**Program/Abstract #66**

**Regulation of mammalian Notch signaling and embryonic development by the protein O-glucosyltransferase Rumi**

Rodrigo Fernandez-Valdivia\(^a\), Hideyuki Takeuchi\(^b\), Amin Samarghandi\(^c\), Mario Lopez\(^d\), Jessica Leonard\(^c\), Robert Haltiwanger\(^e\), Hamed Jafar-Nejad\(^a\)

\(^a\)Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX, USA
\(^b\)Stony Brook University, Stony Brook, NY, USA
\(^c\)Baylor College of Medicine, Houston, TX, USA

The Notch signaling pathway plays important roles in animal development, stem cell biology and homeostasis. Mutations in components of this pathway cause several diseases, including the Alagille syndrome (caused by JAGGED1 mutations). In Drosophila, the protein O-glucosyltransferase (Poglut) Rumi adds glucose to Notch EGF repeats harboring the C1-X-S-X-P-C2 consensus sequence and regulates Notch signaling. To determine the contribution of protein O-glucosylation to mammalian Notch signaling we performed genetic, cell culture and biochemical studies on mammalian homologs of the Drosophila Rumi and found that only one of these, which we also call Rumi, has Poglut activity. Rumi−/− mouse embryos die before E9.5 with posterior axis truncation and severe defects in neural tube development, somitogenesis, cardiogenesis and vascular remodeling. Rumi knockdown in mouse cell lines results in decreased Notch signaling without affecting Notch ligand binding. Overexpression of human Rumi rescues the phenotypes caused by loss of fly Rumi or knockdown of mouse Rumi. Our data indicate that a decrease in Rumi levels results in reduced O-glucosylation of Notch EGF repeats, and that Rumi's enzymatic activity is key to its regulatory role. Genetic interaction studies show that removing one copy of Rumi in a Jagged1+/− background results in severe bile duct morphogenesis defects, similar to Alagille patients. Therefore, addition of O-glucose to EGF repeats is essential for mouse embryonic development and Notch signaling, and Jagged1-induced signaling is sensitive to the gene dosage of Rumi. The severity of Rumi−/− embryonic phenotypes indicates that Rumi is likely to have additional important targets during mammalian development.

doi:10.1016/j.ydbio.2011.05.089

**Program/Abstract #67**

**Requirements for Jag1-Rbpj mediated Notch signaling during early lens development**

Tien Le\(^a\), Kevin Conley\(^b\), Timothy Meada\(^b\), Sheldon Rowan\(^b\), Katherine Yutzey\(^c\), Nadean L. Brown\(^c\)

\(^a\)Cincinnati Childrens Research Foundation, Cincinnati, OH, USA
\(^b\)Harvard Medical School, Boston, MA, USA
\(^c\)Cincinnati Children's Res Fnd Div Devel Biol, Cincinnati, OH, USA

Vertebrate lens development is a multistep process where embryonic ectoderm thickens to form the lens placode, which invaginates into a lens vesicle. After vesicle separation from the surface epithelium, there are two waves of fiber differentiation. In the mouse, multiple labs have demonstrated a critical requirement for Jag1-Notch signaling during the second wave of fiber formation. However, Notch signaling appears to have no role during lens induction or morphogenesis, although multiple pathway genes are expressed. Here we test specifically for roles of Notch signaling at the earliest stages of lens development, using the AP2alpha-Cre driver to delete Jag1 or Rbpj. Somewhat surprisingly, no requirement for Jag1 or Rbpj during lens induction was found. Instead, early loss of Jag1 or Rbpj affected apoptosis during lens vesicle separation from the surface ectoderm. Overexpression of activated Notch1 (NotchIC) also affects apoptosis and vesicle closure and separation. We conclude that in the rodent eye, Notch signaling is not essential for lens induction, although precise levels of Notch signaling are important for vesicle morphogenesis. In addition, AP2alpha-Cre-mediated deletion caused embryonic heart valve and liver abnormalities in Rbpj conditionally mutant embryos.

doi:10.1016/j.ydbio.2011.05.089

**Program/Abstract #68**

**CoREST acts as a positive regulator of Notch signaling in the follicle cells of Drosophila**

Elena Domanitskaya, Trudi Schupbach

HHMI / Princeton University, Princeton, NJ, USA

The Notch signaling pathway has been implicated in the control of a broad array of developmental events. The effects of Notch signaling are highly context specific and depend especially on the presence of positive and negative modulators of Notch signaling. By identifying various factors that regulate Notch signaling, we will gain deeper insights into the mechanisms by which Notch-dependent pathological conditions arise, for instance cancers exhibiting Notch misregulation. To study Notch signaling pathway regulation, we are using the follicular epithelium of the Drosophila ovary as a model system. Follicle cells divide mitotically from stage 2 to stage 6 of oogenesis, followed by the switch from the mitotic cycle to the endocycle triggered upon Notch signaling activation. In order to discover new genes involved in the control of follicle cell patterning, differentiation and morphogenesis, we performed a genetic mosaic screen, and isolated GF60 allele. We showed that GF60 homozygous mutant follicle cells fail to switch from the mitotic cycle to the endocycle. We mapped the lethal GF60 mutation to the gene encoding the transcriptional corepressor CoREST (corepressor for element-1-silencing transcription factor), and we showed that CoREST is a positive regulator of Notch signaling in Drosophila follicle epithelium cells. We show that CoREST acts downstream of Notch proteolytic cleavage and
upstream of Hindsight (Hnt), a Notch target gene that coordinates responses in the follicle cells. Genetic interactions between CoREST and components of the Notch repressor complex, CtBP and Gro, as well as changes in chromatin modifications in CoREST mutant cells, indicate that CoREST is a nuclear modulator of the Notch pathway.

doi:10.1016/j.ydbio.2011.05.090

Program/Abstract #69
Establishment of transgenic lines that report nervous system specific Notch activity based on nort gene regulatory sequence
Joel B. Miesfeld, Brian S. Clark, Brian A. Link
Medical College of Wisconsin Cell Bioloy, Neurobiology, & Anatomy, Milwaukee, WI, USA

Notch signaling is important in development of the vertebrate retina. During zebrafish retinal neurogenesis Notch activity is polarized as demonstrated by the Tg(−3.4 kb her4.3:3:RFP/knu2 transgenic line (Del Bene, 2008). Destabilized fluorescent protein (FP) expression driven by the her4 regulatory sequence was elevated in retinal progenitors with apical nuclei. We are interested in determining if her4.3 FP expression is unique to this transgene or general to other Notch target genes. To investigate this phenomenon we generated additional Notch reporter lines using the previously characterized Tp1 promoter, which consists of 12 Notch responsive RBP-Jk binding sites driving FP (Parsons, 2009). In addition, we established other Notch reporter transgenic lines based on regulatory sequence for the notch-regulated transcript (nort) gene. Expression of nort is augmented by Notch and enriched in neural progenitors, including those of the retina (Tsutsmi and Itoh, 2007). We generated a construct in which FP was expressed from 3.5 kb of genomic sequence directly upstream of nort. Expression of FP from the nort transgene overlapped with endogenous nort expression. Like endogenous nort, we found that FP from the −3.5 kb nort transgene was partially dependent on Notch activity, as demonstrated by morpholino knockdown of Notch receptors or RBP-Jk. Similar to the her4.3:FP transgene, nort:FP and Tp1bglb:FP were elevated in a subset of retinal cells with apical nuclei. Interestingly, while each transgene showed overlapping expression, distinct temporal patterns were noted. We are currently assessing how each expression pattern relates to cell fate within the retina.

doi:10.1016/j.ydbio.2011.05.091

Program/Abstract #70
Neuropeptide signaling in planarian sexual development and regeneration
Amir Saberi, James Collins, Phillip Newmark
University of Illinois at Urbana-Champaign, Urbana, IL, USA

Sexual free-living planarians can dynamically develop or dismantle their reproductive tissues in response to external conditions. We recently showed that a Neuropeptide Y-like peptide secreted by planarian nerve cells is essential for proper sexual development. Specifically, planarians without this hormone fail to develop or maintain reproductive organs. To identify receptors for this peptide, we analyzed spatial expression and RNA interference phenotype of a number of genes predicted to encode G-protein coupled receptors (GPCRs) with similarity to neuropeptide Y receptors from other organisms. This analysis found one gene, referred to as gpcr-b01, RNAi knockdown of which resulted in a block in germ cell differentiation within the testes and failure in the development of accessory reproductive organs. NPY-like hormones are implicated in diverse functions such as feeding behavior, energy homeostasis, and alcohol sensitivity, in species ranging from flies to humans. Our study suggests a role for NPY signaling in the regulation of reproductive physiology in flatworms.

doi:10.1016/j.ydbio.2011.05.092

Program/Abstract #71
Lefty activity is regulated by prodomain-mature lefty interaction
Adrian Vasquez, Amapola Balancio, James Nowakowski, William Branford
Wayne State University, Detroit, MI, USA

Members of the transforming growth factor beta (TGFβ) superfamily of secreted ligands play an integral role in vertebrate embryonic development. One member of this superfamily, Nodal, regulates mesendoderm induction and left-right axial development. Lefty, an atypical member of the TGFβ superfamily, inhibits Nodal signaling by interaction with EGF-CFC Nodal co-receptors and Nodal itself. Without Lefty function, unregulated Nodal signaling severely disrupts embryonic development, yet little is known about how Lefty activity is regulated. Many members of the TGFβ superfamily, including Lefty, are inactive prior to proteolytic cleavage of the prodomain from the mature portion of the protein, as shown by mutants incapable of being cleaved. Based on three results, we propose that in Lefty this inhibition is mediated by interaction between the prodomain and mature Lefty. First, the Xenopus Lefty (Xlefty) prodomain can co-immunoprecipitate mature Xlefty, but not uncleaved Xlefty. Second, the co-expression of the prodomain with full-length Xlefty in the Xenopus embryo antagonizes the effects of Xlefty overexpression. Third, the expression of the Xlefty prodomain in the Xenopus embryo results in exogastrulation, a phenotype which we have previously observed with knockdown of Xlefty. Additionally, we propose that prodomain-mature Xlefty interaction prevents mature Xlefty from interacting with Nodal co-receptors. Consistent with this proposal, preliminary results suggest that Xenopus Cripto-related 1, an EGF-CFC co-receptor, co-immunoprecipitates mature Xlefty, but not uncleaved Xlefty. Future studies will be aimed at determining if a post-cleavage prodomain-mature Xlefty interaction mediates a latent Xlefty complex.

doi:10.1016/j.ydbio.2011.05.093

Program/Abstract #72
Poster Board #B15

Program/Abstract #72 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

doi:10.1016/j.ydbio.2011.05.094

Program/Abstract #73
Notum 1α is a specific inhibitor in Wnt/Beta-Catenin signalling
G. Parker Flowers, Jolanta Topczewska, Jacek Topczewski
Northwestern University, Chicago, IL, USA

Wnts are a large family of secreted proteins crucial for numerous processes in the developing embryo. Proper development requires tight regulation of Wnt signalling both intracellularly and extra-cellularly. Glypicans are a class of heparan-sulfate proteoglycans...