



Monday November 7

3.00pm – 5.30pm

Poster Session 2

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Stem Cells and Development

P-103 FUNCTIONAL ANALYSIS OF NOVEL HOMEBOX GENES EXPRESSED IN THE DEVELOPING PITUITARY GLAND

N M Solomon¹, M L Brinkmeier¹, R H Lyons¹, P Carninci², Y Hayashizaki², S M Faust¹, B C Bjork³, D R Beier³, S A Camper¹

¹ University of Michigan, Ann Arbor, MI, United States, ² RIKEN Genomic Sciences Center, Yokohama, Kanagawa, Japan, ³ Harvard Medical School, Boston, MA, United States

P-104 THE CALCITONIN RECEPTOR LIKE RECEPTOR FUNCTIONS AS THE ADRENOMEDULLIN RECEPTOR DURING EMBRYONIC DEVELOPMENT

R T Dackor, K Fritz-Six, C Gibbons, K M Caron

University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

P-105 DEFECTIVE COMMUNICATION AMONG TA CELLS OF THE MELANOCYTE LINEAGE IN HAIR FOLLICLES OF THE PATCHWORK MUTANT MOUSE

G F Houzelstein¹, N Da Silva¹, F Bernex¹, J Djian¹, D Leroux², J J Panthier³

¹ INRA, Maisons-Alfort, France, ² CEA, Evry, France, ³ Institut Pasteur, Paris, France

P-106 MUTATIONS IN THE GENE ENCODING THE LOW-DENSITY LIPOPROTEIN RECEPTOR MEGF7 (LRP4) CAUSE ABNORMAL LIMB DEVELOPMENT IN THE MOUSE

D Simon-Chazottes¹, S Tutois², M Kuehn³, M Evans⁴, F Bourgade¹, S Cook⁵, M T Davisson⁵, J L Guénet¹

¹ Institut Pasteur, Paris, France, ² Université Blaise Pascal, Aubière, France, ³ NCI-Frederick, Frederick, MD, United States, ⁴ Cardiff University, Cardiff, Wales, United Kingdom, ⁵ The Jackson Laboratory, Bar Harbor, Maine, United States

P-107 IMPORTANCE OF HIP/RPL29 IN MOUSE EMBRYOGENESIS

C B Kirn-Safran¹, R J Focht¹, J L Vivian², D D Carson¹

¹ University of Delaware, Newark, DE, United States, ² University of Kansas, Kansas City, KS, United States

P-108 MICROARRAY ANALYSIS OF PDGFRA⁺ POPULATIONS IN DIFFERENTIATED ES CELLS, AS A MODEL OF MESODERM/MESENCHYMAL CELL LINEAGES

A Takebe, T Era, M Okada, L Jakt, S Nishikawa

Riken center for developmental biology, kobe, Japan

P-109 RESCUE OF THE T-COMPLEX RECESSIVE LETHAL MUTATION *TCLW5* BY A 180KB BAC CLONE

M Sugimoto¹, K Mekada¹, Y Karashima², M Yuzuriha¹, M S H Ko³, R Nagaraja³, S S Tan⁴, N Takagi², K Abe¹

¹ RIKEN BRC, Tsukuba, Japan, ² Hokkaido University, Sapporo, Japan, ³ National Institute on Aging, Maryland, United States, ⁴ University of Melbourne, Victoria, Australia

P-110 ATTENUATION OF GENE EXPRESSION BY NOVEL VECTORS ENCODING SHORT-HAIRPIN RNA IN THE INTRON OF AN RNA POLYMERASE II-TRANSCRIBED GENE

J T Wong, D Fink, A Gertz, C J Ong

University of British Columbia, Vancouver, BC, Canada

P-111 DEFECTS IN GASTRULATION, VASCULOGENESIS AND HEDGEHOG SIGNALING IN THE PLACENTA ARE ASSOCIATED WITH MUTATIONS IN NSDHL, A DEHYDROGENASE THAT FUNCTIONS IN CHOLESTEROL BIOSYNTHESIS

D Cunningham, F Jiang, G E Herman

Children's Research Institute, Columbus, OH, United States

P-112 IDENTIFYING SECRETED PROTEINS INVOLVED IN HUMAN ES CELL SURVIVAL AND DIFFERENTIATION

G Kolle¹, A Topolska², B Gardiner¹, A Laslett², M Pera², S Grimmond¹

¹ Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia, ² Monash Institute of Medical Research, Melbourne, Victoria, Australia

P-113 RECESSIVE GENETIC SCREEN IN ES CELLS BY CONDITIONAL DISRUPTION OF THE BLOOM'S SYNDROME GENE

K Yusa, K Horie, J Takeda
Osaka University, Osaka, Japan

P-114 PROLIFERATION DEFECTS IN EPIDERMAL GROWTH FACTOR RECEPTOR NULL PLACENTAE CAN BE RESCUED IN THE ABSENCE OF CYCLIN-DEPENDENT KINASE INHIBITORS

J Dackor, K E Strunk, D W Threadgill
University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

P-115 GENOMIC MAPPING OF FGF SIGNALLING INPUT TO THE BRANCHING LUNG EPITHELIUM

K A Peterson¹, J E Richardson², I C Welsh¹, C J Bult², T P O'Brien¹
¹ Cornell University, Ithaca, NY, United States, ² The Jackson Laboratory, Bar Harbor, ME, United States

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D E Bergstrom¹, J Masse¹, R J Munroe², J C Schimenti², L H Gagnon¹, K R Johnson¹, U Heinzmann³, G Stumm⁴, R Paffenholz⁴
¹The Jackson Laboratory, Bar Harbor, ME, United States, ² Cornell University, Ithaca, NY, United States, ³GSF-National Research Center for Environmental Health, Nueherberg, Germany, ⁴Ingenium Pharmaceuticals, Martinsried, Germany

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J K Pendola¹, S Sweeney¹, H Lothrop¹, J Schimenti², M A Handel¹, J Eppig¹.
¹ The Jackson Laboratory, Bar Harbor, United States. ² Cornell University, Ithaca, NY, United States

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I Matera^{1,2}, DE Watkins-Chow¹, MS Jones¹, LL Baxter¹, G Elliott¹, C Rivas¹, A Incao¹, WJ Pavan¹
¹National Human Genome Research Institute, NIH, Bethesda, MD, United States, ²Laboratorio di Genetica Molecolare, Istituto Giannina Gaslini, Genova, Italy

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I Aubin¹, CP Adams², S Opsahl³, D Septier³, CE Bishop², N Auge⁴, R Salvayre⁴, A Negre-Salvayre⁴, M Goldberg³, C Poirier², JL Guénet¹
¹ Institut Pasteur, Paris, France, ² Baylor College of Medicine, Houston, Texas, United States, ³ Faculté de Chirurgie Dentaire Université Paris 5, Montrouge, France, ⁴ INSERM CHU Rangueil, Toulouse, France

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H Motaln¹, SC Pells², J McWhir², MM Marques³, S Horvat¹
¹ University of Ljubljana, Biotechnical faculty, Dept. of Animal Science, Domzale, Slovenia, ² Roslin Institute, Division of Gene Expression and Development, Midlothian, Scotland, United Kingdom, ³ Instituto de Desarrollo Ganadero, University of Leon, Campus de Vegazana s/n, Leon, Spain

P-121 STUDENT ORAL ABSTRACT S-12**NEW TRANSCRIPTION FACTORS FOR EARLY EYE FORMATION IN MOUSE**

LY Tang¹, CC Wang¹, KW Choy¹, MS Rogers¹, T Gojobori², K Ikeo², SC Lam¹, CP Pang¹
¹ The Chinese University of Hong Kong, Hong Kong, China, ² Center for Information Biology and DNA Data Bank of Japan, National Institute of Genetics, Japan

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ZIC2-ASSOCIATED HOLOPROSENCEPHALY IS DUE TO A DEFECT IN PRECHORDAL PLATE DEVELOPMENT

NJ Warr, NS Powles-Glover, R Arkell
MRC Harwell, Didcot, Oxfordshire, United Kingdom

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SEARCH FOR HSF1 OOCYTE-SPECIFIC TARGET GENES

A Metchat¹, P Monget², E Christians¹

¹Centre de Biologie du Développement, UMR 5547 and IFR 109
CNRS/UPS, 118 route de Narbonne, 31062 Toulouse cedex 4, France

²Physiologie de la Reproduction et des Comportements, UMR 6175
INRA-CNRS-Université François Rabelais de Tours-Haras Nationaux, 37380 Nouzilly, France

P-103**FUNCTIONAL ANALYSIS OF NOVEL HOMEBOX GENES EXPRESSED IN THE DEVELOPING PITUITARY GLAND**

N M Solomon¹, M L Brinkmeier¹, R H Lyons¹, P Carninci², Y Hayashizaki², S M Faust¹, B C Bjork³, D R Beier³, S A Camper¹

¹ University of Michigan, Ann Arbor, MI, United States, ² RIKEN Genomic Sciences Center, Yokohama, Kanagawa, Japan, ³ Harvard Medical School, Boston, MA, United States

Pituitary gland development involves the differentiation of five hormone-producing cell types. Humans and mice with inactivating mutations in *PROP1* exhibit pituitary hypoplasia and failed hormone-producing cell differentiation. To understand the molecular basis of this process we prepared cDNA libraries from pituitary primordia of normal and *Prop1* mutant embryos at critical embryonic times (e12.5 and e14.5), sequenced over 30,000 clones and established a searchable database using gene ontology terminology. Seventeen novel homeobox genes were identified in this database and their expression in the developing pituitary was evaluated by in situ hybridization. *Ash2l* was among these genes and it is related to the *Drosophila Ash2* gene, **a**bsent, **s**mall, or **h**omeotic discs-**2**, a regulator of homeotic selector genes and a member of the trithorax gene family. *ASH2L* is a component of a transcriptional regulatory complex that includes *MEN1*, which is mutated in the human syndrome **M**ultiple **E**ndocrine **N**eoplasia Type **1**. The functional association of *ASH2L* with *MEN1* implicates *ASH2L* in the pathogenesis of pituitary adenomas, the most common type of intracranial tumor in humans. We are investigating the role of *Ash2l* in pituitary organogenesis and pituitary adenoma pathogenesis using loss of function models and gene expression profiling. We are also developing a high throughput method to assess the function of other novel homeobox genes using RNAi technology and transient transgenics. These studies are likely to uncover the molecular basis for congenital pituitary hormone deficiencies, the majority of which have unknown etiology and yield a mechanistic understanding of the etiology of pituitary tumors.

P-104**THE CALCITONIN RECEPTOR LIKE RECEPTOR FUNCTIONS AS THE ADRENOMEDULLIN RECEPTOR DURING EMBRYONIC DEVELOPMENT.**

R T Dackor, K Fritz-Six, C Gibbons, K M Caron

University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Adrenomedullin (AM) is a 52-amino acid peptide that serves as a vasodilator, angiogenic factor and growth factor. AM is essential for life, since *AM*^{-/-} embryos die at mid gestation with extreme hydrops fetalis and cardiovascular defects. To date, numerous *in vitro* studies have suggested that AM can mediate its biological effects through at least three different receptors. To provide an *in vivo* model of the most likely candidate receptor, calcitonin receptor like receptor (CLR), a gene targeted knockout model of this gene was generated. Mice heterozygous for the targeted *Calcr1* allele appeared normal at birth and did not differ from wild-type controls in several physiological parameters tested. However, heterozygote matings failed to produce viable *Calcr1*^{-/-} pups, demonstrating that *Calcr1* is essential for survival. Timed heterozygous matings confirmed that CLR is important in embryonic development since *Calcr1*^{-/-} embryos die between E13.5 and E14.5. *Calcr1*^{-/-} embryos dissected as early as E12.5 exhibited extreme hydrops fetalis, thin vascular walls and smaller hearts, similar to our previous observations in *AM*^{-/-} embryos at E13.5. BrdU and TUNEL staining of *Calcr1*^{-/-} and *AM*^{-/-} hearts indicates that this signaling system is important in regulating cardiac proliferation and apoptosis. The *Calcr1* gene targeted mice provide the first *in vivo* evidence that CLR can function as an AM receptor during embryonic development. Moreover, the fact that *Calcr1*^{-/-} embryos develop a similar phenotype an entire day earlier in development than *AM*^{-/-} embryos also suggests that a more complex signaling mechanism may be involved.

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DEFECTIVE COMMUNICATION AMONG TA CELLS OF THE MELANOCYTE LINEAGE IN HAIR FOLLICLES OF THE PATCHWORK MUTANT MOUSE

G F Houzelstein¹, N Da Silva¹, F Bernex¹, J Djian¹, D Leroux², J J Panthier³

¹ INRA, Maisons-Alfort, France, ² CEA, Evry, France, ³ Institut Pasteur, Paris, France

The niche for stem cell melanocytes has been located within the bulge of the hair follicle, beneath the sebaceous gland. Why and how the melanocyte stem cells are recruited to exit the niche and colonize the bulb of hair follicles, where they terminally differentiate, is largely unknown. To address these issues, we study mice homozygous for the recessive patchwork (*pwk*) mutation. The distribution pattern of melanoblasts in the epidermis of *pwk/pwk* fetuses indicated that *pwk/pwk* melanoblasts have normal capacities until they colonize the developing hair follicles. It is only at E18.5 that apoptotic melanoblasts were detected within the bulb of hair follicles. We showed that melanocyte stem cells and transit amplifying (TA) cells are present in *pwk/pwk* hair follicles producing white hairs at P8. However, no melanoblasts or melanocytes were ever seen within the hair bulb of *pwk/pwk* white hair follicles. Hence, we propose that defective communication among *pwk/pwk* TA cells prevents their migration and/or differentiation before they reach the hair bulb.

We mapped *pwk* within a 0.33 cM interval and identified *Strawberry notch* (*mSno*) as a candidate gene. *mSno* expression is enhanced in the skin of *pwk/pwk* embryos at E17.5. Targeted expression of *mSno* in the melanocyte lineage led to a transgenic mouse line that exhibits the patchwork phenotype. In *Drosophila*, *sno* links the EGFR and Notch pathways. We are investigating an implication of the Notch pathway in melanocyte lineage development and are testing whether the patchwork mutation alters the Notch pathway in melanoblasts and/or melanocytes.

P-106

MUTATIONS IN THE GENE ENCODING THE LOW-DENSITY LIPOPROTEIN RECEPTOR MEGF7 (LRP4) CAUSE ABNORMAL LIMB DEVELOPMENT IN THE MOUSE

D Simon-Chazottes¹, S Tutois², M Kuehn³, M Evans⁴, F Bourgade¹, S Cook⁵, M T Davisson⁵, J L Guénet¹

¹ Institut Pasteur, Paris, France, ² Université Blaise Pascal, Aubière, France, ³ NCI-Frederick, Frederick, MD, United States, ⁴ Cardiff University, Cardiff, Wales, United Kingdom, ⁵ The Jackson Laboratory, Bar Harbor, Maine, United States

We have identified and characterized two allelic mutations that occurred in a previously unknown gene (*Lrp4* - formerly *Megf7*) encoding a protein of the low-density lipoprotein receptors (LDLRs) family and that have a strong phenotypic effect on limb (mainly digit) differentiation. The first mutation was generated by a MuLV retroviral insertion in the first intron of the gene, the second is the consequence of a A → T transversion in a splicing donor site leading to exon skipping. The LDLRs are a family of trans-membrane proteins involved in endocytosis of specific ligands. Ligands have been identified and functions have been documented for several, although not all, members of this family indicating that these genes play an important role in the embryonic development and physiology of living organisms including mammals. Our *Lrp4* mutant alleles represent new models that should be helpful for understanding mammalian limb patterning and provide new insight on the yet unclassified human polysyndactylies. Our findings also help in the annotation of the gene *Lrp4*, whose functions might be to up-regulate the expression of the gene encoding fibroblast growth factor 4 (Fgf4), either directly or via the mediation of Hedgehog genes (*Shh/Ihh*).

P-107**IMPORTANCE OF HIP/RPL29 IN MOUSE EMBRYOGENESIS**C B Kirn-Safran¹, R J Focht¹, J L Vivian², D D Carson¹¹ University of Delaware, Newark, DE, United States, ² University of Kansas, Kansas City, KS, United States

HIP is a multifunctional, heparin/heparan sulfate-interacting protein identical to ribosomal protein L29. Originally, HIP/RPL29 protein was purified through its polyanion binding properties from human uterine epithelial cells where it is believed to participate in implantation processes at the embryo-uterine interface. In addition, HIP/RPL29 is thought to contribute to proper association between the two ribosomal subunits and, consequently, in translational efficiency. Because high levels of HIP/RPL29 are found in embryonic stem (ES) cells and in all types of proliferating and developing tissues, we hypothesize that the presence of HIP/RPL29 is essential for normal embryonic growth.

To investigate the *in vivo* functions of HIP/RPL29 in the mouse, we targeted *Hip/Rpl29* gene in mouse W4 ES cells of the 129S6 background. The effect of *Hip/Rpl29* monoallelic expression was assessed *in vivo* by breeding chimeras obtained after microinjection of *Hip/Rpl29* +/- ES cells into C57BL6/N host blastocysts with wild type mice of different strains. Because there is increasing evidence that gene disruption of specific ribosomal protein genes is lethal in embryos prior to implantation, we anticipate that intercrosses between heterozygous progeny will not lead to viable *Hip/Rpl29* -/- offspring. For this reason additional studies are currently focusing on developing subtle allele variants of the *Hip/Rpl29* gene. Specific genetic alterations of *Hip/Rpl29* wild type allele(s) and the study of its subsequent effects on embryonic development will shed light on its putative function as a promoter of early pregnancy. (Supported by NIH COBRE starter grant 5P20RR15588 to C.B.K.S and NIH grant HD25235 to D.D.C.).

P-108**MICROARRAY ANALYSIS OF PDGFRA⁺ POPULATIONS IN DIFFERENTIATED ES CELLS, AS A MODEL OF MESODERM/MESENCHYMAL CELL LINEAGES**

A Takebe, T Era, M Okada, L Jakt, S Nishikawa

Riken center for developmental biology, kobe, Japan

An inherent difficulty in using DNA microarray technology on the early mouse embryo is its relatively small size. We investigated whether use of ES cell differentiation culture, which has no theoretical limit in the number of cells, can improve this situation. 7 distinct populations generated from the ES cell differentiation culture were analyzed by DNA microarray and examined for genes whose distribution patterns are similar to those of PDGFRA, a gene implicated in differentiation of mesoderm/mesenchymal lineages. Using software developed in our lab to compare the expression patterns of particular genes in multiple data points, we formed a group of 30 genes which showed the highest similarity to PDGFRA, 18 of these genes were shown to be involved in development of either mesodermal or neural crest cells. This list also contains several genes whose role in embryogenesis has not yet been fully identified. One such molecule is mARID3b, originally identified as Rb1 binding protein. As expected, mARID3b expression is found in the paraxial mesoderm and cranial mesenchyme and overlaps with the area of PDGFRA expression. mARID3b-null mouse showed an early embryonic lethality and most phenotypes of this mutant appear to develop from a failure to generate a sufficient number of cranial mesenchymal cells. These results demonstrate the powerful potential of ES cell differentiation culture to identify novel genes playing an indispensable role in embryogenesis.

P-109**RESCUE OF THE *T*-COMPLEX RECESSIVE LETHAL MUTATION *TCLW5* BY A 180KB BAC CLONE**

M Sugimoto¹, K Mekada¹, Y Karashima², M Yuzuriha¹, M S H Ko³, R Nagaraja³, S S Tan⁴, N Takagi², K Abe¹

¹ RIKEN BRC, Tsukuba, Japan, ² Hokkaido University, Sapporo, Japan, ³ National Institute on Aging, Maryland, United States, ⁴ University of Melbourne, Victoria, Australia

The mouse *t*-complex is a naturally occurring variant of the proximal region of chromosome 17. The *t*-complex contains four tandem inversions relative to the wildtype, and therefore, recombination in *t*-heterozygotes is severely suppressed along the entire *t*-complex. The variant form of the *t*-complex found in wild mice is known as *t*-haplotypes, which usually contain a recessive lethal mutation. In spite of remarkable progress in genetics since the first discovery of *t*-haplotypes in 1932, the nature of the lethality for any *t*-haplotypes is unclear. *tc/w5* is a *t*-complex recessive mutation in the *t^{w5}*-haplotype, which causes embryonic lethality at the gastrulation stage. In the homozygotes, extensive death of the embryonic ectoderm was observed, whereas the extraembryonic ectoderm and the visceral endoderm were less affected. However, our tetraploid rescue experiments have shown that the product of *tc/w5* likely functions in extraembryonic tissues and supports proliferation/differentiation of embryonic ectoderm through cell-cell interactions. These results point to the *tc/w5* product as an important regulator of pluripotent cell development in mammalian embryos. Our previous genomic studies delimited the *tc/w5* critical region to 750kb. In this study, we carried out transgenic rescue using five BACs, and successfully rescued the lethality of *t^{w5}/t^{w5}* embryos by one BAC, indicating that *tc/w5* is located within a 180kb BAC, harboring 15 transcription units.

P-110**ATTENUATION OF GENE EXPRESSION BY NOVEL VECTORS ENCODING SHORT-HAIRPIN RNA IN THE INTRON OF AN RNA POLYMERASE II-TRANSCRIBED GENE**

J T Wong, D Fink, A Gertz, C J Ong

University of British Columbia, Vancouver, BC, Canada

Exogenously induced RNA interference (RNAi) causes post-transcriptional silencing of specific genes, and promises to therapeutically target genes relevant to diseases. Commonly used vectors with RNA polymerase III promoters driving transcription of short hairpin RNAs (shRNAs) do not allow direct tissue-specific RNAi. Here we describe novel shRNA vectors, in which a self-complementary direct repeat sequence corresponding to a target gene is encoded within an intron of the human growth hormone (hGH) gene, driven by an RNA polymerase II (pol II) promoter. The basic vector comprises the cytomegalovirus (CMV) promoter driving expression of the hGH gene containing within its second intron a self-complementary sequence corresponding to firefly luciferase (pCMV-hGH-luc). When transiently cotransfected with a firefly luciferase reporter vector in 293T epithelial cells, pCMV-hGH-luc caused over 70% attenuation of luminescence. A lentiviral vector comprising the CMV-driven hGH shRNA construct fused to the enhanced green fluorescent protein (EGFP) gene (pLenti-CMV-hGH-luc-EGFP) was also generated. Expression of the construct as an EGFP fusion protein allows ready identification of cells expressing the shRNA construct, while the lentiviral vector backbone facilitates rapid and efficient production of transgenic mice. A third vector, designed for tissue-specific shRNA expression, comprises the lentiviral backbone containing the hGH-luc-EGFP construct driven by the prostate-specific promoter ARR2PB (pLenti-ARR2PB-hGH-luc-EGFP). Production of transgenic mice harbouring the intron containing shRNA driven by tissue specific and systemic RNA pol II promoters are underway. Insertion of sequences against target genes into introns of RNA pol II transcribed genes could allow efficient *in vivo* delivery of shRNAs for tissue-specific gene knock-downs.

P-111

DEFECTS IN GASTRULATION, VASCULOGENESIS AND HEDGEHOG SIGNALING IN THE PLACENTA ARE ASSOCIATED WITH MUTATIONS IN NSDHL, A STEROL DEHYDROGENASE THAT FUNCTIONS IN CHOLESTEROL BIOSYNTHESIS

D Cunningham, F Jiang, G E Herman
Children's Research Institute, Columbus, OH, United States

The X-linked *Nsdhl* gene encodes a sterol dehydrogenase involved in cholesterol synthesis. A series of mutant alleles of *Nsdhl* produce phenotypes of varying severity, but all are embryonic male-lethal. The *Bpa^{1H}* null allele exhibits the most severe phenotype, with affected male embryos rarely surviving beyond E9.5. Morphological abnormalities are visible during gastrulation (E7.5). The *Bpa^{8H}* allele is less severe, with affected males surviving to E10.5-12.5. Mutant embryos display an abnormally thin labyrinth layer of the placenta with reduced invasion of allantoic mesoderm to form the endothelium of placental blood vessels. A pale yolk sac, with poorly organized vasculature is also commonly seen in *Bpa^{8H}* male embryos. To investigate a possible primary defect in vasculogenesis, allantoic explants from E8.5 embryos were cultured *in vitro*. Wild type explants proliferated and endothelial cells differentiated within the cell mass to form a network of vessels, while *Bpa^{8H}* explants attached and grew, but did not form a vascular network. Since Hedgehog (HH) proteins require covalent modification by cholesterol for their normal signaling function and are known to play an important role in vasculogenesis, we asked whether HH signaling is disrupted in *Bpa^{8H}* placentae using a *Ptch-lacZ* reporter transgene to detect HH signaling *in vivo*. In wild type placentae, *lacZ* expression was seen in the allantoic mesoderm beginning around E10, while *Bpa^{8H}* littermates showed little or no expression of the reporter. Moreover, expression of Indian hedgehog was detected by *in situ* hybridization in clusters of yolk sac-derived endoderm cells within normal allantoic mesoderm at this stage. These data represent the first evidence of a role for HH proteins in placenta development, and suggest that the placental defects arising from *Nsdhl* deficiency may be due to perturbation of HH signaling.

P-112

IDENTIFYING SECRETED PROTEINS INVOLVED IN HUMAN ES CELL SURVIVAL AND DIFFERENTIATION

G Kolle¹, A Topolska², B Gardiner¹, A Laslett², M Pera², S Grimmond¹

¹ Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia, ² Monash Institute of Medical Research, Melbourne, Victoria, Australia

Embryonic Stem cells have the potential to form all the tissues of the adult body. While progress towards maintaining and differentiating embryonic stem cells in a directed manner has been rapid, there are still fundamental issues to be dissected. Secreted proteins provide one of the most robust ways of influencing the growth and differentiation of ES cells. Previous work performed by us and others has defined the mammalian secretome - the compliment of secreted proteins in the genome (Grimmond *et al.*, Genome Res. 2003,13:1350-9). Using this dataset and differential display techniques, we are investigating the secreted compliment of mammalian ES cells and how these can be used to affect ES cell growth and differentiation. We have undertaken microarray analysis to define potential secreted (and other) factors that are differentially expressed between differentiating human ES cells. A set of highly expressed secreted factors including those already known to play a role such as the nodal co-factors Cripto-1 and lefty were identified. Further to this, we have developed a reverse transfection platform to screen the effects of secreted proteins on human ES cell survival and differentiation. Using the FANTOM2 full length clone dataset, we have produced a library of secreted factors, including several classes of growth factors as well as other known and novel proteins. The results of the pilot screens will be discussed.

P-113**RECESSIVE GENETIC SCREEN IN ES CELLS BY CONDITIONAL DISRUPTION OF THE BLOOM'S SYNDROME GENE**

K Yusa, K Horie, J Takeda

Osaka University, Osaka, Japan

Forward genetic screen is a powerful strategy to identify genes responsible for phenotypes of interest. But this has been hampered in mammalian systems because of the difficulties to introduce null mutation to both alleles and to construct a comprehensive mutant library that covers all genes. In order to facilitate the forward genetic screen in mammals, we focused on the characteristic of Bloom's syndrome (BS), a rare genetic disorder associated with cancer predisposition. BS cells show high frequency of recombination between homologous chromosomes as well as sister chromatids, resulting in mitotic recombination and sister chromatid exchange (SCE), respectively. We developed a novel system for a recessive genetic screen in mouse ES cells.

To regulate the expression of the BS gene (*Blm*), we generated a conditional allele of the *Blm* using a tet-off system. In targeted homozygous ES cells, *Blm* protein disappears quickly and elevated level of SCEs was observed upon administration of doxycycline. The rate of mitotic recombination was elevated 27 folds. Combining with ENU mutagenesis, we made an ES cell library bearing bi-allelic mutation and screened mutants lacking for GPI-anchor biosynthesis. Mutants corresponding to half of 23 known genes and 2 novel mutants were obtained from the library, indicating the effective generation of the bi-allelic mutant library.

Our new method for comprehensive isolation of bi-allelic mutants should have a major impact on the analysis of molecular mechanism of pluripotency and differentiation of ES cells.

P-114**PROLIFERATION DEFECTS IN EPIDERMAL GROWTH FACTOR RECEPTOR NULL PLACENTAE CAN BE RESCUED IN THE ABSENCE OF CYCLIN-DEPENDENT KINASE INHIBITORS.**

J Dackor, K E Strunk, D W Threadgill

University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

The Epidermal Growth Factor Receptor (EGFR) is a receptor tyrosine kinase known to activate multiple signaling pathways and lead to diverse cellular responses such as proliferation, differentiation, migration and invasion. Reduced EGFR signaling in the placenta has been associated with intrauterine growth retardation (IUGR) in humans, and mice homozygous for a null allele of *Egfr*, *Egfr^{tm1Mag}*, display a strain-dependent placental phenotype that results in embryonic lethality on many genetic backgrounds. Most strains examined to date exhibit a reduced spongiotrophoblast layer but strains dying before mid-gestation are distinguished by a disorganized labyrinth layer. Previous studies using BrdU have shown that e10.5 *Egfr* null placentae contain fewer proliferating trophoblasts than wildtype placentae. We have intercrossed mice heterozygous for *Egfr^{tm1Mag}* and null alleles for genes regulating the cell cycle in order to characterize interactions between EGFR and cell cycle machinery in murine trophoblasts. *Egfr* nullizygous embryos were rescued 30% of the time in a *Cdkn1a* (*p21*)- but not *Trp53*- or *pRb*- deficient background. Histological analysis of the *p21*, *Egfr* nullizygous placentae showed a less severe labyrinth defect compared to *Egfr* nullizygous placentae with wildtype *p21*. To better understand the P53-independent interaction between EGFR and P21 we have set up intercrosses with mice heterozygous for null alleles of *Smad3*, *Cdkn1b* (*p27*) and *Cdkn2c* (*p18*). Data from *Egfr^{tm1Mag}* crosses with SMAD3-deficient mice may identify a role for TGF- β signaling. Intercrosses with P27- and P18-deficient mice will reveal whether the relationship between EGFR and cyclin-dependent kinase inhibitors (CKIs) is specific for the CIP family of CKIs or is a more general interaction with CIP (P21, P27, P57) and INK4 (P16, P17, P18, P19) CKIs.

P-115**GENOMIC MAPPING OF FGF SIGNALLING INPUT TO THE BRANCHING LUNG EPITHELIUM**K A Peterson¹, J E Richardson², I C Welsh¹, C J Bult², T P O'Brien¹¹ Cornell University, Ithaca, NY, United States, ² The Jackson Laboratory, Bar Harbor, ME, United States

Reciprocal signalling interactions between epithelial and mesenchymal cells coordinate the process of lung branching morphogenesis by differentially regulating the expression of downstream target loci. A conserved set of signalling pathways facilitate this intercellular communication by transmitting instructive cues carried out by the activation of transcription factors. *cis*-Regulatory modules made up of clusters of multiple transcription factor binding sites (TFBS) are "hard-wired" in the genome and establish a combinatorial code for orchestrating tissue specific gene expression. In order to better understand the underlying genomic regulatory architecture, we constructed a signalling network model from the point-of-view of the branching epithelium to interpret the incoming FGF signal from the mesenchyme. A morphoregulatory network of 20 loci was selected based upon gene expression pattern and perturbation studies demonstrating an essential contribution to the lung branching process. The PEA3 subfamily of ETS transcription factors contains *Etv4* and *Etv5*, both of which are expressed in the branching epithelium and respond to FGF10 stimulation. Using the consensus PEA3 binding site in combination with cell-type specific TFs such as NKX2.1, FOXA1 and GATA6, we scanned each locus for combinatorial clusters of TFBS. The resulting *cis*-regulatory modules were prioritized using comparative genomics and experimentally tested for enhancer activity in a cultured mouse lung epithelial cell line. These studies address the question of how regulatory information is patterned within the genomic landscape and investigate its relationship to dynamic gene expression during development.

P-116**THE NADPH OXIDASE COMPLEX GENES *NOX3* AND *NOXO1* ARE REQUIRED FOR NORMAL VESTIBULAR FUNCTION AND DEVELOPMENT**D E Bergstrom¹, J Masse¹, R J Munroe², J C Schimenti², L H Gagnon¹, K R Johnson¹, U Heinzmann³, G Stumm⁴, R Paffenholz⁴¹ The Jackson Laboratory, Bar Harbor, ME, United States, ² Cornell University, Ithaca, NY, United States,³ GSF-National Research Center for Environmental Health, Nueherberg, Germany, ⁴ Ingenium Pharmaceuticals, Martinsried, Germany

The ability of the mammalian inner ear to detect linear acceleration and gravity is dependent upon the proper formation, localization, and maintenance of crystalline deposits known as otoconia. Otoconia act as inertial masses affixed to stereocilia of the saccular and utricular maculae. Deflection of otoconia/stereocilia in response to linear acceleration and/or gravity generates vestibular-evoked potentials that are transmitted to the brain. In humans, aging, trauma, and ototoxic drugs can interfere with normal otoconial localization and maintenance, resulting in vestibular dysfunction. To better understand the development of the macular/otoconial system, we have been studying the head tilt (*het*) and head slant (*hslt*) loci of mice. Both *het* and *hslt* mutant mice display a complete absence of saccular and utricular otoconia and display several behaviors consistent with vestibular impairment.

Recombination- and deletion-based positional cloning strategies have identified *Nox3* and *Noxo1* as the causative genes underlying the *het* and *hslt* phenotypes, respectively. Sequence analysis shows that *Nox3* is paralogous to *gp91phox* (*Nox2*), an NADPH oxidase of immune cells that generates reactive oxygen species as a bacteriocidal weapon against invading microorganisms. Similarly, *Noxo1* is paralogous to *p47phox* (*Ncf1*), a cytosolic component of the same oxidase complex.

Based on these results, we have hypothesized that a novel NADPH oxidase complex, composed of immune complex paralogs, is present in the vestibular system and acts to initiate otoconial development from components in the endolymph including otoconin 90, calcium, otopetrin 1, and bicarbonate.

Current experiments are directed at identifying additional components of the vestibular NADPH oxidase complex.

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MOUSE MODELS OF INFERTILITY: AN ENU-INDUCED MUTANT MOUSE RESOURCE FOR GENE DISCOVERY IN REPRODUCTIVE BIOLOGY

J K Pendola¹, S Sweeney¹, H Lothrop¹, J Schimenti², M A Handel¹, J Eppig¹.

¹ The Jackson Laboratory, Bar Harbor, united States. ² Cornell University, Ithaca, NY, United States.

The ReproGenomics Program at The Jackson Laboratory utilizes ENU mutagenesis and phenotype screening to generate mutant mouse models of infertility for the scientific community. The phenotypes assessed in these mutant lines include gonad histology, gametogenesis, fertilization and pre-implantation development. In the past 3 years, 36 mutant mouse lines have been identified with recessive mutations leading to infertility (determined by natural mating). Thus far, 29 infertility mutations have been mapped to distinct chromosomal regions. Together, these mouse models represent a wide range of reproductive phenotypes including meiotic arrest, sperm morphology and motility defects, absence of growing oocytes and follicles, poor pre-implantation development and unexplained infertility. These valuable and unique reproductive mutant mouse resources are available at no cost through the program web site at <http://reprogenomics.jax.org>. Dissection of gene function using these mutant models will deepen understanding of known reproductive pathways, facilitate identification of new pathways, and lead to the discovery of novel contraceptive targets and therapies for infertile couples. Supported by the NIH PO1 HD42137

**See page 26 for P-118 and P-119
27 for P-120 and P-121
28 for P-122 and P-123**