

**DEVELOPMENT/CANCER BIOLOGY/AGING****ORAL PRESENTATION****Tuesday October, 30****11.30 – 11.45am****O5-1****DISTRIBUTION OF CTCF BINDING SITES ALONG THE X CHROMOSOME IN RELATION TO X INACTIVATION IN MOUSE EMBRYOS AND ADULTS**Fan Yang<sup>1</sup>, Galina Filippova<sup>2</sup> and Christine Disteche<sup>1</sup><sup>1</sup>Department of Pathology, University of Washington, Seattle WA 98195, USA, <sup>2</sup>Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle WA 98109, USA

X inactivation silences most genes on one X chromosome in early female development; however, some genes escape X inactivation in a species-specific manner. The mechanisms of escape from X inactivation are still elusive. Previously, we found that CTCF, a CCCTC-binding factor and a chromatin insulator, binds at the 5' end of two escape genes adjacent to an inactivated gene, suggesting that CTCF acts as an insulator to separate escape and inactivated domains. Here, we compare the chromosome-wide distribution of CTCF binding sites along the mouse X chromosome in male and female adult livers and 12.5dpc embryos by using chromatin immunoprecipitation combined with high-resolution DNA tiling arrays (ChIP-chip). Our results indicate that the majority of CTCF binding sites are highly conserved between males and females and that the distribution of CTCF binding sites is also very similar between adult and 12.5dpc embryos. In addition, CTCF does bind to transition regions between five known escape genes (*Eif2s3x*, *Jarid1c*, *Ikbkg*, *Ubelx*, and *Utx*) embedded within inactivated domains, further supporting the insulator function of CTCF in escape from X inactivation.

**DEVELOPMENT/CANCER BIOLOGY/AGING****ORAL PRESENTATION****Tuesday October, 30****11.45 – 12.00pm****O5-2****MOLECULAR CROSS-TALK DURING EARLY NEURONAL DIFFERENTIATION: COMPARATIVE ANALYSES USING THE DLL1, NOTCH1 AND MIND BOMB1 KNOCKOUT SYSTEMS**Rashmi Rajendra<sup>1</sup>, Gerhard K. H. Przemeck<sup>1</sup>, Karen Artzt<sup>2</sup>, Martin Hrabe de Angelis<sup>1</sup><sup>1</sup>Institute of Experimental Genetics, GSF - National Research Center for Environment and Health, Neuherberg (Munich) Germany, <sup>2</sup>Institute for Molecular and Cellular Biology, University of Texas at Austin, Texas, USA

The murine embryo is ideal for understanding early vertebrate neurogenesis at the molecular level. Delta-Notch signaling plays an iterative role during embryonic development. In this study, we have focused on the analyses of ligand, receptor and effector in parallel, by using the Dll1, Notch1 and Mind bomb1 (Mib1) knockout systems. The similarities in all three CNS, are premature neuronal differentiation, loss of contact inhibition, premature neuronal cell death and down-regulation of Delta-Notch down-stream effectors. The main difference is temporal, wherein the Dll1 knockout embryos show similar phenotypes one day later as compared to Notch1 and Mib1. Temporal changes can be analyzed using embryonic development timing and molecular markers for early nervous system. Further, we show in detail a loss of neuroepithelial integrity within these mutant embryos and the molecular status of these cells. One interesting observation accompanying the loss of neuroepithelial integrity is the changes in sub-cellular localization of activated  $\beta$ -Catenin, along with altered localization of activated Notch1, within the Dll1 and Mib1 embryos. This study also aims at understanding molecular correlations between Notch-Delta and  $\beta$ -Catenin signaling, within this context.

**DEVELOPMENT/CANCER BIOLOGY/AGING****ORAL PRESENTATION****Tuesday October, 30****12.00 – 12.15pm****O5-3****HARNESSING TRANSPOSONS FOR CANCER GENE DISCOVERY**

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Cancer gene discovery continues to drive current cancer research with the promise of identifying new diagnostic markers and therapeutic targets by elucidating novel genetic interactions that promote or sustain tumor formation. *Sleeping Beauty* (SB) transposon-mediated insertional mutagenesis has emerged as an exciting approach to identify novel cancer-causing genes in the mouse. The SB transposon faithfully “hops” throughout the genome by a cut-and-paste mechanism mediated by the ubiquitous expression of the SB transposase. Initial tumor data generated using an SB transposon harboring the MSCV promoter demonstrated a bias towards hematopoietic tumors. More recently, experiments using a modified SB transposon containing the CAG promoter have generated cohorts of mice with solid tumors, primarily carcinomas, which in some cases metastasize. Many animals also develop multiple, independent primary tumors. These data demonstrate the utility of the SB transposition system for cancer gene discovery across organ systems. Recently, our lab has also developed an inducible expression system for the SB transposase that allows for saturation mutagenesis in any given tissue of interest in both wild type and sensitized mutation backgrounds. We are currently using this inducible SB transposition system to interrogate the genetic pathways involved in the initiation and progression of several solid tumor types, including skin, liver, GI and pancreas.

**DEVELOPMENT/CANCER BIOLOGY/AGING****ORAL PRESENTATION****Tuesday October, 30****12.15 – 12.30pm****O5-4****HM1 NEGATIVELY MODULATES MOUSE SONIC HEDGEHOG (SHH) SIGNAL TRANSDUCTION AND AFFECTS INTRAFLAGELLAR TRANSPORT**

Pamela V. Tran<sup>1</sup>, Courtney J. Haycraft<sup>2</sup>, Tatiana Y. Besschetnova<sup>3</sup>, Rolf W. Stottmann<sup>1</sup>, Bruce J. Herron<sup>4</sup>, Jagesh V. Shah<sup>3</sup>, Bradley K. Yoder<sup>2</sup>, David R. Beier<sup>1</sup>

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Characterization of intraflagellar transport (IFT) mouse mutants has led to the proposition that normal primary cilia are required for mammalian cells to respond to the Sonic Hedgehog (SHH) signal. We describe an ENU-induced mutant mouse, alien (aln), which is the first mutant reported with both abnormal primary cilia and derepression of the SHH pathway. aln mutants exhibit preaxial polydactyly, craniofacial abnormalities and ventralization of the neural tube. The aln locus encodes a novel protein, THM1 (Tetratricopeptide repeat (TPR)-containing Hedgehog modulator 1), which localizes to cilia and is an ortholog of Chlamydomonas Flagellar Associated Protein 60 (FAP60). aln cilia have bulb-like structures at their tips which have abnormal accumulations of IFT proteins, characteristic of the ciliary phenotypes of *Caenorhabditis elegans* retrograde IFT mutants. In aln, full-length glioblastoma (GLI) proteins properly localize to cilia, which does not occur in previously described IFT mutants. To define a role for THM1 in IFT, we have knocked down Thm1 using RNAi in IMCD cells that stably express IFT88-YFP and are currently examining anterograde and retrograde IFT velocities using live-cell imaging. Preliminary results show that different levels of Thm1 knock-down produce two ciliary phenotypes: 1) severely affected cilia with abnormal accumulations of IFT88-YFP, recapitulating the aln ciliary phenotype and 2) moderately affected cilia which are significantly longer than in wild-type. We propose that loss of THM1 causes defective retrograde transport resulting in accumulation of IFT proteins, while maintaining functional anterograde IFT, enabling entry of SHH signaling components into cilia and thereby accounting for the increased SHH signaling phenotype in aln which contrasts with other mutations that affect primary cilia.

## DEVELOPMENT/CANCER BIOLOGY/AGING

ORAL PRESENTATION

Tuesday October, 30

12.30 – 12.45pm

O5-5

**CTHRC1 SELECTIVELY ACTIVATES PLANAR CELLULAR POLARITY PATHWAY OF WNT SIGNALING BY STABILIZING WNT-RECEPTOR COMPLEX**

Shinji Yamamoto, Osamu Nishimura, Kazuyo Misaki, Shigenobu Yonemura, Hiroshi Tarui and Hiroshi Sasaki  
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Wnt ligands activate several distinct pathways, but the mechanism of pathway selection is not known. Here we show that Cthrc1 (collagen triple helix repeat containing 1) is involved in the selective activation of planar cellular polarity (PCP) pathway by Wnt ligands. Cthrc1 is a secreted glycoprotein with 12 GXY repeat. Cthrc1 is strongly expressed in tissues with active PCP signaling including notochord and the sensory hair cells of the inner ear. Although its null-mutants showed no apparent abnormality, introduction of a heterozygous mutation of a PCP gene, *Vangl2* (*Loop-tail* allele) (*Cthrc1*<sup>-/-</sup>; *Vangl2*<sup>Lp/+</sup>) resulted in the abnormalities characteristic to PCP mutations: open brain and randomized orientation of sensory hair cells of the organ of Corti. In 293T cells, Cthrc1 acted non-cell autonomously and suppressed activation of the canonical Wnt pathway at the downstream of Dishevelled and upstream of b-catenin. On the other hand, Cthrc1 activated RhoA, Rac1 and JNK, the downstream components of the Wnt-PCP pathway. Cthrc1 bound to various extracellular components of Wnt signaling including Wnt, Frizzled, Ror2 and LRP5/6. However, when these components were combined, Cthrc1 stabilized the receptor-ligand complex for the Wnt-PCP pathway (Wnt-Frizzled-Ror2) but not the complex for the canonical Wnt pathway (Wnt-Frizzled-LRP5). Taken together, these results suggest that Cthrc1 is a novel Wnt signaling component, which selectively activates the PCP pathway, and its underlying mechanism is enhancement of Wnt-receptor interaction by forming a stabilized complex of Cthrc1-Wnt-Frizzled-Ror2.

**DEVELOPMENT/CANCER BIOLOGY/AGING****ORAL PRESENTATION****Tuesday October, 30****12.45 – 13.00pm****O5-6****IDENTIFICATION AND CLONING OF EMBRYONIC LETHAL GENE, *tw5*, MAPPED WITHIN THE T/t COMPLEX OF THE MOUSE**Michihiko Sugimoto<sup>1</sup>, Misako Yuzuriha<sup>1</sup>, Michiko Hirose<sup>2</sup>, Atsuo Ogura<sup>2</sup>, Karen Artzt<sup>3</sup>, Kuniya Abe<sup>1</sup><sup>1</sup>Mammalian Cellular Dynamics, RIKEN BRC, Tsukuba, <sup>2</sup>Bioresource Engineering Division, RIKEN BRC, Tsukuba,<sup>3</sup>Inst. Cell Mol. Biol., Univ. Texas Austin

The mouse T/t-complex is a genetic region spanning about 15 cM of mouse chromosome 17, harboring a number of mutations that affect tail length, embryonic development, and germ cell functions, which have long intrigued developmental geneticists. Despite of more than 80 years history of the t-complex research, so-called “t-lethal” has not been cloned to date. Here we report identification of one of the t-lethal genes, *tw5*. Mutation in *tw5* causes embryonic lethality at the gastrulation stage with extensive death of the embryonic ectoderm while extraembryonic tissues are less affected. Our tetraploid complementation study indicated that the *tw5* likely functions in extraembryonic tissues to support proliferation/differentiation of embryonic ectoderm through cell-cell interactions. Thus the *tw5* gene product should represent an important developmental regulator of pluripotent cells in mammalian embryos. The *tw5* gene is mapped near the H-2K region, and we have narrowed down a critical region to 750 kb covered by 5 overlapping BAC clones. We successfully rescued the lethality by introducing one of these BAC clones. The BAC clone was further fragmented, and a 20 kb genomic fragment carrying a single gene finally rescued the lethality. In the exon 2 of this gene, 2 bp insertion that caused a frame shift mutation was found in *tw5*-haplotype genome, but not in other t-haplotype genome, including spontaneous revertant of the *tw5*, clearly indicating that the gene is responsible for the *tw5* mutation.