoblasts during fin regeneration.

To investigate the developmental origin of OSCs, we conducted the somite transplantation analysis. Donor somites from the *Tg(mmp9:eGFP; beta-actin:DsRed2)*, which ubiquitously expresses DsRed2, not only produced the osteoblasts in the caudal fin, but also gave rise to the *mmp9*-expressing OSCs, indicating that the OSCs were originated from the somites. However, the early OSCs labelled by the Cre-loxP recombination at 4 weeks post fertilization neither produced osteoblasts nor new OSCs in the fin, suggesting that *mmp9*-negative precursors produce the BSCs and osteoblasts during infant stage. On the other hand, at later stages after 3 months, the OSCs at the fin joints gave rise to the fin ray osteoblasts by the homeostatic cell turnover. These observations suggest that the OSCs are a reserved population of stem cells that are dedicated to the homeostasis and regeneration of calcified bones.

Z6255C Melanocyte stem cell dynamics in wound healing. *Christina D. Carnevale*, David M. Parichy. University of Washington, Seattle, WA.

Humans cannot regenerate lost or damaged appendages and organs, with all of their complexity, whereas several ectothermic organisms, such as salamanders and teleost fishes, can. After severe cutaneous injuries resulting from burns or other trauma, humans develop discolored scars due to the failure of melanocytes to regenerate at the pre-wound density; such discoloration of scar tissue can be psychologically debilitating even after the acute injury has healed. By contrast, zebrafish can regenerate their melanocyte complement perfectly, even after full-thickness skin wounds. Here, we use high resolution imaging with photo-convertible fluorophores to document the dynamics of individual melanocyte progenitors as they migrate into the wound and populate regenerative tissue, as well as assess roles for candidate pathways in these events. Understanding melanocyte stem cell behavior in an organism that does not develop discolored scars may suggest novel ways to stimulate pigment regeneration in human patients.

Z6256A Regeneration after zebrafish traumatic brain injury is dependent upon microglia. *J. Y. Chen*[1], K. Skaggs[2], Y. Qadeer[1], D. Goldman[1], J. M. Parent[1]. 1) University of Michigan, Ann Arbor, MI; 2) University of Findlay, Findlay, OH.

Unlike mammals, adult zebrafish are capable of virtually full restoration of damaged brain tissues after injury. This repair is characterized by the lack of glial scar formation and the ability of adult-born neurons to establish long-distance projections to the contralateral hemisphere. The remarkable regenerative capacity of zebrafish allows for exploration of the cell types and molecular pathways necessary for effective regeneration. Brain injury induces a widespread neuroinflammatory response that recruits activated microglia to damaged regions. This is followed by proliferation of telencephalic ventricular zone (VZ) radial glia, whose neural progenitor cell progeny migrate to areas of injury and give rise to new neurons – a process that is complete in roughly 21 days post lesion (dpl). We generated a right telencephalic stab lesion in adult zebrafish using a 30-gauge Hamilton syringe. To determine the role of microglia in the injury response, we ablated these cells by injecting liposomal clodronate at the time of stab lesioning. The loss of microglia after injury was associated with a pronounced decrease in regenerative potential, as clodronate-treated animals displayed persistent tissue damage even at 90 dpl when compared to controls, suggesting that microglial signaling plays a key role in the regenerative process. Using EdU pulse labeling of proliferating cells, we found that peri-injury clodronate-induced microglial ablation significantly reduced proliferation in the parenchyma, but not the VZ. In separate experiments, we performed RNAseq to examine a brain injury model, quinolinic acid (QA)-induced excitotoxic lesioning, with even more robust regeneration than stab wound alone. Comparing injured, with and without QA, and uninjured brains allowed us to identify stab lesion-induced altered expression of many genes, a subset of which were further upregulated in response to QA injury. We have chosen candidates known to be secreted by microglia for further analysis. These findings highlight a microglial-dependent repair mechanism in the zebrafish brain that may provide strategies for promoting mammalian brain repair.

Z6257B A novel role for miR-9 and Argonaute proteins in balancing quiescent and activated neural stem cell states. *M. Coolen*[1], S. Katz[1], D. Cussigh[1], N. Urban[2], I. Blomfield[2], F. Guillemot[2], L. Bally-Cuif[1]. 1) NEUROPSI CNRS, Gif-sur-Yvette, FR; 2) Francis Crick Institute, London, UK.

Adult neurogenesis is the process by which adult neural stem cells (NSCs) produce new neuronal and glial cells throughout an animal life. Over the past two decades, studies in vertebrate animal models have unveiled the crucial importance of this phenomenon for neural tissue homeostasis and proper brain function. Fundamentally, this process is a balance between maintaining a quiescent NSC pool and recruiting them into the neurogenesis cascade. Using the adult zebrafish telencephalon as a model, we aim at deciphering the molecular mechanisms governing this balance. We place special focus on microRNA-9 (miR-9), which we previously showed to control the transition between commitment states in embryonic neural progenitors. Our recent work shows that miR-9 expression in the adult telencephalon is restricted to NSC residing in a quiescent state (qNSCs) and is anchoring them in this state, at least in part through maintaining high levels of active Notch signaling. Moreover miR-9 expression highlights a striking heterogeneity within qNSCs, which we could link to the division history of the cells. Unexpectedly, we also could observe that miR-9 concentrates into the nucleus of qNSCs together with Argonaute proteins, effector proteins of microRNAs. Manipulating the subcellular localization of miR-9/Ago complexes impacts the quiescent vs activated state of NSCs, thus implying that miR-9 is regulating quiescence through a non-canonical nuclear activity. Interestingly, miR-9/Ago nuclear localization is a specificity of adult NSCs, as opposed to embryonic and juvenile NSCs, where we find it exclusively in the cytoplasm; it thus reveals and highlights a marked age-dependent change in NSCs properties and microRNA regulation.

Z6258C Development and Regeneration in the Zebrafish Lateral Line System. *Ivan Cruz*, David Raible. University of Washington, Seattle, WA.

The zebrafish lateral line system is an excellent model system to investigate tissue development, as well as tissue regeneration. The lateral line system is composed of external sensory organs called neuromasts that are located on the head and trunk of the fish. Each neuromast has centrally located mechanosensory hair cells that are surrounded by non-sensory support cells. These hair cells are structurally and functionally similar to the hair cells located within mammalian ears that detect fluid fluctuations caused by sound. However, unlike their mammalian counterparts, zebrafish are able to replace lost hair cells by symmetric divisions of underlying support cells. Our lab has previously shown that adult zebrafish replace hair cells and support cells after multiple ototoxin-induced hair cell deaths. Currently, the mechanism preserving hair cells and support cells during regeneration and development is unknown. Using the Zebrabow transgenic line, we are able to permanently and mosaically label different support cell populations to perform lineage tracing of support cells under different conditions. We observe that neuromasts, initially of a multicolored support cell arrangement, drift towards clonality over an extended period of time. This suggests that neuromasts are maintained by a small long-lived population of progenitor support cells to produce all the support cells in the neuromast.

Z6259A Leukocyte Itga4 Signaling Regulates Heart Regeneration in Zebrafish. *J. Diao*[1], D. Li[2], X. Zhu[1], W. Pan[2], JW Xiong[1]. 1) Institute of Molecular Medicine, Peking University, Beijing, Beijing, CN; 2) Shanghai Institute of Biological Sciences, Shanghai, China.

Different from the human heart, zebrafish are capable of fully regenerating its damaged heart after ventricular resection, cryoinjury or genetic ablation of cardiomyocytes, but the underlying mechanisms are not completely understood. In this study, we report that Itga4, a subunit of integrin heterodimer, is necessary for adult zebrafish heart regeneration. Both mRNA and protein of Itga4 were dynamically expressed during heart regeneration. Homozygous Itga4 mutants showed deficiency in the recruitment of leucocytes and angiogenesis in the injury site, and therefore had severe cardiac fibrosis and compromised myocardial regeneration. Itga4 was found to mainly colocalize with Lcp1-positive and coronin1a-EGFP-positive inflammatory cells, suggesting Itga4 regulates heart regeneration mainly through its function in leucocytes. Taken together, our study has obtained novel insights of Itga4-mediated leukocyte signaling into zebrafish heart regeneration.

Z6260B Defining the Progenitor Population in Adult Zebrafish Jaw Bone Regeneration. *D. Giovannone*, S. Paul, S. Schindler, F. Mariani, G. Crump. University of Southern California, Los Angeles, CA.

Approximately 8 million bone fractures occur annually and 10% of these are delayed and nonunion fractures which fail to heal despite therapeutic intervention. Current therapeutic strategies are severely hindered by the limited supplies of autograft bone and the poor mechanical properties of artificial materials. Zebrafish are masters of regeneration, and I have found that adult zebrafish can rapidly regenerate up to half of its lower jawbone after resection. Craniofacial bone development is highly conserved between zebrafish and humans, and hence understanding large-scale bone regeneration in zebrafish may lead to novel treatments in patients. A unique feature of zebrafish jawbone regeneration is the involvement of an unusual bone-producing chondrocyte population. In this proposal, I aim to identify the progenitor population that generates these ossifying chondrocytes during large-scale bone regeneration, as well as the role of macrophages in stimulating this progenitor population. Utilizing Cre-based transgenic lineage tracing strategies, I will test that a Runx2+/Sp7- population of pre-osteolasts in the periosteum generate the cartilage callus during regeneration. Using a genetic ablation strategy, I will then test that macrophages are required for the earliest events in bone repair, namely the shift of periosteal cells from making bone during homeostasis to making cartilage during repair. Lastly, I propose to utilize a *RUNX2*:GFP transgene to isolate these periosteal cells for deep sequencing of expressed mRNAs, which will help identify macrophage-dependent genes activated in periosteal cells after injury. Together, positive findings will illuminate the role of the immune system in shifting the fate of periosteal cells from bone to cartilage during bone repair, with knowledge gained in the zebrafish model being used in the future to develop new bone repair strategies in patients.

Z6261C Zebrafish fin fold regeneration requires proper control of inflammation via macrophage. *T. Hasegawa*[1], C. Hall[2], P. Crosier[2], A. Kudo[1], A. Kawakami[1]. 1) Tokyo Institute of Technology, Yokohama, Japan; 2) University of Auckland, Auckland, New Zealand.

Elucidation of tissue regeneration mechanism is necessary for the realization of regenerative medicine. Analysis of its mechanism in regenerative animal models such as zebrafish is one of important approaches.

We have adopted the larval fin fold regeneration model and found that the *cloche* (*clo*) mutant, which lacks the hematopoietic and endothelial cells, displays a unique phenotype that apoptosis is induced in the regenerative blastema cells. Our analysis suggested that the blastema cells are normally maintained by a factor from the myeloid cells.

Here, we firstly examined which type of the myeloid cell is crucial for blastema maintenance. Knockdown of macrophage differentiation by the morpholino antisense oligo (MO) against *interferon regulatory factor 8 (irf8)* showed an increase of apoptosis during regeneration. On the other hand, knockdown of neutrophil differentiation by the colony stimulating factor 3 receptor *(gcsfr)* MO didn't effect survival of blastema cells. These results suggested that macrophage may play crucial role in the maintenance of blastema.

Next, we performed a transcriptome analysis and found that *interleukin1b (il1b),* a molecule that plays a central role in inflammatory response, is highly expressed in the *clo* for a prolonged time. Furthermore, treatment with dexamethasone, an anti-inflammatory reagent, or the *il1b* knockdown by MO significantly decreased the apoptosis in the *clo* mutant, suggesting that an excessive and prolonged inflammation is actually a cause for apoptosis in the *clo*.

Together, our study suggests a scenario that the tissue inflammation is properly regulated by a factor from the macrophage during regeneration.

Z6262A Manipulating hair cell regeneration in zebrafish lateral line neuromast. *D. LIU*[1], Y. WU[1], X. MI[1], H. GUO[1], Z. ZHU[1], D. W. Raible[2]. 1) School of Life Sciences, Peking University, Beijing, CN; 2) Department of Biological Structure, University of Washington, Seattle, WA, USA.

Hearing or balance diseases, due to inner ear hair cell (HC) loss, are a global problem. While mammals including humans cannot regenerate hair cells, other vertebrates can restore lost HCs through mitotic division or by direct conversion of precursors. It remains elusive how these two modes of regeneration are regulated and whether both modes share precursors. In this study, we find that HC regeneration in zebrafish posterior lateral line (pLL) neuromasts uses two sequential modes: direct conversion (resulting in new HCs within 24 hours of damage) and mitotic regeneration (occurring 24-72 hours after damage). The relative proportion by which each mode regenerates HCs is amendable. In brief or persistent low Notch activity and high Wnt signaling, direct conversion becomes major regeneration mode. Mitotic HC regeneration is also regulated by low early Notch activity and late Wnt signaling. In homeostatic neuromasts, newly divided, *atoh1a*$^-$ support cells (SCs at G1 phase of mitosis) are competent to respond to direct conversion cues, while a few *atoh1a*$^-$ SCs in early cell cycle (S phase) are ready to proliferate upon damage. In conclusion, the cell cycle states of a few *atoh1a*$^-$ SCs in homeostatic neuromasts determine by which mode they become nascent HCs in damaged neuromasts, and both modes are differentially regulated by Notch and Wnt signaling.

Z6263B Role of Neuropilins in Zebrafish Heart Regeneration. *V. J. Lowe*, C. Pellet-Many, Ian Zachary. University College London, London, GB,.

Background Zebrafish are able to fully regenerate their hearts after injury by resolving the initial scar and replenishing the injury site with functional cardiomyocytes. Transforming growth factor β and platelet-derived growth factor BB are known to be important for zebrafish cardiac regeneration. They are also ligands that trigger intracellular responses *via* receptor complexes including neuropilins (NRPs). NRP1 and NRP2 are co-receptors for vascular endothelial growth factors and other cytokines, essential for developmental angiogenesis and implicated in diverse pathophysiological processes, but little is known of their role following myocardial injury.

Hypothesis Because several NRPs binding partners are involved in zebrafish heart repair, we hypothesised that neuropilins play a role in zebrafish heart regeneration. We used the zebrafish cryoinjury model of myocardial infarction to characterise neuropilin spatio-temporal expression in the regenerating heart.

Results Quantitative PCR shows n*rp1a, nrp1b* and *nrp2a* are significantly upregulated at 1 and 3 days post cryoinjury (dpci), whereas *nrp2b,* the mostly highly expressed isoform in the heart, does not change after cardiac damage. *In situ* hybridisation shows expression of *nrp1a* mRNA localised to the activated epicardium, proximal to the injury. Immunofluorescence imaging also indicated strong upregulation of NRP1 by epicardial cells overlaying the injured region, and expression by the endocardium. Furthermore, epicardial NRP1 expression was demonstrated in epicardial cells *in vitro.* NRP2 is expressed in leucocytes during the initial inflammatory phase following injury and by cardiomyocytes migrating into the injured zone during the regenerative phase.

Conclusions Neuropilin isoforms exhibit marked regional differences in expression following cryoinjury. Our results indicate a novel role for NRP1 in the activation of the epicardium, a region of the heart with an essential role in cardiac regeneration, whereas NRP2 may be more important for inflammation and cardiomyocyte replacement.

Z6264C Unravelling the molecular mechanisms of myocardial de-differentiation during zebrafish heart regeneration. *C. J. Onderisin*[1,2], I. C. Scott[1,2]. 1) The Hospital for Sick Children, Toronto, Ontario, CA; 2) Department of Molecular Genetics, Univ. of Toronto, Toronto, Canada.

Adult mammalian hearts are incapable of regenerating lost cardiomyocytes (CMs) after injury, replacing them instead with akinetic scar tissue that negatively impacts cardiac function. The adult human heart is notorious for its limited regenerative potential; evidence from multiple studies suggests that the CM turnover rate may be as little as 1% or less per year. For this reason, decades of research efforts have focused on stimulating CM proliferation following trauma. To date, delivery of stem and progenitor cells to the injured mammalian myocardium has had limited success in improving cardiac function. A promising alternative approach involves activating endogenous regeneration programs that are conserved through evolutionary history, present in vertebrates such as the zebrafish. The zebrafish heart can fully regenerate following partial resection of the adult cardiac ventricle, or genetic ablation of CMs in adult or embryonic hearts. Virtually all regenerated myocardium derives from pre-existing CMs that sequentially de-differentiate and proliferate. However, a mechanistic understanding of how this process occurs has yet to be described in detail, and specific molecular markers for CMs in the early stages of de-differentiation and cell-cycle re-entry are undefined. Following chemical-genetic ablation of ventricular CMs in embryonic zebrafish hearts, it has previously been demonstrated that atrial CMs de-differentiate and proliferate to re-populate ventricular myocardium (Zhang, R. et al, Nature, 2013). Importantly, a transient population of atrium-derived de-differentiated CMs was identified that had terminated expression of the atrium-specific marker *amhc*, but had not yet adopted a ventricular CM fate, as measured by expression of the ventricle-specific marker *vmhc*. Here I present the embryonic zebrafish heart as a model to study the process of CM de-differentiation in the context of myocardial regeneration. By combining cell lineage tracing techniques with a temporally-sensitive reporter for heart chamber-specific gene expression, I present a strategy for the isolation of de-differentiated CMs for further transcriptional analysis.

Z6265A Hair cell regeneration in the zebrafish lateral line is impared by crude root extracts of *Valeriana officinalis*. *R. E. Rodriguez Morales*, A. Santana, M. Behra. University of Puerto Rico, MSC, PR.

Hair cells (HCs) are sophisticated mechanoreceptors found in sensory tissues of all vertebrate inner ears that transduce sound waves into electrical signals. Mammals, unlike birds and fish cannot regenerate them, making HCs loss the leading cause for hearing impairment in humans. We are interested in finding new agents that can trigger or hamper HC regeneration, but sensory tissues are deeply buried in the skull and therefore hard to reach and manipulate. We took advantage of the fact that HCs are also found in a fish/amphibian mechanosensory organ which is called the lateral line (LL). It is composed of stereotypically and superficially distributed sensory patches, the neuromasts (NMs), which contain HCs surrounded by supporting cells (SCs). Cilia of HCs are directly exposed to and deflected by currents of surrounding waters. HCs of the LL are sensitive to a range of ototoxic drugs (antibiotics, metals…) which are present in the water and can be easily and quite specifically destroyed this way. It was shown that HC destruction will trigger proliferation and differentiation of SCs into new functional HCs in all NMs over the course of 3 days. To ablate HCs, we treated 5 day post fertilization (dpf) larvae with copper and observed HC regeneration during the following 3 day post treatment (dpt) in the presence of crude plant extracts. One of them, *Valeriana officinalis* (Val) crude root extracts did significantly delay HC regeneration. Moreover, several regenerating NMs seemed unstable and starting at 2 dpt they presented apoptotic-like features. This was never observed in animals which had not been copper treated, suggesting that the presence of the extract had no effect on LL development but was specifically affecting its regeneration. Val contains more than 150 active components. One of them is Valeric acid which led to the synthesis of an FDA approved drug, valproic acid (VPA). One of the postulated roles of VPA is to be a potent inhibitor of histone deacetylase (HDAC). VPA and Trichostatin A, another HDAC, were both shown to affect HC regeneration possibly by affecting SCs division and survival. We are currently exploring this tantalizing lead to understand the mode of action by which Val is hampering HC regeneration. Our work shows for the first time that a crude root extract of *Valeriana officinalis* is interfering with HC regeneration.

Z6266B Thyroid hormone coordinates zebrafish pigment cell lineages during post-embryonic development and homeostasis. *Lauren M. Saunders*, Cole Trapnell, David M. Parichy. University of Washington, Seattle, WA.

Global, circulating endocrine factors are important for coordinating differentiation and morphogenesis of numerous stem cell derived lineages during post-embryonic development and later adult homeostasis. One such factor, thyroid hormone (TH), plays a major role in the diversification and maintenance of neural-crest derived pigment cell lineages in zebrafish. Different classes of pigment cells—including black melanophores and yellow xanthophores—have markedly different responses to TH signaling. TH represses melanophore population expansion, and fish lacking TH have twice the normal number of melanophores. By contrast, TH promotes xanthophore differentiation and proliferation. We do not yet understand how this single factor, TH, differentially impacts the differentiation and morphogenesis of these cell lineages. Because TH has potentially hundreds of downstream effectors, we are taking whole-transcriptomic approaches to understand its different roles in these two cell types, including the extent to which TH specifies alternative fates of multipotent progenitors or has cell-type specific effects on morphogenesis and terminal differentiation after fates have been specified. We are also using epigenomic analyses to test whether TH differentially impacts chromatin organization in melanophore and xanthophore lineages. We anticipate that our unusually tractable system will provide novel insights into how a common endocrine signal is translated into alternative cellular outcomes across lineages during the development and maintenance of adult form.

Z6267C The induction of radial glial cell proliferation after stab injury in the optic tectum of adult zebrafish. *Y. Shimizu*, Y. Ueda, T. Ohshima. Dept. of Life Sci. and Med. Bio-Sci. Waseda University, Tokyo, JP.

Adult neurogenesis is a highly conserved phenomenon that neural stem cells (NSCs) produce new neurons, astrocytes or oligodendrocytes in the adult brain. In the adult mammalian brain, neurogenesis is restricted to the subventircular zone (SVZ) and the subgranular zone (SGZ). In contrast, zebrafish have 16 NSCs niches and can continue to produce new neurons through life. In the optic tectum where optic nerves project, radial glial cells (RGCs) located in the deeper layer express several stem cell markers as Sox2 and msi1, and are quiescent, while RGCs in the telencephalon are proliferative and work as NSCs. We confirmed that the number of proliferative RGCs in the optic tectum was fewer than that in telencephalon.

Zebrafish have also great ability to regenerate brain after the traumatic brain injury compared to mammalian. In this study, we established the stab injury of the optic tectum and analyzed the proliferation of RGCs after stab injury. We confirmed that the induction of RGC proliferation after stab injury. Then we analyze the differentiation of RGCs by BrdU labeling for 24 hours and confirmed that the number of HuC, neuronal marker, and BrdU-double positive cells was increased in the injured optic tectum at 1 week post injury compared with those in the intact side.

To analyze molecular mechanisms that control the proliferation of RGCs in response to stab injury, we injected zymsoan A into cerebrospinal fluid using cerebroventircular microinjection and confirmed that the proliferation of RGCs was also induced at 1day post injection. We treated zebrafish with dexamethasone (Dex), anti-inflammatory drug, 2 days before and after stab injury. The proliferation of RGCs in the Dex-treated groups was decreased at 2dpi. These results suggest that inflammatory responses have a key role in the induction of RGC proliferation after the tissue damage in the PGZ. Wnt signaling is well known to regulate stem cell proliferation and differentiation during development and also contributes to the regenerative response after traumatic brain injury. To elucidate contribution of Wnt signaling to the proliferation of RGCs, we analyzed the Wnt reporter zebrafish after the stab injury. We confirmed that the expression of GFP was detected in the BLBP-positive cells after the stab injury in optic tectum. Then we treated injured-zebrafish with the Wnt inhibitor and analyzed the proliferation of RGCs. The number of proliferative RGCs was significantly decreased compared with that of DMSO-treated zebrafish. These results suggested that the up-regulation of Wnt signaling may induce the proliferation of RGCs in response to stab injury.

Z6268A Systemic and local signaling interfaces of zebrafish bone regeneration. *Scott Stewart*[1,4], Benjamin Armstrong[1,2,4], Kryn Stankunas[1,3,4]. 1) Institute of Molecular Biology; 2) Department of Chemistry; 3) Department of Biology; 4) University of Oregon, Eugene, OR.

Zebrafish tissues and organs display exceptionally robust self-repair capabilities. For example, damaged fins are readily restored, which entails perfectly regenerating bony ray structures, each comprising two semi-cylindrical bones that encase mesenchymal cells, nerves, and blood vessels. Bone regeneration in fin rays is mediated by injury-induced progenitor osteoblasts (pObs) that later redifferentiate to produce replacement bone. Two opposing signaling pathways, Wnt/β-catenin and BMP, balance competing demands for pOb renewal and differentiation, respectively, until regeneration is complete. To identify additional factors that directly influence Obs, we performed an in vitro screen using a pOb-like cell line derived from regenerating fins. Unexpectedly, we found that insulin or insulin-like growth factor (IGF), combined with dexamethasone, is sufficient to stimulate Ob differentiation. mTOR signaling is required for insulin/IGF-driven differentiation both in vitro and in regenerating fins. Using a novel O-propargyl-puromycin and click chemistry-based approach, we determined that re-differentiating fin Obs dramatically and specifically upregulate mTOR-dependent protein anabolism. We suggest that during an initial “burst” phase of bone regeneration, locally produced IGF2b stimulates full Ob differentiation. Concomitant with re-vascularization, regenerating bone then enters a “steady-state” phase, when the restored blood supply delivers circulating insulin to sustain Ob maturation. At both phases, we propose bone maturation integrates Ob-autonomous BMP signaling, which maintains a specific differentiated Ob transcriptional program, with insulin/IGF environmental cues that promote sufficient mTOR-dependent protein synthesis to generate a robust bone matrix.

Z6269B The contribution of biliary epithelial cells to hepatocytes in the developing liver with *tomm22* knockdown. *J. Wu*[1,2], T. Choi[1], D. Shin[1]. 1) University of Pittsburgh, Pittsburgh, PA; 2) Tsinghua University School of Medicine, Beijing, China.

The liver has an elegant regenerative capacity. After mild liver injury, hepatocytes proliferate to restore the lost liver mass and

function. However, when hepatocyte proliferation is impaired, which occurs upon severe liver injury or in chronic liver diseases, biliary epithelial cells (BECs) activate and give rise to hepatocytes. The only effective therapy for patients with severe chronic liver diseases is liver transplantation, but the shortage of donor livers necessitates alternative therapies. Studying BEC-driven liver regeneration may provide insights into how to augment innate liver regeneration in the liver patients as the alternative therapy. Here, we presented a new liver regeneration model in which BECs contribute to regenerated hepatocytes upon hepatocyte damage and loss. The hepatocyte damage results from temporary knockdown of the mitochondrial import gene *tomm22* by morpholino antisense oligonucleotides (MO) as reported by Curado et al., 2010. We found that all hepatocytes in *tomm22* MO-injected larvae at 7 days post-fertilization (dpf) weakly expressed *Tp1:*H2B-mCherry, a BEC marker, suggesting BEC origin of these hepatocytes. To unequivocally determine the origin of the regenerated hepatocytes, we permanently labeled BECs with a BEC Cre line, *Tg(Tp1:CreER^{T2}),* and traced their lineage in the MO-injected larvae. Surprisingly, we found maximum 60% of the hepatocytes were derived from BECs, suggesting that both BECs and survived, pre-existing hepatocytes contribute to the regenerated hepatocytes in the MO-injected larvae. In addition, we found that in the MO-injected larvae, survived hepatocytes weakly induced *Tp1:*H2B-mCherry expression, suggesting hepatocyte dedifferentiation. Furthermore, we found that both macrophage ablation and the suppression of Wnt/β-catenin signaling in the MO-injected larvae increased the number of *Tp1:*H2B-mCherry-negative hepatocytes and concomitantly decreased the number of *Tp1:*H2B-mCherry-positive hepatocytes at 7 dpf. This new liver regeneration model will help one better understand the mechanisms of liver regeneration upon hepatocyte damage and loss.

Z6270C miRNAs function to limit vascular development flexibility. *S. Nicoli*. Yale Cardiovascular Research Center, New Haven, CT.

miRNAs confer genetic robustness by precisely regulating protein expression. Studies in invertebrate models support that miRNA regulation of developmental gene networks provides phenotypic reproducibility under genetic or environmental perturbations. Whether miRNAs ensure uniform phenotypes in vertebrate development is unclear, as most mammalian miRNA gene knockouts do not have embryonic defects. Using a multifaceted, single-cell resolution screen of zebrafish embryonic blood vessels, we found that TALEN and CRISPR/Cas9 mutagenesis of single and multi-gene endothelial-expressed miRNA families significantly altered natural phenotypic variation of vascular cells. Genome-wide analysis of endothelial miRNA target genes revealed that each miRNA functions in distinct specification and morphogenesis signaling networks. Upon pharmacological network perturbation, the vascular system becomes further sensitized to aberrant malformations with progressive loss of miRNA gene copies. Our data establish that miRNAs act individually or in concert with other members in the phylogenetic family to faithfully reproduce tissue architecture complexity and to stabilize specific phenotypes in the face of variable perturbations. These discoveries demystify the cryptic function of miRNA-gene regulation in vertebrate development, and establishes it as a pivotal mechanism in defining an individual's phenome and disease susceptibility.

Z6271A Syndecan4 facilitates FGF signaling in trailing cells and cell migration in the zebrafish lateral line primordium. *C. M. Fox*[1,2], A. B. Chitnis[1]. 1) NICHD, Bethesda, MD; 2) Johns Hopkins University, Baltimore, MD.

The posterior lateral line (PLL), a sensory system that zebrafish use to sense water movement, is an extraordinarily tractable system in which to study cell migration and pattern formation. The posterior lateral line primordium (PLLP) spearheads the early development of this system, migrating from the ear to the tail, depositing rosettes of cells that will develop into mechanosensory neuromasts. Two signaling systems work in concert to achieve this pattern in the PLLP: Wnt signaling maintains a population of mesenchymal cells in the leading domain and fibroblast growth factor (FGF) signaling organizes cells into protoneuromasts in the trailing domain. Heparan sulfate proteoglycans (HSPGs), found in the extracellular matrix and on the cell surface, are known to regulate both Wnt and FGF signaling in a variety of developmental processes. Several core proteins for HSPGs are expressed within the PLLP and have been shown to be generally important for FGF signaling during PLLP migration. We show that the HSPG syndecan4 (SDC4) is expressed in the most trailing cells of the PLLP, where FGF signaling depends on a diminishing supply of FGF ligand. Knockdown of *sdc4* interferes with protoneuromast formation, slows migration speed, and weakens FGF signaling output. Likewise, in *sdc4* mutants with compromised FGF signaling, we observe slower migration. Overexpression of *sdc4* induces dorsalization, a common effect of ectopic FGF signaling. Together, these results show that SDC4 maintains FGF signaling in the trailing cells of the PLLP, facilitating protoneuromast development and collective cell migration.

Z6272B The Sec14-like Phosphatidylinositol Transfer Proteins Act as GTPase Proteins to Mediate Wnt/Ca^{2+} Signaling. *S. Jia*, B. Gong, W. Shen, W. Xiao, Y. Meng, A. Meng. Tsinghua University, Beijing, CN.

The non-canonical Wnt/Ca^{2+} signaling pathway plays important roles in embryonic development, tissue formation and diseases. However, it is unclear how the Wnt ligand-stimulated, G protein-coupled receptor Frizzled activates Phospholipases for calcium release. We report that the zebrafish/human phosphatidylinositol transfer protein Sec14l3/SEC14L2 act as GTPase proteins to transduce Wnt signals from Frizzled to phospholipase C (PLC). Depletion of Sec14l3 attenuates Wnt/Ca^{2+} responsive activity and causes convergent and extension (CE) defects in zebrafish embryos. Mechanistically, Sec14l3-GDP forms complex with Frizzled and Dishevelled; Wnt ligand binding of Frizzled activates Sec14l3 on the plasma membrane and the resulted Sec14l3-GTP binds to and activates phospholipase Cδ4a (Plcδ4a); Subsequently, Plcδ4a tethers and cleaves phosphatidylinositol-4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol 1,4,5-triphosphate ($InsP_3$), ultimately stimulating calcium release. Furthermore, Plcδ4a can act as a GTPase-activating protein to accelerate the hydrolysis of Sec14l3-bound GTP to GDP. Our data provide a new insight into GTPase protein coupled Wnt/Ca^{2+} signaling transduction.

Z6273C Atrazine Affects Cartilage and Heart Development in Zebrafish (*Danio rerio*). *C. S. Lassiter*, B. S. Walker. Roanoke College, Salem, VA.

Atrazine is a commonly used herbicide that has been implicated as an endocrine disrupting compound. Atrazine's role in cartilage and bone formation has yet to be established. Craniofacial cartilage and bone deformities account for a large number of birth defects in the United States. Zebrafish treated with 1 µM atrazine had gross morphological defects in their cartilage at 5 days post fertilization, while embryos treated with concentrations below 1 µM had cartilage elements with increasingly wide angles, resulting in a shorter, fatter face with increasing atrazine concentration. Further investigations into the heart rate and hatch rate of larvae revealed atrazine leads to increased heart rates and decreased hatch rates in embryonic zebrafish. Future experiments into the effects of atrazine on bone need to be performed in order to gain a better understanding of the overall effects of this chemical.

Z6274A Wdr68 modulates TGFβ interference with BMP signaling for lower jaw patterning. *A. J. Martinez*, T. Whitman, G. Alvarado, R. Shang, M. Yousefelahiye, Y. Yu, A. Pham, B. Wang, E. Alvarado, R. M. Nissen. California State University Los Angeles, Los Angeles, CA.

Birth defects are among the leading causes of infant mortality and contribute substantially to illness and long-term disability. Craniofacial anomalies, excluding cleft lip and palate, occur in 1 out of every 1600 births in the United States. Many craniofacial syndromes are caused by defects in signaling pathways that pattern the cranial neural crest cells (CNCCs) along the dorsal-ventral axis. For example, defects in Bone Morphogenetic Protein (BMP) signaling are associated with cleft lip/palate, auriculocondylar syndrome is caused by impaired Endothelin-1 (Edn1) signaling, and Alagille syndrome is caused by defects in Jagged-Notch signaling. The BMP, Edn1, and Jag1b pathways intersect because BMP signaling is required for ventral edn1 expression that, in turn, restricts jag1b to dorsal CNCC territory. In zebrafish, wdr68 is required for both the ventral Meckel's (M) cartilage and the dorsal Palatoquadrate (PQ). Previously, we identified the wdr68 gene as essential for edn1 expression in the zebrafish. Here we show that wdr68 activity is required between the 17-somites and prim-5 stages through experiments using an inducible Tg(hsp70l:GFP-Wdr68) zebrafish line. This identified developmental window overlaps with the known onset of edn1 expression at the 18-somites stage. Using an RNA rescue assay and in situ hybridization (ISH), we also found that ectopic edn1 mRNA can rescue the expression of the downstream target dlx6a in wdr68 mutants. We similarly examined the expression patterns of jag1b, hey1, and grem2 and found ventrally-expanded expression of these otherwise normally dorsally-restricted genes. Strikingly, we found that the BMP agonist ISL could partially rescue lower jaw formation and edn1 expression in wdr68 mutants. To elucidate the mechanism of action, we generated wdr68 deletions by CRISPR/Cas9 gene targeting in the BMP-responsive mouse C2C12 cell line. However, we found no defects in pSmad1/5 accumulation or BMP reporter induction in wdr68 mutant cells relative to non-target (NT1) controls. The Transforming Growth Factor Beta (TGFβ) signaling pathways are also important for several patterning events during early embryonic development, including proper craniofacial development. Notably, TGFβ can interfere with BMP signaling via Smad3 displacement of Smad4 from pSmad1/5 complexes. We examined this potential mechanism and found that TGFβ interference with BMP signaling was greater in wdr68 mutant cells relative to NT1 controls. To determine whether the interference mechanism might also act in vivo, we treated wdr68 mutant zebrafish embryos with the TGFβ signaling inhibitor SB431542 and found a partial rescue of craniofacial development. Together these data suggest an indirect role for Wdr68 in the BMP-Edn1-Jag1b signaling hierarchy through modulating TGFβ interference with BMP signaling.

E8001A *Tetrahymena* in the classroom: An example of the use of model organisms in K-12 education. *D. Cassidy-Hanley*, T. Clark. Cornell University, Ithaca, NY.

Model organisms can play an important role in K–12 science education. Engaging students in hands-on activities featuring real organisms adds a dimension of interest and excitement that cannot be obtained with other teaching methods, and encourages students to creatively explore scientific ideas and concepts. Here we present a successful educational outreach program that utilizes the resources of the national *Tetrahymena* Stock Center to foster the use of a safe, easy-to-grow protozoan for hands-on inquiry-based educational activities in K-12 classrooms. It is our hope that sharing our approach for supporting educational use of a model organism may provide useful insight and information to others interested in increasing the use of model organisms in K-12 classrooms. ASSET (Advancing Secondary Science Education through Tetrahymena), an NIH SEPA funded program at Cornell University, has worked with teachers and students to develop inquiry-based K-12 biology curricula that feature hands-on manipulation of live *Tetrahymena* cells, and that are ideal for demonstrating many of the basic principles of biology in novel and engaging ways. Teachers and student input during curriculum development insures that the self-contained modules are grade appropriate and user friendly, and rigorous testing and evaluation confirm that the educational content increases student understanding of fundamental biological concepts and knowledge of the scientific method. Grade appropriate cross-curricula activities engaging students in the inter-relatedness of science and society are used to make science more relevant to real life issues and to increase overall student interest in science. A unique independent high school research program is also being developed to provide students, especially at low-resource schools, with the means of carrying out a small independent research project in their classroom. All ASSET modules contain comprehensive teacher guides, detailed student protocols, unit-related questions, and answer keys. Guides to relevant literature are also provided, with links to additional source material available on the website. To support the use of ASSET modules at schools serving minority and low-income students, the basic materials needed to carry out the experimental protocols, including live cells and growth media, are available without charge using an on-line request system. In addition, an equipment lending library provides basic equipment for free short-term loan to under-resourced schools to insure equitable access to ASSET curricula. ASSET offers opportunities for collaborative educational outreach to interested *Tetrahymena* researchers around the country, and provides a model for the integration of model organism stock center resources with K – 12 educational outreach.

E8002B 'Moving' AP Biology forward: Using *Drosophila*-optimized wrMTrck to examine muscle mutants. *Nicole M. Green*[1], Rebecca Steiger[2], David Brooks[1], Erika R. Geisbrecht[1]. 1) Kansas State University, Manhattan, KS; 2) USD 475 Geary County Schools, Junction City, KS.

As scientific technology and techniques advance at an ever-increasing rate, researchers should be mindful to aid in the development of foundational concepts useful to the next generation of researchers. Current techniques are often underrepresented in pre-baccalaureate level programs due to lack of funding, equipment and expertise. However, technological advances in the lab and classroom have extended our ability for collaboration on topics of increasing complexity through guided experimental inquiry. The fusion of engineering solutions and scientific investigation forms the backbone of the Next Generation Science Standards (NGSS) that drives K-12 curriculum. This creates a need for more inventive and technology-inclusive laboratory modules and opens the door to broader impact projects to aid in recruitment of the next generation of researchers. Through our collaboration with the USD 475 Geary County school district, we have integrated our lab's research on muscle development into an inquiry-driven lab in which students use the model *Drosophila melanogaster* to examine the consequences of defective muscle proteins on overall organismal locomotion. Recently our lab has optimized a freely available ImageJ plugin for *C. elegans*, known as wrMTrck, to function as a tool for tracking the path and velocities of *Drosophila* L3 larvae. Biological structure-function relationships can easily be seen by using a genetic mutant for *thin/abba*, an E3 ubiquitin ligase found in muscle. Loss of proper protein turnover causes progressive degeneration of L3 somatic musculature and resulting locomotory handicaps. This laboratory module exposes students to the power of *Drosophila* for screening genes involved in muscle development and maintenance, as well as how to conduct and creatively analyze mutant phenotypes. We have adapted phases of experimental design to function in any normal high school lab via the use of iPhone/Android video capture and freely downloadable software installed on either PC or Mac computers. By providing accessible K-12 protocols modified from our own experimental procedures, we can motivate students to think critically, internalize scientific practices, and engage in authentic research experiences prior to their entry into institutes of higher education.

E8003C DNA barcoding: engaging students in molecular biology and bioinformatics through authentic biodiversity research. *E.Bruce. Nash*, David Micklos, Sharon Pepenella, Cristina Fernandez-Marco. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Just as the unique pattern of bars in a universal product code (UPC) identifies each consumer product, a "DNA barcode" is a unique pattern of DNA sequence that identifies each living thing. Using tools developed by Cold Spring Harbor Laboratory's DNA Learning Center (DNALC), educators can engage their students in the process of science, using molecular biology, ecology, and bioinformatics to design and answer questions about biodiversity.

The DNALC has a longstanding commitment to simplifying complex biochemical and bioinformatics workflows for use in biology education. Our mitochondrial DNA sequencing service and *BioServers* analysis workflow (http://www.bioservers.org) was the first distributed project to allow students to analyze their own DNA sequences. Over 110,000 student sequences have been uploaded since the program's inception in 1997, and the *BioServers* website has logged 1.6 million user sessions.

As educational lead for *CyVerse* (formerly iPlant Collaborative), NSF's major cyber-infrastructure for life sciences, the DNALC formally moved into the world of biological big data. Since 2010, DNALC staff have introduced *CyVerse* tools to 1,700 researchers and college educators at 2-day workshops conducted around the U.S. Under this project the DNALC also developed *DNA Subway*, an educational interface to bioinformatics workflows – including DNA barcode data analysis. Compementing these bioinformatics tools, the DNALC has also developed streamlined biochemistry and a sample database for student DNA barcoding research.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

Meeting the need for authentic research experiences, students can use the same tools and data as seasoned biologists to: 1) design their own research questions 2) sample organisms and collect metadata; 3) isolate DNA and amplify barcoding regions; 4) submit DNA for sequencing; 4) process and analyze sequences; and 5) use sequences to study phylogenetics, diversity, and evolution. Students use simple, affordable DNA isolation protocols and the DNALC's online analysis tools as they discover novel barcodes and track biodiversity - results that they can enter into the project database and publish. These scalable hands-on experiments and participation in a global biodiversity initiative immerse students in the process of science, providing relevance and ownership that enhance learning and attitudes towards science.

The poster will summarize the DNALC's efforts to introduce student DNA barcoding to diverse students, the results of these student efforts, and the effect on student learning and attitudes toward science and science careers.

E8004A SMART research collaborations to foster K-12 STEM development. *M. A. Pickart*[1], D. LaFlamme[2], X. Xing[1]. 1) Concordia University Wisconsin, Mequon, WI; 2) Saint Dominic Catholic School, Brookfield, WI.

Fostering STEM education and career paths remain a national priority. Despite the acknowledged importance of mentorship and support networks for student professional development, STEM enriching activities often do not foster lasting relationships with science professionals nor provide needed opportunities for youth to participate in professional science meetings, publications, and other activities. This is true for all educational levels, but is particularly challenging for elementary and middle schools where the complexity of language and concepts may hinder communication and learning when young students work together with science professionals. To meet these challenges, this work has centered on creating a continuum of STEM educational experiences through authentic, life science focused collaborative research for teams of students, teachers, and science professionals. The Milwaukee School of Engineering SMART (students modeling a research topic) Team Program that facilitates student abstraction of complex chemical concepts needed to understand structure function relationships of proteins by creating 3D printed models has been used for the basis of developing collaborative research teams. With an emphasis on building both relationships and models, the SMART Program provides the unique focus on scientific communication and process necessary for team development. Subsequently, teams have worked together to address specific research questions aimed at validating and extending scientific knowledge through experimentation in zebrafish. Over the past few years, proteins investigated include Collagen 8a1, T (Brachyury), and Factor X. Importantly, students have been welcomed into professional zebrafish conferences such as ZDM8, Boston, 2015 to present their findings and participate in a professional science meeting. In summary, this overall approach provides a unique way of addressing dual needs for both STEM education and scientific advancement in a fun, engaging, and meaningful way while fostering professional development for all involved.

E8005B Disruption of Sortilin-related receptor (sorl1) gene causes severe malformations, apoptosis and stunted structure in newly TALEN Knockout zebrafish *Danio rerio* model: Construction and molecular characterization. *T. Saleh*[1,2], J. Lee[1], S. Chang[1], H. Oh[1], K. Kim[1], T. Sakuma[3], T. Yamamoto[3], J. Park[1]. 1) Seoul National University, Seoul, Korea; 2) Suez Canal University, Egypt; 3) Hiroshima University, Japan.

Tamer Said Abdelkader[1,2], Ji-Min Lee[1], Seo-Na Chang[1], Hanseul Oh[1], Kyung-Sul Kim[1], Tetsushi Sakuma[3], Takashi Yamamoto[3], and Jae-Hak Park[1]*

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The sorl1 gene is a neuronal apolipoprotein E receptor predominantly expressed in the central nervous system. It is genetically associated with Alzheimer's disease (AD) as a switch in the Amyloid precursor protein (APP) processing pathway. Significant reduction in the gene expression has been found in brain tissue of AD patients. TALEN plasmids were constructed using the Platinum Gate TALEN Kit. Sets of plasmids designed to disrupt the first exon of zebrafish sorl1 gene. DSB-inducing activities of constructed TALEN plasmids were evaluated using a human cell-based reporter assay. Synthesized TALEN mRNAs were injected into cytoplasm of 1-cell stage embryos 1nl containing about 100-300 ng/ul mRNAs. Microinjected eggs were maintained in E3 medium at 28ºC for further analysis. Targeted genomic loci were amplified using primers designed to anneal approximately 150–200 base pairs upstream and downstream from the expected cut site. The test analysis showed high activity of generated TALEN plasmids than positive control ZFN. The fold change of sorl1_A TALENs with sorl1_A reporter recorded value was about 4 times. Analysis of genomic DNA extracted from TALEN microinjected embryos demonstrated three mutations in the spacer sequence; alteration of Cytosine nucleotide (C) to Guanine nucleotide (G) and addition of two thymine nucleotides (T) bases on different locations. Abnormalities like bent tail, stunted structure, cardiac edema, and apoptotic cells were found in defected individuals. We therefore conclude that disruption of first exon of sorl1 gene by TALENs mRNAs causes severe malformation suggesting after reduction of App function.

Keywords: TALEN, Sorl1, knock-out, zebrafish, *Danio rerio.*

E8006C *Drosophila* cancer model used to introduce research to freshman biology majors. *J. Ahlander*. Northeastern State University, Tahlequah, OK.

Model organisms are an excellent way to introduce students to research. The purpose of this work was to use a *Drosophila* cancer model in a freshman biology majors laboratory course as the experimental foundation for learning scientific thinking and research methodology. The *eyeful* cancer model exhibits an eye tumor phenotype caused by hyperactivation of the Notch and Akt signaling pathways. In a classroom laboratory, I used the *eyeful* cancer model to help students to study the relationship between genetics and the environment on the *eyeful* tumor phenotype. We discovered that altering the nutrient density of the fly food affected the proportion of flies exhibiting the tumor phenotype, where caloric density positively correlated with tumor formation. I modified this experiment for use in a freshman majors biology lab using a simple low versus high calorie food comparison. Within a 2-hour laboratory period, freshman students were asked to distinguish

between "normal" and "cancerous" phenotypes of flies raised on either low or high calorie media. Although they had never participated in such research beforehand, most students found a statistically significant difference between the experimental conditions. The students were then allowed to design and carry out their own experiements over subsequent weeks of the semester to determine whether other dietary changes could affect tumor formation in this experimental model. Using this approach, several benefits were gained by both faculty and students. Student motivation to participate in the class was high because of a general interest in diet and cancer, and they were able to test their own hypotheses regarding this relationship. In a short period of time a large number of students were trained in a classroom setting on some of the most critical aspects of biological science education - how to think about, design, and conduct research experiments. Finally, as several of these students continued to participate in independent laboratory research after the semester had finished, this approach can help students to discover early in their education whether research is something they may want to pursue.

E8007A Approaches and assessment of incorporating authentic research experiences into an undergraduate genetics course. *D. P. Aiello*. Austin College, Sherman, TX.

Mentoring of undergraduates by faculty in the research lab is a high impact teaching practice that improves a range of students' disciplinary skills, data acquisition and application skills, and writing and oral presentation skills, among others. These formative experiences can also provide a clarification of purpose for undergraduates with regard to career path. However, faculty are often limited in the number of students they can effectively mentor in a traditional research lab setting, thereby limiting the number of student participants. Incorporating authentic undergraduate research experiences into the teaching laboratory is a high-impact teaching practice that can achieve many similar outcomes as mentored research lab experiences, but provides for a broader engagement of students. Presented here is an approach taken over the last several years to incorporate authentic research experiences into a sophomore-level genetics class at a private liberal arts college. The laboratory combines two distinct, semester-long, research projects, one of student-design and one of instructor-design based on ongoing work in the research lab. The laboratory takes advantage of the relative ease of working with the model organism *Saccharomyces cerevisiae* (budding yeast) to introduce students to the foundational principles of transmission and molecular genetics. Three years of assessment using the Classroom Undergraduate Research Experience (CURE) Survey, developed by David Lapatto at Grinnell College, indicates significant learning gains by students participating in the course. The goals of this presentation are to engage and share ideas with colleagues from other institutions using this pedagogical approach, and to share ideas with others seeking to include authentic research into their own classrooms.

E8008B Promoting leadership development within undergraduate STEM curricula. *D. P. Aiello*, L. F. Barton, S. L. Gould, K. S. McCain, K. E. Reed, J. M. Richardson. Austin College, Sherman, TX.

All thoughtful, scientifically trained people are called to lead at some point in their lives and should be prepared to do so. The STAR Leadership Program is designed to intentionally develop five leadership behaviors (Collaborative Work, Interpersonal Communication, Problem Solving, Foresight and Planning, and Moral Consciousness) amount undergraduate STEM students. The curricular component of the program integrates intentional instruction on leadership theory and activities to promote the development of leadership skills in existing science courses. By having multiple opportunities to learn and practice these skills within different disciplinary contexts, and at different levels of instruction, students are given opportunities to develop their awareness, abilities, and commitment to leading others. Thus far, leadership instruction has been integrated into 25 courses from within biology, chemistry, environmental studies, and physics, ranging from introductory to advanced course levels. Behavior competencies are directly assessed using rubrics that were developed for all five leadership behaviors, derived from the AAC&U VALUE Rubrics, and modified to fit the needs of the STAR Leadership Program.

E8009C An undergraduate laboratory class using CRISPR/Cas9 technology to mutate Drosophila genes. *Richard Cripps*[1], TyAnna Lovato[1], Vanesa Adame[1], Holly Chapapas[1], Marilyn Cisneros[1], Carol Deaton[1], Sophia Diechmann[1], Chauncey Gadek[1], Maria Chechenova[1], Paul Guerin[2]. 1) Department of Biology, Univ New Mexico, Albuquerque, NM; 2) Institute for Social Research, University of New Mexico, Albuquerque, NM.

CRISPR/Cas9 genome editing technology is used in the manipulation of genome sequences and gene expression. Due to the ease and rapidity with which genes can be mutated using CRISPR/Cas9, we sought to determine if a single-semester undergraduate class could be successfully taught, wherein students isolate mutants for specific genes using CRISPR/Cas9. Six students were each assigned a single Drosophila gene, for which no mutants currently exist. Each student designed and created plasmids to encode single guide RNAs that target their selected gene; injected the plasmids into *Cas9*-expressing embryos, in order to delete the selected gene; carried out a two-generation cross to test for germline transmission of a mutated allele and generate a stable stock of the mutant; and characterized the mutant alleles by PCR and sequencing. Three genes out of six were successfully mutated. Pre- and post- survey evaluations of the students in the class revealed that student attitudes towards their research competencies increased, although the changes were not statistically significant. We conclude that it is feasible to develop a laboratory genome editing class, to provide effective laboratory training to undergraduate students, and to generate mutant lines for use by the broader scientific community.

E8010A *Drosophila* and zebrafish in undergraduate teaching laboratories and student-driven independent research projects. *M. Daggett*, H. Babcock. Missouri Western State University, St. Joseph, MO.

Drosophila and zebrafish have been incorporated into undergraduate teaching laboratories and independent research projects at Missouri Western State University. Both of these model organisms are accepted model organisms for the study of embryonic development, genetic based diseases and in toxicology. This poster outlines and presents specific protocols and practical information for how these two model

organisms are used at a predominately undergraduate institution with limited available resources. Examples of recent student projects will be presented, including the use of *Drosophila* in student-driven studies that test the effects of diet on the regulation of metabolism and physiological function.

E8011B Experiments in Inclusive Education. *E. A. De Stasio*, L. Stinson, B. T. De Stasio. Lawrence Univ, Appleton, WI.

We describe here concerted efforts to support and encourage persistence of members of marginalized groups in STEM education at our institution. These efforts include formation of a formal group jointly led by students and two science faculty members in which issues of inclusion, allyship, and instances of discrimination and micro-aggressions are discussed anonymously but openly in a safe space. Anecdotes revealed by students of color in particular were eye-opening for faculty members and have sparked change. For example, did you know that Think-Pair-Share pedagogy can lead to isolation in your classroom unless it is carefully choreographed? Attention to students' experiences led to experimentation with pedagogy and inclusion of topics that support the experiences of marginalized groups in an undergraduate genetics course that will be outlined. Lastly, we describe a version of supplementary instruction for introductory biology, the goals of which are community formation and practice with a variety of study skills, with content drilling as a tertiary goal. Though these experiments are being implemented in a small liberal arts college, the lessons learned should be transferable to other educational settings.

E8012C Teaching Experimental Design through Worm Picking. *N. C. Evans*. Purdue University Calumet, Hammond, IN.

At many primarily undergraduate institutions, research opportunities in biology can be difficult for students to obtain. Yet, due to the nature of science and the careers students will seek, research is an important part of their educational experience. The Experimental Design course at PUC is intended to allow undergraduate students to experience research projects from conception to completion within in the context of a semester long course. This course takes advantage of the short reproductive cycle of *C. elegans* to perform student led experiments focusing on genetic, molecular, and microscopy techniques - but could easily be modified based on the goals, skills, and equipment availability of any institution. The course is broken down into two projects. In the first project each student pair utilizes both forward and reverse genetics to analyze a unique mutant *C. elegans.* During the course of the genetic analysis additional skills are gained including animal husbandry, light and fluorescence microscopy, generation of recombinant DNAs, use of RNAi, DNA sequencing, and qRT-PCR. In the second project, students research, propose, and carry out experiments to test the effect of environmental factors on a physiological process (for example aging). This exposes students to the process of literature searches and design of well controlled experiments. Unlike, the traditional laboratory course, it is anticipated that students may not complete some portions of the project or may not obtain results. Efforts are then made to help students understand why and to design future experiments to address these issues. Furthermore, the layout of the class is ideal for teaching various forms of scientific communication including grant writing, journal article preparation, and oral/poster presentations. Due to the time limitations of this lab intensive course, digital tools can be integrated using a Learning Management System. For example, some course material is delivered via video tutorials, collaborative tools are used to edit and improve student writing, and discussion boards are utilized to promote student communication of ideas and results, and to encourage students to help each other with experimental difficulties.

E8013A Integrating professional development opportunities during graduate education. *Joyce Fernandes*. Miami Univ, Oxford, OH.

National dialogs such as Vision and Change, Future of the Research Enterprise, Engage to Excel, Achieving Systemic Change, have called for changes in instructional approaches that revitalize the learning environment at academic institutions. Supporting faculty development and leadership are key to sustaining pedagogical reform. This poster will present a case for including professional development opportunities for graduate students in a seminar format that allows the integration of research/teaching responsibilities. Such an approach, with a focus on improving educator skills can benefit graduate students irrespective of whether they enter academic or non-academic career paths. A Graduate seminar on "Trends in Undergraduate Biology Education" was offered for a 15-week period. The structure of this seminar and the outcomes will be presented. All studnets participatd in a survey at the end of the semester. With a focus on retention in Introductory Biology courses, students read and discussed at least 12 papers in the field. $1/5^{th}$ of the seminar time was used to engage students in an ongoing intervention to measure the impacts of a supplemental course on the success of undergraduates in an introductory biology class. The graduate students participated in data collection, which involved a quantitative analysis of assignments [concept maps and outlines], and a discussion of the rationale for the particular approach. 17/18 students enrolled in the seminar wished to teach at a 2- or 4-year primarily undergraduate institution; 18/18 indicated (1) the lack of adequate training to prepare them as TAs (2) the need for getting formative feedback about their output as TAs, and (3) to provide opportunities beyond the usual TA experiences to develop their skills not only in teaching but also in communicating the essence of the topic being taught. These and additional data from the survey will be presented such as preparation of TAs, how they develop thier teaching style, and what they found most useful by attemnding hte seminar.

E8014B Microscopic image analysis of zebrafish pigmentation in an undergraduate cell biology laboratory. *A. M. Henle*. Carthage College, Kenosha, WI.

I developed a new zebrafish laboratory module to introduce undergraduate cell biology students to animal handling, microscopic image acquisition, and analysis. This laboratory module addresses several of the core competencies outlined by the Partnership in Undergraduate Life Science Education *Vision and Change in Undergraduate Biology Education*, including integration of quantitative reasoning, the process of science, and modeling and simulation. Students work with two or more strains of zebrafish to compare pigmentation in the caudal fin. Adult zebrafish are anesthetized and the caudal fin is amputated. Melanophores, which are the melanin-containing pigmented skin cells, are then imaged in the caudal fin via microscopy. Students analyze their acquired images on the computer using the freeware FIJI (Fiji Is Just ImageJ,

NIH) to calculate the area of pigment dispersal (an estimate of cell size) and total pigmentation in the caudal fin. These measurements are compiled as class data and are compared across multiple lines of zebrafish to allow students the opportunity to work with authentic datasets. Students in this lab are excited to work with zebrafish and gain a deeper understanding of model organism research. This lab module is ideal for the undergraduate setting in that it provides students with an opportunity to design and perform a novel experiment, it uses limited resources, can be completed in as few as two lab sessions, and can be performed with many lines of zebrafish.

E8015C STEAM (Science Technology Engineering Arts and Math) approaches in the undergraduate classroom. *M. Kaplow*. New York Univesity, New York, NY.

New pedagogical approaches have recently emerged in higher education to make scientific learning more active and engaging in the classroom. While the curriculum of many colleges have started to use active learning techniques such as case study analysis, analysis of scientific literature, the emergence of STEAM, a method for integrating creativity and the arts with STEM fields is largely absent in the classrooms of undergraduate education. STEAM was initially developed at the Rhode Island School of Design as a way to foster collaboration between artists and engineers. The purpose of STEAM was to solve engineering problems through the creative approaches of design. While STEAM teaching practices have primarily flourished and have been utilized in elementary school education, STEAM remains to be explored in the undergraduate classroom. The following study investigates utilizing STEAM pedagogy in undergraduate upper elective science courses. Results show that students majoring in both biology and neuroscience are unfamiliar with combining creative arts with scientific ideas and concepts. While many students are familiar with reading and analyzing scientific papers most have never integrated art in STEM classrooms. Student assessments revealed students general reluctance to use “drawings” “schematics’’ during classroom assignments. Students were only willing to use creative arts approach to questions that were more challenging to answer. Results analyze the relationship between STEAM teaching techniques and student learning outcomes. The ongoing study proposes methods on improving student learning through STEAM techniques. The study also outlines approaches that make STEAM more accessible to undergraduate students in higher education.

E8016A Yeast orphan gene project: Finding a place for ORFans to GO. *J. B. Keeney*[1], E. Strome[2]. 1) Juniata College, Huntingdon, PA; 2) Northern Kentucky University, Highland Heights, KY.

When the genomic sequence of the model eukaryote *Saccharomyces cerevisiae* was completed in 1996, the expectation was that an understanding of the integrated functioning of the collection of genes in this single-celled eukaryote would shortly follow. Despite almost 20 years of intense collaborative effort among yeast researchers, nearly 10% of open reading frames (ORFs) are considered uncharacterized. Determining the function of these orphan genes (ORFans) will require mining the current yeast genomic data, compiled in the *Saccharomyces* genome database (SGD), to most effectively design ORF-specific experiments in cell and molecular biology, and comparative genomics. A network of yeast researchers/educators with a focus on teaching experimental design could help overcome the challenges and absorb the risks of researching individual ORFans. The goal of the proposed Yeast Orphan Gene Project is to organize a consortium of undergraduate researchers and faculty at primarily undergraduate institutions (PUIs) to coordinate resources and design strategies to assign molecular functions to *S. cerevisiae* ORFans. Ultimately, the yeast orphan gene project aims to use the process of determining orphan gene function as a tool to teach undergraduate students key concepts in bioinformatics, genomics, molecular biology, and genetics and impart valuable experience in scientific collaboration and leadership. Based on analysis of SGD information, students will design and execute ORF specific experiments for defining gene function. The network will facilitate collaboration between students at different institutions so that students can share strategies and technique solutions while developing experience in on-line collaborations. These skills, including the ability to (1) apply the process of science, (2) use modeling and simulation, and (3) communicate and collaborate, are core competencies in “Vision and Change”. The proposed network will prepare undergraduates for careers in STEM, provide tools for faculty at PUIs to expand research experience for undergraduates, and incorporate research experiences into undergraduate courses. It will also be a mechanism to distribute a tested model of an authentic course-based research experience to a diverse set of institutions by providing workshop and assessment support. The networking activities will expand the expertise of faculty, as well as provide undergraduates tools and resources for collaboration. Defining the function of yeast ORFans will advance the goal of the yeast community to determine the function of the entire set of annotated yeast ORFs.

E8017B The Genomics Education Partnership: Assessment of Key Elements of a Course-based Undergraduate Research Experience (CURE). *Judith Leatherman*[1], Anna Allen[2], Justin DiAngelo[3], M. Logan Johnson[4], Chris Jones[5], Lisa Kadlec[6], Hemlata Mistry[7], Alexis Nagengast[7], Don Paetkau[8], Susan Parrish[9], Laura Reed[10], Cleo Rolle[11], Jamie Sanford[12], Ken Saville[13], Chiyedza Small[14], Joyce Stamm[15], Matthew Wawersik[16], Leming Zhou[17], David Lopatto[18], Sarah Elgin[19], The Genomics Education Partnership. 1) University of Northern Colorado; 2) Howard University; 3) Penn State Berks; 4) Notre Dame College; 5) Moravian College; 6) Wilkes University; 7) Widener University; 8) St. Mary's College-IN; 9) McDaniel College; 10) University of Alabama Tuskegee; 11) Capital Community College; 12) Ohio Northern University; 13) Albion College; 14) Medgar Evers College-CUNY; 15) University of Evansville; 16) College of William & Mary; 17) University of Pittsburgh; 18) Grinnell College; 19) Washington University, St. Louis.

The Genomics Education Partnership (GEP) is a consortium of faculty members from over 100 colleges and universities who are involving students in classroom-based undergraduate research experiences (CUREs) in bioinformatics. Our project uses comparative genomics to study the unusual properties of the *Drosophila* Muller F element (dot chromosome). Students participate in two research areas: sequence improvement and gene annotation, working from recently sequenced *Drosophila* species. Student results are reconciled and pooled for final analysis; our recent paper in *G3* had 940 undergraduate co-authors (Leung *et al.* 2015 *G3* 5(5): 719-40). Our previous educational research

shows that students who participate in this project show attitude and knowledge assessment gains, irrespective of the type of institution or the implementation strategy used by the faculty member (ranging from short lab modules to stand-alone research courses and independent research projects). However, the level of student gains is strongly correlated with the amount of time spent on the project. GEP faculty members report that implementation of this research-based curriculum is strongly facilitated by core computational and pedagogical support from the Washington University team, and that a shared curriculum and organizational structure can mitigate issues caused by varying campus-level support. Our current educational research focuses on two questions: first, how active pedagogical strategies impact student learning gains, and second, how reminding students that they are performing original research impacts students' attitudes toward science. We are also developing new curriculum to make the GEP project more accessible to beginning students, including those at community colleges. Our consortium continues to recruit new faculty collaborators, particularly those interested in CUREs for first and second year undergraduate students. Supported by HHMI grant #52007051, NSF grant #1431407, and Washington University in St. Louis.

E8018C Open Genetics Lectures (OGL): An Open Source Introductory Genetics Textbook. *J. Locke*, L. Canham, M. K. Kang, M. Harrington, M. Deyholos. Univ Alberta, Edmonton, AB, CA.

Commercial introductory genetics textbooks are expensive for students and often don't cover topics or recent discoveries desired by instructors. At the University of Alberta we have written an open source college/university level introductory genetic textbook titled Open Genetics Lectures (OGL). This text contains ~128,000 words organized into 41 chapters that correspond to lecture sized topics typically covered in an introductory genetics course. Topics include "DNA is the Genetic Material" through to "CRISPR-Cas9 Technology". Each chapter includes many figures and tables that can be used in lectures, and ends with questions about its content. Sample answers are provided. Because it was written under the Creative Commons Copyright (open source), all text, figures, tables, and questions can be used without restrictive copyright concerns. The text chapters can be downloaded as Microsoft Word files or PDF format. Each chapter can be individually deleted or edited, or additional ones can be added to customize the text for any course.

All text, tables, images and questions can be accessed through an archive site:
https://dataverse.library.ualberta.ca/dvn/dv/OpenGeneticsLectures.

E8019A Zebrafish lateral line as an inquiry-based lab model for cell biology. *Jason Meyers*. Colgate University, Hamilton, NY.

As a bridge between a foundations-level survey of biology and upper level electives, we have recently developed a tier of courses that focus more on the process of doing biology rather than being content driven. As part of this tier, we are developing multi-week investigative labs that allow students to take an active role in experimental design, while introducing them to key methods within the field, and focus on iterative student-led exploration rather than obtaining specific expected results. For one of these courses focused at the cellular level, the majority of the laboratory portion utilized the zebrafish lateral line as a model. The zebrafish lateral line is a series of small sensory organs, called neuromasts, along the surface of the fish. These sensory organs are made up of clusters of sensory hair cells, homologous to those in in the inner ear, surrounded by supporting cells. These sensory organs are formed from a migratory primordium that travels along the embryonic fish's body depositing small clusters of cells, with zones of proliferation and differentiation coordinated by well-known signaling cascades including Wnt, FGF, and notch. As the development of the lateral line involves cell migration, cell cycle and proliferation, differentiation and cell cycle exit, cytoskeletal alterations, mesenchymal-to-epithelial transitions, and cell-cell signaling utilizing several different pathways, this system encapsulates many of the broad themes of cell biology. The rapid development, widely available transgenic lines that label key cell types or serve as reporters, and ease of genetic and pharmacological manipulation make it possible for young undergraduate students to easily explore many different aspects of these cells in an inquiry-based fashion. At the end of the term, students developed their own three-week research project building on aspects of their earlier investigations. The structure of the lab exercises and simple variations, examples of student data, and student outcomes will be discussed.

E8020B Using the Yeast Mating Response to Study Genetics and Cell Biology: From the Biology Lab to the Computer Lab and Back. *Michelle A. Mondoux*[1], Lara K. Goudsouzian[2], Patricia Riola[2], Karen Ruggles[2], Pranshu Gupta[2]. 1) College of the Holy Cross, Worcester, MA; 2) DeSales University, Center Valley, PA.

We have developed and implemented a laboratory exercise appropriate for a sophomore or junior-level Biology course. The laboratory reinforces and integrates several topics, including the cell cycle, the effects of checkpoint activation, signal transduction, and the cytoskeleton. Depending on the themes emphasized by the instructor, the laboratory can be adapted for either a Genetics or Cell Biology course.

Students evaluate the growth of *Saccharomyces cerevisiae* cultures of opposite mating types and observe the effects of adding mating pheromone on cell division and cell shape. Growth and morphology are assayed at regular intervals over the course of several hours. By the end of the laboratory, students have become adept at identifying the stages of the yeast cell cycle via their distinct morphologies by performing "bud counts". Students also become practiced in the use of a spectrophotometer to determine the concentration of cells in growth medium. Students can then analyze the data by generating growth curves and plotting changes in morphology over time. Both analyses lead to the demonstration that yeast cells will respond in characteristic ways to mating pheromone, but only in the presence of the opposite-sex mating factor.

This year, we have entered into a collaboration with a Computer Science seminar course on campus. The students in this course have been assigned the task of creating supporting materials for the yeast mating labs, with the Cell Biology professor as the group's formal "client". In accordance with the client's requests, the students are generating simulations of the laboratory, appropriate for use by those who take Cell

Biology lecture without enrolling in the laboratory. The Computer Science students have also been asked to generate games (both two-dimensional and three-dimensional) to accompany the laboratory, to reinforce the learning outcomes of the experiment as well as increase interest and engagement of the students.

Our preliminary assessment of this novel hybrid laboratory measures how the laboratory and the computer games and simulations, both individually and in conjunction, influence student learning outcomes and student attitudes and engagement in Cell Biology.

E8021C A Simple HPC Workflow for RNA-Seq in the Classroom. *E.Bruce. Nash*, Spector Mona, Williams Jason, Ghiban Cornel, Lauter Sue, Yang Chun-hua, Micklos David. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

RNAseq – an entryway to Undergraduate Big Data Analysis:

A Simple HPC Workflow for RNA-Seq in the Classroom

Nash, E. Bruce, Spector M., Williams, J., Ghiban, C., Lauter, S., Yang, C., and Micklos, D.

In the last decade, "next-generation" sequencing (NGS) methods have decreased DNA sequencing costs by 10,000-fold. This means that undergraduate biology students could potential work with the same data and tools as high-level researchers. Surveys by the Cyverse (formerly *iPlant Collaborative*), an NSF-funded national cyberinfrastructure for biology, reveal that 95% of graduate students and researchers say they are currently, or soon will be, using large sequence datasets. However, two-thirds have little or no experience with bioinformatics and only a third have access to sufficient computational resources needed to analyze these data.

We have created an extensible infrastructure and training program that enables faculty to integrate NGS into undergraduate instruction. Free access to these tools democratizes NGS, allowing faculty at any undergraduate institution to engage their students in cutting-edge biological research.

Many biology faculty are also attempting to scale research projects into course-based undergraduate research experiences (CUREs). To allow NGS CURES, we developed the Green Line of *DNA Subway* (http://www.dnasubway.org) as a user-friendly graphical interface that allows access to compute resources of NSF's Extreme Scientific and Engineering Discovery Environment (XSEDE) to perform computationally intense RNA-Seq analysis through a web browser.

The project focuses on RNA-Seq as a tractable whole genome analysis for those new to NGS. The Green Line supports analysis of transcriptomes to measure gene expression or novel transcripts.. Collaborating with a faculty Working Group, we developed week-long workshops to introduce RNA-Seq to faculty. These workshops are now being adapted into online training materials.

Details of the program, online tools, and the results and effects of implementation in CURES and independant student research will be presented.

E8022A No lectures here: How an active and problem-based learning classroom in genomics transformed the confidence, creativity and communication skills of all students. *A. R. Skop*, S. Neumann, B. Minkoff. Univ Wisconsin-Madison, Madison, WI.

Effective use of active learning techniques remains a challenge in STEM disciplines. We will present our success with Genetics 564, a 100% active learning-based undergraduate capstone course on Genomics and Bioinformatics. Genetics 564 is devoid of 'traditional' lecture-based teaching. Students learn about bioinformatic techniques through the reading and presentation of primary literature, and apply this knowledge to their research project as they analyze a human disease gene. The culmination of students' research is the writing of a set of specific aims and the publication of a website detailing their results. Students also engage in an iterative process of peer review throughout the semester. This course uses active learning to provide students with presentation, research, and peer review experiences similar to those used by scientists. In Genetics 564, we have observed that, with direction, undergraduate students can utilize both primary literature and bioinformatic databases to design experiments and test hypotheses. Additionally, students constantly peer- and self-review their presentations and scientific writing, a second layer of active learning that challenges them to not only produce high-quality research but also to critique their work, an important aspect of a truly active classroom. Tasks such as these are usually reserved for graduate level students and higher. The success of the students in our course has demonstrated its utility for teaching students scientific presentation, writing, research, and review skills, and student responses to the course have been overwhelmingly positive. Altogether, this suggests that the active and project-based learning we have employed contributes to both students' success and motivation within our course setting. Given this, we propose that multiple courses across disciplines would benefit from incorporating aspects of the Genetics 564 structure into their undergraduate classroom. All course materials are freely available on the Genetics 564 website (http://genetics564.weebly.com), where one can also find an archive of past student final projects.

E8023B A Multi-Course Inquiry-based Science Laboratory Module Approach Integrates Research and Teaching through functional annotation of the *Tetrahymena thermophila* geneome. *J. J. Smith*. Missouri State University, Springfield, MO.

In 2006 the ciliated protozoan *Tetrahymena thermophila* genome was sequenced and predicted gene annotation has been completed. *Tetrahymena* is a unicellular model organism that has been used for molecular and cellular biological discoveries such as telomeres, histone modifications, and catalytic RNA. Now experimental research must be done to further functionally annotate the *Tetrahymena* genome, which will increase the usefulness of this model organism for many areas of study. In order to further annotate the genome of *Tetrahymena* and start functional and proteomic annotation of this organism we have taken on a multi-course inquiry-based approach to class laboratories in cell and molecular biology curriculum at Missouri State University. In this approach the predicted *Tetrahymena* genes are cloned into entry plasmids in an introductory level biomedical science lab (performed by Missouri Sate University honors students). The cloned genes can then be used in upper division molecular biology classroom labs in order to characterize the expression, localization, and protein interactions. Students in all classes conduct bioinformatics and gene expression experiments in order to

further characterize and confirm the annotation of the predicted genes. Through this multi-classroom inquiry-based laboratory approach students learn the basic techniques required for laboratory research in science as well as aid in the further genomic and proteomic annotation of the *Tetrahymena thermophila* genome. The students classroom laboratory experience culminates in the submission of their results for publication on the Student/UnPublished Results database (SUPRdb) website (http://ciliate.org/suprdb/; for student classroom research data). This allows any scientific researcher in the field to see what genes have been studied and the most current results and resources for each gene.

E8024C Research/education partnerships to develop course-based undergraduate research experiences. *R. M. Spell*[1], P. Hanson[2], M. E. Miller[3], C. W. Beck[1]. 1) Emory University, Atlanta, GA; 2) Birmingham-Southern College, Birmingham, AL; 3) Rhodes College, Memphis, TN.

Partnerships between research and education provide a powerful resource in the creation of course-based undergraduate research experiences (CUREs) for students. A national faculty survey about authentic research experiences in introductory laboratory courses found: 1) More than half of the intro courses incorporated **little or no** authentic research exposure; 2) **Lack of time** to develop new research experiences was the biggest barrier to implementation of authentic research in laboratory courses (4 out of 4 on the Likert scale); and 3) Faculty definitions of authentic research revealed non-overlapping constructions of course-based authentic research that emphasized either **the process of science** or **novel questions** (**Spell *et al*. 2014**). To bridge the divide between disciplinary researchers and education specialists and between separate emphases on the development of scientists (Process of Science) or the development of science (Novel Questions), our NSF-funded REIL–Biology RCN-UBE has recruited disciplinary researchers as part of institutional teams to develop curriculum for authentic research experiences in introductory laboratory courses. We have held workshops at both education and research-oriented conferences. Our first workshops generated applications from over 30 different institutions including faculty from diverse institutions including community colleges and minority-serving institutions and massive online college systems, in addition to liberal arts colleges and research universities. We discuss this effort in the context of a continuum of strategies to foster faculty and curriculum development and to provide examples for faculty creating their own CURE.

E8025A Research based learning in bioinformatics using yeast experimental evolution. *Laurie Stevison*[1], Molly Burke[2]. 1) Auburn University; 2) Oregon State University.

I recently taught an Introductory Bioinformatics course for advanced undergraduate and graduate students using the text 'Practical Computing for Biologists'. As a new course in an active learning classroom, I designed the course to follow a research study from start to finish to simulate real world challenges of data analysis and dissemination. I searched NCBI for a public dataset using yeast, due to their small genome size, and found an evolve and resequence experiment based on multiple genome sequences. Throughout the semester I corresponded with the first author, Dr. Burke, who provided advice and access to datasets. Early in the semester, students were each assigned an accession of a yeast genome. Then, in concert with teaching genomics and the GATK best practices workflow in class, the students analyzed datasets within research teams to understand the challenges of data analysis. I split the analysis into steps to scaffold the analysis to help the students hone their bioinformatics skills. In class, they learned several bioinformatic tools, and were required to use these tools to assess data quality and submit group reports. The reports reinforced their understanding of the tools and allowed them to see how the data quality improved at each step. While they were required to examine data in multiple ways, they were asked only to report findings relevant to their conclusions. After completing data analysis, I transitioned the class to statistical analysis. For this, I combined their samples into a larger class dataset moving forward. Each group performed a different statistical analysis done in the original paper. The students were introduced to Github earlier in the class, which has built-in educational assessment tools. Although this class is still ongoing, for their statistical analysis, they will make a 'readme' file and upload the associated scripts and graphs to a data repository to teach them that research should be repeatable. Dr. Burke will also be available to students in case they have any questions for the author. Then, they will read the manuscript to compare/contrast their findings to the original study. Since the class only analyzed 5 of 12 replicate populations, this comparison is meant to reinforce the large effort of one scientific paper. After a few lectures on effective science graphics and communication, they will present their work in an auditorium open to the biology department. The final step will be peer assessment where they evaluate each other's presentations and Github pages. While the students were not required to have prerequisites besides genetics, a statistics prerequisite was highly recommended. Future iterations may require some expertise in command line prior to taking the course as proficiency in this area is needed to handle the required workload.

E8026B F.I.R.E. lab: A full immersion research experience in an undergradate laboratory course. *C. L. Van Buskirk*, students of CSUN BIOL447/L. California State University Northridge, Northridge, CA.

CSUN biology majors are eager for opportunities to work closely with faculty members and gain research experience. However, there are not enough spaces in faculty labs to meet this need. The course BIOL447/L: Full Immersion Research Experience (F.I.R.E. lab)[1] helps to bridge that gap, allowing up to 18 students to participate in original, student-directed research. In contrast with many undergraduate lab courses that introduce students to a series of techniques, F.I.R.E. lab instead focuses on introducing students to the full process of scientific inquiry within the confines of a limited set of techniques.

During the first half of the course, students are introduced to a series of research articles that familiarize them with a specialized topic, in this case sleep behavior in *C. elegans*. During this phase of the course they also become familiar with nematode handling, behavioral assays, and RNAi. During the second half of the course, students perform a series of experiments that they have designed to address a unique question, and have the option of working individually or in groups. Prior to starting their projects, students apply for 'funding' by writing a grant proposal that undergoes rounds of peer review and instructor feedback. Student proposals must include an experimental strategy that is amenable to

the twice-per-week class schedule.

In the final weeks of the course, students analyze data, prepare figures, and assemble their work into a poster that they present at a mock conference on the final day of class. Several students go on to present their work at a campus-wide symposium the following semester. Some student projects contribute significantly to peer-reviewed publications[2]. Moreover, positive outcomes from the initial offering of this course contributed to a successful NSF:CAREER award application. This poster presentation is aimed at faculty seeking to better integrate their research and educational activities, and will include examples of student projects, challenges and successes, and assessment tools.

1. https://sites.google.com/site/biol447/

2. Hill, A.J., Mansfield, R., Lopez, J., Raizen, D.M., and C. Van Buskirk. (2014) Cellular Stress Induces a Protective Sleep-like State in *C. elegans*. Curr. Biol. 24; 2399-2405.

E8027C DNA Subway – An Educational Bioinformatics Platform for Genomics and Course-based Research. *J. J. Williams*[1,2], Sheldon McKay[3], Cornel Ghiban[1,2], Uwe Hilger[1,2,4], Mohammed Khalfan[5], Sue Lauter[1,2], David Micklos[1,2], CyVerse. 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) CyVerse, T.W. Keating Bioresearch Building, University of Arizona, Tucson, AZ; 3) Ontario Institute for Cancer Research, MaRS Centre, Toronto, ON, Canada; 4) BIO5 Institute, T.W. Keating Bioresearch Building, University of Arizona, Tucson, AZ; 5) Center for Genomics and Systems Biology, New York University, New York, NY.

DNA Subway is an educational bioinformatics platform developed by CyVerse (formerly iPlant Collaborative - NSF #DBI–0735191). *Subway* bundles research-grade bioinformatics tools, high-performance computing, and databases into web-based, classroom-friendly workflows. "Riding" *DNA Subway* lines, students can predict and annotate genes in up to 150kb of DNA (Red Line), identify homologs in sequenced genomes (Yellow Line), identify species using DNA barcodes and phylogenetic trees (Blue Line), and examine RNA-Seq datasets for differential transcript abundance (Green Line).

The American Association for the Advancement of Science and other research and science policy groups have reached similar conclusions about reforming undergraduate Science, Technology and Engineering, and Math (STEM) education: 1) Focus on the first two years of college education is critical to recruiting and retaining STEM majors. 2) Foster conceptual understanding, higher level thinking, and practice of STEM rather than memorization of terms, facts, and techniques. 3) Adopt inquiry and student-centered approaches that begin with students' own questions. 4) Increase opportunities for interdisciplinary and collaborative work. *Subway* promotes these principles by combining bioinformatics with wet-lab 'hooks,' offering educators a paths to using student-generated data in course-based research experiences (CURES).

Since 2010, *Subway* has supported more than 90,000 student projects, and longitudinal data documents 19,418 student exposures collected from undergraduate faculty trained at CyVerse 'Genomics and Education' and NSF Advanced Technological Education workshops. Student using *Subway's* streamlined tools (e.g. 'Red Line' genome browsers) demonstrate increased confidence when using these tools in the same contexts as researchers (e.g. through online databases such as *UCSC Browser*). At the secondary level, high school students participating in research projects using *Subway* demonstrate comparable level of gains on attitudinal and behavioral measures to the aggregate results of undergraduate students assessed using the Survey of Undergraduate Research Experience (SURE).

Examples from The *Urban Barcode Project* (http://www.urbanbarcodeproject.org) and *RNA-Seq for the Next Generation* project (http://www.rnaseqforthenextgeneration.org) highlight how products of student research can be exported and utilized in follow-up experiments, including direct publication of sequence data to NCBI GenBank. *DNA Subway* is freely accessible at dnasubway.org.

E8028A *CourseSource*: a journal of evidence-based teaching resources for undergraduate biology education. *R. L. Wright*. University of Minnesota, Minneapolis, MN.

Colleges and universities nationwide are changing the ways they teach undergraduate biology courses. These changes reflect a growing understanding and application of evidence-based education practices, including active learning, aligned assessments, and inclusive teaching strategies. Implementation of these evidence-based practices will support recruitment and training the next generation of scientists, and also enable greater gains in scientific literacy among the non-scientists in our biology classes.

This educational transformation is slowed by the time and energy that an educator must devote to learn about evidence-based strategies and to develop teaching materials that implement those strategies. In response to this need, a national convocation (Vision and Change in Undergraduate Biology Education: A Call to Action) recommended creating a peer-reviewed, open access journal of student-centered, evidence-based biology education resources. *CourseSource,* now in its second year, is a response to that recommendation.

Built on collaborations with scientific societies, including the Genetics Society of America, *CourseSource* is an open-access journal of peer-reviewed college biological teaching materials that:

- Incorporate student-centered, evidence-based pedagogy.
- Focus on learning goals and objectives determined by scientific societies.
- Can be easily replicated or adapted for other classrooms.

CourseSource enables authors to publish teaching materials in a high-quality format that documents their scholarly teaching efforts, accomplishments, and innovations. A key feature of *CourseSource* is organization into "courses" with learning goals and objectives developed by the related scientific society. To see the genetics learning framework, go to http://coursesource.org/courses/genetics.

Just 12 months after the journal went live, each article had been downloaded an average of 25 times, suggesting that many readers are finding the articles to be useful. We will describe *CourseSource* publication standards, provide examples, and consult with colleagues about their ideas for *CourseSource* articles.

E8029B Course-Based Undergraduate Research in Molecular Biology. *D. Zies*. University of Mary Washington, Fredericksburg, VA.

The Biology Department at the University of Mary Washington recently revised our curriculum to focus on the research process. In addition to other requirements, Biology majors are now required to complete a two course sequence designed to improve their understanding of how scientific knowledge is acquired. First they take BIOL260, The Research Process, in which they are introduced to scientific method and the statistical analysis of data. Then they enroll in one of several upper level courses designated as research intensive. These courses require that small groups of students carry out a short research project and present their results in a research forum. I redesigned my molecular biology course to meet the new requirement and taught the course in the fall of 2014 and 2015. My course was designed around the use of yeast as a model organism. The lecture material was taken entirely from review articles and primary literature. In the laboratory portion of the course, student groups chose one gene from the lecture topics as the focus of their research project. Early on, students learned molecular techniques that they used later in the semester to characterize the mutant phenotype of their gene and to show normal gene expression under cellular conditions of their choice. Students had to research their gene of interest, write a proposal, design their own set of experiments, carry out the experiments and present their results to the rest of the class. In general, the course design was successful. Student comments from the first offering suggested that they enjoyed the topics and felt real ownership of their research projects. They did, however, struggle with designing and carrying out their projects. It was clear that our students were not well prepared for research and their comments confirmed the need for this type of course in our curriculum. In the second offering of the course, I administered the CURE pre-course and post-course surveys and received a more formal evaluation of the course. These results show that while students made significant gains in several areas of their appreciation for research, there is still room for improvement. In addition to evaluating my own course, my department has begun an evaluation of our two course sequence. We administered the CURE pre-course survey during the first two weeks of BIOL260 in fall 2015 and spring 2016. At the end of this semester, we will collect matched post-course surveys from students that enrolled in a research intensive course this spring. We will continue to collect these data over the next few semesters. Preliminary data from the first students that complete the survey in the two course sequence will be presented.

E8030C Learning how to teach: Using the PALM fellowship to design a student-centered instructional unit for a large-enrollment genetics classroom. *Christopher L. Baker*[1], Michelle K. Smith[2]. 1) The Jackson Laboratory, Bar Harbor, ME, USA; 2) The University of Maine, Orono, ME, USA.

The Promoting Active Learning & Mentoring (PALM) Network, a partnership between several communities including GSA, supports the incorporation of student-centered, evidence-based learning techniques into undergraduate education by partnering PALM fellows with experienced faculty. As founding members of this fellowship, we worked together to design an instructional unit on meiotic recombination and genetic linkage for a large-enrollment undergraduate genetics course. We selected meiotic recombination and linkage to capitalize on the research experience of the fellow in meiotic recombination and the faculty mentor's expertise in genetics education research and access to a large-enrollment class. Furthermore, previous work has shown that undergraduate genetics students struggle to integrate how linkage, recombination, and chromosome dynamics fit together in a unified model for heredity. In addition, the process of recombination is often introduced only as the mechanism for allowing gene mapping, rather than a fundamental biological process required for proper completion of meiosis and a critical driver of evolutionary diversity required for natural selection. Our instructional unit addresses these concepts through clicker questions with peer discussion, chromosome modeling, and in-class small group activities. To assess the impact of this instructional unit we: 1) examined student pre/post performance on the Genetics Concept Assessment, which contains questions about linkage and the arrangement of alleles on chromosomes, 2) used student responses to clicker questions to provide formative assessment feedback, and 3) measured student performance on summative assessments including homework and exams. We also examined instructional practices of the fellow using the Classroom Observation Protocol for Undergraduate STEM (COPUS), which records the behaviors of the instructor and students. Results of these assessments and plans to further improve the learning unit will be presented along with feedback on the effectiveness of the lesson from student surveys.

E8031A An Undergraduate RNAi-Based Genetic Screen Reveals a Novel Component of the Polyamine Transport System. *Michael Haney*[1], Corey Seavey[1], David Brown[1], Cole Washington[1,2], Adam Foley[1,2], Pascale Lubbe[1,2], Melissa Vega[1,2], Michael Dieffenbach[1,2], Connor Michalski[1,2], David Abad[1,2], Fiona Quigley[3], Laurence von Kalm[1]. 1) University of Central Florida, Orlando, FL; 2) Undergraduate Author; 3) High School Author.

Polyamines are small organic molecules required for multiple cellular functions including proliferation, growth, control of chromatin structure and transcription. Cancer cells require an abundance of polyamines through biosynthesis and transport to maintain their proliferative phenotype. An FDA approved drug (DFMO) that targets synthesis is often ineffective because malignant cells circumvent the therapy by up-regulating transport. Thus a combination therapy targeting both synthesis and transport simultaneously is highly desirable. Though synthesis is thoroughly explained, import through a polyamine transport system (PTS) remains undefined. Since polyamine transport is observed in all organisms this represents a major gap in our knowledge of a basic cellular process and hinders the development of effective drugs targeting the PTS.

Utilizing a team of undergraduate students supervised by graduate students, our lab conducted an RNAi-based genetic screen to identify components of the polyamine transport system. Starting with a previously identified gene encoding a family of P-type 5B ATPases we identified 29 genes with similar spatial and temporal expression profiles. These genes were then tested in a novel RNAi-based survival assay developed in our laboratory. As a result of the screen we identify RabX6, which is involved in endosomal transport as a novel component of the PTS.

E8032B Plumbing STEM education: Designing a "Pipeline" CURE for a small teaching-focused college. *K. L. Schmeichel*[1], T. W. Lee[2], C. May[1], D. J. Katz[2]. 1) Oglethorpe University, Atlanta, GA; 2) Emory University, Atlanta, GA.

Course-embedded undergraduate research experiences (CUREs) improve student learning, engagement, and retention in STEM disciplines. However, due to limited research support, undergraduate educators at primarily-teaching institutions are at a disadvantage when implementing such state-of-the-art practices. Nation-wide models provide accessible CURE options that address this disparity, but alternative models that benefit from the intimate faculty-student relationships that exist at teaching-focused colleges are also possible. A collaboration between an investigator at an R1 institution, Emory University, and a nearby teaching-focused college, Oglethorpe University, resulted in the design of a novel "Pipeline" CURE strategy that is a fusion of the apprentice- and cohort-style of undergraduate research and that can be implemented with basic laboratory infrastructure. Because Oglethorpe Biology majors take more than one course with an instructor, the faculty can build concept sophistication over time in a deliberately developmental manner. The "Pipeline" CURE applies the same sensibilities to laboratory work: over the course of four-years, students are incrementally trained through laboratory activities themed around the R1 investigator's laboratory focus: stem cell reprogramming in C. elegans. Students are introduced to nematode husbandry and behavior in Introductory Biology; as sophomores they use C. elegans in a linkage project in Genetics. In an upper-level class, students apply these skills in performing novel genetic screens germane to the R1 laboratory's research agenda. Under these circumstances, the R1 investigator can pilot high-risk inquiries that could spin-off into fruitful projects for graduate students/fellows. Although we have yet to produce a cohort that has completed a full pipeline, the foundations for this model are in place. In the fall of 2015, C. elegans modules were included in Introductory Biology and in Genetics with the idea that a fully-trained cohort will emerge from the pipeline in 2017. We have also incorporated class-wide RNAi screens for candidate enhancers/suppressors of a sterility defect, into the laboratory portions of two Developmental Biology offerings (2014 and 2016; 14 and 19 students, respectively). Anecdotal remarks indicated that students were engaged and enthusiastic about their involvement in authentic research. We also learned several logistical lessons for more fluid implementation in future iterations. The results of the 2016 cohort, including responses to indirect attitudinal surveys and direct performance evaluations are pending completion of the current academic term. If these trials prove effective at supporting students' interactions with the Biology curriculum, the "Pipeline" CURE approach may prove applicable to other teaching-focused/research-based academic alliances.

E8033C Fostering critical thinking skills via analysis of primary literature. *E. Tour*, C. Abdullah, R. Lie, J. Parris. Univ California, San Diego, La Jolla, CA.

Primary literature offers rich opportunities to teach students how to "think like a scientist." Calls for increased incorporation of original scientific literature into science education have been issued by a variety of educational organizations (e.g., "Vision and Change", AAAS, 2011). We describe a Master's-level course that offers a structured analysis of four recent papers from diverse fields of biology: one flawed paper, one exemplary paper, and a pair of conflicting papers. Students who took this course self-reported a significant increase in a variety of skills associated with critical engagement with primary literature (e.g., being able to critically analyze a paper's data, independently draw conclusions, propose a follow-up experiment). However, objective measures of the same students' skills detected only an improved ability to design experiments. We will also present the first analysis, to our knowledge, of what these recent biology undergraduates perceive as the most challenging aspects of engaging with the primary literature. We analyzed 69 pairs of pre- and post-course free responses to the question: "What aspects of reading and analyzing primary literature do you find most challenging?" Thematic analysis of these data was conducted by three raters who were blind to both the identity of the students and the pre-/post- status of the response. Before instruction, the challenges that students reported centered around unfamiliar experimental techniques, background, and jargon-rich scientific language. After instruction, drawing independent conclusions and evaluating authors' conclusions became the most frequently identified challenge. In addition, after taking this course, the frequency of challenges aligned with the Higher Order Cognitive Skills (Analysis, Synthesis, Evaluation) increased significantly. Together, these changes are consistent with a more competent, critical approach to reading scientific papers. We discuss the implications of our findings to instructional programs that utilize scientific primary literature.

E8034A Compatibility between learning and examination styles – analysis of the performance of students in advanced genetic courses. *Krassimir Yankulov*. University of Guelph, Guelph, ON, CA.

Learning styles and their effect on the efficacy of education is an important topic in educational research. Consequently, there have been numerous attempts to adjust lecture styles and course offerings in a fashion that is equally compatible with students who prefer the writing/reading style and students who prefer visual learning. However, the question on how to fit examination to the preferred learning styles of students has rarely been addressed.

Here I report the learning outcomes of students who prefer a presentation assignment versus students who prefer a writing assignment. Both groups have been students in a 4th year molecular genetics course. The performance of these two cohorts has been measured by their marks in the midterm and final exams and by their engagement in class discussions. Statistical analysis of the data indicates that the writers have slightly but consistently outperformed the presenters. Considerations of teaching style variations could not explain the higher marks of the writers. These observations suggest that strong writing skills can provide a sizeable gain to the overall success at university level. However, the possibility that the writers outperform the readers because of the predominant writing exams is not excluded.

Some recent data on this issue will be provided to initiate discussion on this intriguing topic.

E8035B Student peer review: an educational and assessment tool for upper year genetic courses. *Krassimir Yankulov*. University of Guelph, Guelph, ON, CA.

Peer reviews are the generally accepted mode of quality assessment in scholarly communities, however they are rarely used for evaluation at college levels. Over a period of seven years I have performed a peer review simulation at a senior level course in molecular genetics at the University of Guelph. Briefly, I have asked groups of two authors to write research proposals on one of four topics that are announced in the beginning of the semester. On average 10-12 proposals are written each year. Specific guidelines on how to compose the proposal are given ahead of time to the whole class. These proposals are submitted to a dedicated web tool available at our university (PEAR, Peer Evaluation, Assessment and Review) and anonymously directed to the class members for a peer review. This simulation of grant submissions and review has a significant educational value and is very well accepted by the students. The actual PEAR site is designed to fully protect the identity of the authors and the reviewers and meets the highest standards of double-blind review process.

I have used the data in PEAR to analyze the metrics of this simulation exercise. I show that student peer marks are highly variable and not suitable as a precise performance evaluation tool. Subsequent analyses have shown ways to improve precision of student evaluation, but their applicability is yet to be established. Interestingly, student peer reviews can clearly recognise substandard performance, but the peers struggle to distinguish between good and excellent performance. These finding provide provocative insight on the process of peer review in general.

E8036C ***Fungal infections Aspergillossis and Cryptococcal meningitis in C H U Oran .*** *Z. B. Benmansour*. Chu University of Medecine Oran, Algeria, oran, DZ.

Objective: The invasive fungal infections in hospital is a subject of concern both for the healthcare professionals .The infection at the hospital is a major risk for the patients the invasive acts, in first row of morbidity, mortality.

Materials and methods A retrospective study was conducted from september 2013 to Mars 2015, fungal infections immunocompromised patients and the occurrence of Cryptococcus neoformans admitted with Aspergillosis was diagnosed in 23 individuals. The median age of the patients under study was39.25 years With male preponderance .(27 cases/36), neck stiffness (16/36), altered consciousness (14/36), fever (12/36) and convulsions (9/36).Cryptococcal meningitis highly contributes to mortality in HIV-infected patients. A need exists to improve strategies for clinical management of AIDS patients and systhematique Aspergillosis 430 samples from different departments .Cryptococcal meningitis in HIV-infected patients is animportant fungal pathogen in immunocompromised patients. High mortality was related to delayed diagnosis.

Results: prevalence of the meningo-encephalitis cryptococcose at the patients infected by theHIV was 0,4 %. frequency practically decreased while it was 2,09 %**Discussion**: Theevolution of the symptomatologie before hospitalization represented 1 week. disease is progressive,fever, headache,immunosuppressed patient, with a rate of CD4 lower than200 / mm3,

Conclusion: the fungal infection relegated to the last rows neuromeningeal cryptococcose Aspergillosis in systematic study.

E8037A Fellowships in Research and Science Teaching (FIRST): An integrative postdoctoral experience that generates effective researchers and educators. *Joanna Wardwell-Ozgo*[1], Teresa Lee[1], Gandhy Pierre-Louis[1], Arri Eisen[1], J. K. Haynes[2], Doug Eaton[1]. 1) Emory University School of Medicine, Atlanta, GA; 2) Morehouse College, Atlanta, GA.

The Fellowship in Research and Science Teaching (FIRST) at Emory University is a three-year postdoctoral training program designed to provide a quality foundation for successful careers in academia. Supported for 16 years by an Institutional Research and Career Development Award (IRACDA) from the NIH, FIRST combines interdisciplinary research training with instruction in teaching pedagogies, classroom technology, course development, and undergraduate mentoring. As with traditional postdoctoral positions, fellows work under the direction of research mentors at Emory University, a nationally recognized research institute. As a crucial addition, however, fellows also receive training in innovative pedagogical methods like active learning and technology-based teaching. Fellows apply these skills by teaching and mentoring students at one of three Historically Black Colleges or Universities in Atlanta: Morehouse College, Spelman College, or Clark-Atlanta University. FIRST also aims to increase the overall representation of minority scientists within the biological/biomedical sciences by maintaining a diverse population of fellows, and thus inspiring minority undergraduates to pursue the same path. In 16 years, FIRST has trained ~170 fellows. In comparison to their peers in traditional postdoctoral positions or fellowships, FIRST fellows publish at the same rate, receive comparable external funding, and importantly, are more successful at obtaining academic positions after completion of postdoctoral training. Impressively of 145 alumni, 64% are faculty at research intensive, liberal arts, or minority serving institutions. By combining teaching and research training, FIRST produces scholars who are successful, independent researchers and effective educators that will inspire the next generation of scientists.

E8038B ***Developing Future Biologists*****: a dev-bio lab course for outreach, diversity recruitment, and professional development.** *S. Barolo*[1], B. Carpenter[1,2], A. Chin[1], E. Dulka[1], M. Echevarria-Andino[1], D. Lorberbaum[1], J. Martinez-Marques[1], L. Marty Santos[1], J. Pinskey[1], S. Raj[1], B. Allen[1], L. Bricker[1], L. Gruppen[1], D. Gumucio[1], D. Wellik[1], E. Suárez-Martínez[3], A. Ramos[1]. 1) University of Michigan Medical School, Ann Arbor, MI; 2) Emory University, Atlanta, GA; 3) University of Puerto Rico, Ponce, PR.

Future advances in human health will continue to depend on basic discovery research in developmental biology, and on the recruitment of a strong and diverse pool of talented researchers to that field. *Developing Future Biologists* is a one-week intensive laboratory course developed by doctoral and postdoctoral trainees at the University of Michigan (U-M), with the advice and assistance of faculty members. The DFB course

introduces modern concepts and techniques in developmental biology to undergraduate students in underserved populations. The course, which was created by a diverse group of graduate and postdoctoral trainees, aims to recruit talented students into the field, especially those who would not otherwise be aware of the excitement of developmental biology research; to increase diversity among trainees in the field; to develop professional skills in the trainees who develop, plan, and teach the course; and to improve the climate of diversity and cultural awareness among trainees and faculty at the University of Michigan. The *DFB* course was taught in 2015 and 2016 in Ponce, Puerto Rico. Assessment of the impact of *DFB* on the students who took the course, on the Michigan trainees and faculty who desgined and taught it, and on graduate recruitment at U-M, will be discussed.

E8039C The DNA Day Network: Integrating career training and outreach into trainee development. *S. Hall*[1], L. Villafuerte[2]. 1) University of Massachusetts Medical School, Worcester, MA; 2) University of Kansas, Lawrence, KS.

Careers in science require an important understanding of effective communication that can reach far from the bench, into the voting community, and to individuals that will be the future workforce. It is recognized that the ability to progress the scientific enterprise requires strong intellectual and financial support from the community. The Massachusetts and Kansas DNA Day programs work to develop early career scientists to strengthen their communication skills beyond the doors of the research institution and to integrate outreach into their research programs throughout their careers. Ambassador training provides tools to build an understanding of how to merge basic principles of science with the ambassadors' research interests. In addition to ambassador development, a major goal of the DNA Day programs is to link high school course content with real life research applications - building bridges between students, the textbook, and the bench. During DNA Day ambassador visits, students not only hear about the relevance of the work from the ambassadors, but also engage in an activity that allows them to experience simplified techniques that are used to explore and answer research questions. We put the tools in the hands of the students in hopes that this creates a sense of ability within the students, encouraging them to envision themselves pursing a career in STEM. Additionally, as part of the DNA Day experience, ambassadors discuss their research interests and the paths they have navigated to reach their current position. This opens the floor to questions from students that allow them to better visualize themselves as part of the future STEM workforce.

E8040A The Effects of Myrrh and Rosemary Extract on Cancer Cell Lines. *Areej Alanazi*. Tennessee State University, Nashville, TN.

The National Cancer Institute defines cancer as a collection of related diseases. We know that if there are any changes in our cells' function, such as how cells grow or divide, these changes include mutations in the deoxyribonucleic acid (DNA). From this point, a body's cell will divide without stopping, and it will be an abnormal cell that will produce a tumor. This study has tested the hypothesis that myrrh and rosemary have compounds that will inhibit the growth of cancer cells. In this experiment, four different types of cancer cells— which are lung cancer (A549), colon cancer (SW620), breast cancer (MCF7), and cervical cancer (HeLa)—were exposed to myrrh and rosemary extractions. The alamarBlue Cell Viability assay protocol and fluorescent analysis were used to evaluate the cytoxicity of the extracts. The findings indicate that the more we have a concentration of myrrh extraction, the more we get the inhibition of lung cancer cell growth. Also, rosemary appears to inhibit cervical cancer (HeLa).

E8041B The Effects of *Lepidium sativum* on Four Tumor Cell Lines. *Afnan Felimban*. Tennessee State University, Nashville, TN.

Cancer is considered as multiple diseases generally because there is not a single treatment for all disease forms. It is important to develop mechanisms to treat cancer that will not harm normal cells and possibly inhibit the reoccurrence of the disease. Natural compounds have been a valuable source for the treatment of human diseases. We are currently studying a medicinal plant to determine its effect on tumor cell lines. The plant is *Lepidium sativum*. Methanol extractions from the seeds of this plant produced the secondary compounds use to analyze cell growth. We used AlamarBlue® to measure cell viability. The cancer cell lines testing are breast cancer (BT549), lung cancer (A549), breast cancer (MCF7) and cervical cancer (HeLa). Our analysis shows a significant inhibition of the cancer cells growth after exposure for 24 hour. These results indicate that *Lepidium sativum* should be studied more to determine its effect on tumor cells.

E8042C Alterations induced in ovarian follicular kinetics of adult zebrafish on long term exposure to environmental estrogenic contaminants. *B. B. Goundadkar*, P. A. Katti. Karnatak University, Dharwad, Karnataka, IN.

The aquatic environment is the ultimate destiny for chemical contaminants of anthropogenic origin. Present study is an attempt to assess the alterations induced in the ovarian follicular composition of adult female zebrafish (*Danio rerio*) exposed to environmental estrogenic contaminants (EECs) at a concentration lower than environmental recorded levels for long-term (80 days) in the laboratory. Adult zebrafish (n=20) were maintained in a medium containing environmental estrogens such as, 17α-ethynil estradiol (EE2) (5 ng/L and 10ng/L) or diethylstilbestrol (DES) (5 ng/L and 10ng/L) or bisphenol (BPA) (5 ng/L and 10ng/L). Corresponding negative controls were maintained for comparison. On the 81st day the fish were sacrificed, body and ovarian weights were recorded and ovaries were processed for histology, sections of 3 µm thick were cut and stained with hematoxylin and eosin. All the follicles were counted and quantified from alternative serial sections. Growing follicles were classified into 6 stages based on their functional status and size. Atretic follicles were counted separately. The results reveal that exposure of female fish to estrogenic compounds even at a very low dose for long-term leads to increased body weights and size, with an increase ($P<0.05$) in the gonadosomatic index (GSI). There was a significant decrease in the number of previtellogenic follicles in all EEC exposed groups. Vitellogenic follicle number was greater in EE2 group compared to other EEC exposed groups, while atretic follicles were relatively greater in DES and BPA groups. It is suggested that long-term exposure to EEC even at lower concentrations alters follicular composition of adult zebrafish ovary.

The letter preceding the number is the community. A, B, C after the number is presentation time.
W – *C. elegans*, C – Ciliates, D – Drosophila, M – Mouse, P – PEQG, Y – Yeast, Z – Zebrafish

E8043A Genetic modifiers compensating for loss of epidermal growth factor receptor. *S. Howe*, D. Threadgill. Texas A&M Health Science Center, College Station, TX.

Genetic compensation usually occurs when polymorphic modifier genes provide robust compensation for the loss of a gene of interest. The epidermal growth factor receptor (EGFR) is a critical gene for early development. Its disruption can cause abnormal embryonic and placental development, and it is implicated in tumorigenesis. Mouse embryos lacking *Egfr* have reduced proliferation in trophoblast stem cell lineages that form the spongiotrophoblast layer of the placenta. Depending on severity, this can impact the labyrinth layer, the size of the placenta, and the timing and frequency of embryonic lethality. These placental phenotypes are strongly dependent on genetic background, indicating the presence of *Egfr* modifier genes. We crossed the ALR/LtJ mouse strain, a robust survivor with *Egfr* loss, to the 129S1/SvImJ, which have a mid-gestation lethality phenotype due to placental dysfunction, to show that hybrid ALR.129 *Egfr*-null embryos survive into late gestation. Using a phenotypic congenic approach, we generated evidence suggesting the presence of three dominant loci. We are using a reverse genetics approach to identify the *Egfr* modifiers by investigating dysfunction of known trophoblast stem (TS) cell lineage pathways. We utilized 129S1 and ALR mice that are homozygous for a conditional *Egfr* allele ($Egfr^{tm1.1Dwt}$) to generate TS cells. The introduction of Cre recombinase into TS cells by electroporation induces recombination at the loxP sites deleting *Egfr*, which provides genetically matched TS cell lines with and without EGFR. We are using these cell lines to elucidate candidate *Egfr* modifier genes capable of compensating for loss of EGFR. The findings will aid in the identification of modifier genes of *Egfr* that may influence the severity of conditions related to malformed placentas, such as intrauterine growth restriction, and may impact the effectiveness of EGFR-targeted cancer chemotherapies.

E8044B Nutritional Regulation of Oogonial Proliferation and Differentiation into Primary Oocytes in the Adult Ovary of Zebrafish (*Danio rerio*). *P. A. Katti*, P. A. Deshpande, S. S. Narvekar, M. S. Kanetkar. Karnatak University, Dharwad, Karnataka, IN.

Nutritional stress is known to block stem cell multiplication and cause delay germ cell divisions. In the present study nutritional regulation of oogonial proliferation and differentiation into primary oocytes was studied in zebrafish (*Danio rerio*) by subjecting adult fish to different feeding regimes. Adult female zebrafish (wild) after acclimatization to the laboratory (temperature: 26 ± 1°C and photoperiod: 11.30L: 12.30D) for one week, were divided into five groups and maintained under different feeding regimes. Group-I daily four times fed; Group-II daily twice fed; Group-III daily once fed; Group-IV every alternate day fed and Group-V every fourth day fed. The experiment lasted for two months; all experimental fish were fed on commercial pellets and *Artemia nauplii ad libitum*. At the end of experiment, body mass and body size of each fish were recorded, ovaries were excised, weighed and processed for histology. Serial sections (3µm thick) were cut and stained with hematoxylin and eosin. Oogonia, primary oocytes, vitellogenic oocytes and atretic follicles were quantified from serial histological sections. Our observations reveal that there was an increase in body mass, ovary mass, number of oogonia and primary oocytes in daily four times, daily twice and daily once fed fish over initial controls. While they remained comparable to initial controls in alternate day fed fish, and were significantly lower in every fourth day fed fish. These results indirectly suggest that overfeeding increased body mass of fish without causing a corresponding increase in body size, oogonial population and their rate of proliferation while, underfeeding depleted oogonial number. It is concluded that nutrition regulates germ cell mitosis optimally in zebrafish ovary, overfeeding may not increase rate of proliferation linear but under feeding curtails it.

E8045C Effect of heat stress on condesin II levels and localization. *V. Rana*, H. Nguyen, G. Bosco. Dartmouth College, Hanover, NH.

In interphase, the genome is known to organize into topologically associated domains (TADs) of transcriptionally active and inactive gene clusters. Gene expression during this phase is dynamic and chromatin must reflect structural organization but maintain a level of plasticity in order to respond to different stimuli through gene expression changes. Stimuli like stressors can globally change the chromatin landscape in very short time scales. One such stressor is heat stress which can lead to reorganization of topologically associated domains in cultured insect cells. Interestingly, heat shock results in an increase of chromatin bound structural proteins and changes in TAD arrangements. However CAP-H2, a subunit of condensin II and regulator of genomic topology, levels are reduced on chromatin after heat shock. This contradictory result presents many questions such as what the role of CAP-H2 and therefore condensin II is during heat shock and what the fate of CAP-H2 is during this time. Investigation into the turnover of CAP-H2 using cell fractionation and western blots revealed that CAP-H2 is degraded after release from chromatin due to heat stress. Recovery after heat stress does not require CAP-H2 as knockdown with RNAi does not indicate significant levels of cell death compared to the control. Despite these results many questions still remain such as what the role CAP-H2 in re-establishing or restructuring of chromatin is during recovery? We hypothesize that condensin II may prevent long range interactions necessary during heat shock and facilitate short range interactions as cells recover.

E8046A Effect of genetic variations on various post translational modifications (PTMs) and its role in protein regulation. *M. Saleem*[1], Rabia Arif[1], Siu Lee[2]. 1) University of the Punjab, New Campus, Lahore, Punjab, PK; 2) University of Melbourne, Australia.

High resolution melting analysis together with Real time PCR was tested for quick identifications of cellobiohydrolase I (cbhI), Ketosynthase, beta tubulin and two ribosomal proteins (RpS29 and RpS26) in eight strains of *Sordaria fimicola* (S1, S2, S3, N5, N6, N7, SW17.2 and SW92.1). HRM analysis successfully reported these (RpS29 and RpS26) genes in *Sordaria fimicola first time*. All strains of *Sordaria fimicola* were authenticated by targeting ITS2 region. Above mentioned proteins were targeted to predict three dimensional structures based on various post translational modifications (PTMs). Post translational modifications are very important to understand the regulatory mechanisms of cytoplasmic and nuclear proteins. Different post translational modifications (PTMs) such as phosphorylation, glycosylation, acetylation, and methylation control the structure, function regulation of proteins. In this work the role of different modifications and their interplay, is investigated by using various bio-informatics tools.

E8047B Acute heat shock leads to loss of polarity in *C. elegans* embryos. *D. Singh*, C. Pohl. Institute of Biochemistry II, Goethe University, Frankfurt am Main, DE.

Cortical flows are indispensable for *C. elegans* development. Anterioposterior (A/P)[1], dorsoventral (D/V)[2] and left-right[3] asymmetries rely on differential regulation of cortical actomyosin. Our work has shown how a previously un-described rotational cortical flow polarized orthogonal to the A/P axis repositions the cytokinesis midbody remnant (MB^r) onto the future ventral side of an otherwise D/V symmetric embryo. The MB^r acts as a polarizing cue by serving as a cortical landmark for F-actin accumulation. Upon subjecting the embryos to a transient heat shock we found that the cortical flows are severely diminished[4]. Acute heat shock abolishes cortical flows leading to loss of all other discernable signs of A/P and D/V polarity. Hallmarks of cell polarity in *C. elegans* include mutually exclusive polarity domains and asymmetric cell division; both these processes fail in heat stressed embryos. We observed mass internalization of cortical domains loaded with polarity markers (PAR-2/6), indicating that lack of cortical domain recycling leads to polarity loss. Taken together, our work reveals heat-labile aspects of actomyosin regulation, asymmetric cell division and polarity.

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