mice have prenatal eye phenotypes (1–3), and Alagille Syndrome patients with *Jagged1* mutations exhibit anterior eye deformities (4). But, very little is known about the roles of *Notch* signaling in eye induction, growth or morphogenesis. We found that the ligand *Jagged1*, and two receptors, *Notch1* and *Notch2*, are expressed during lens placode invagination, vesicle morphogenesis and fiber cell differentiation. To determine the requirements for *Notch* signaling in the lens, we are conditionally deleting *Notch* pathway genes in this tissue. First we examined loss of the vertebrate *Su(H)* orthologue, *RBP-JK1*, which complexes with the *Notch* intracellular domain to transduce canonical signaling via *Hes1* transcriptional activation. When *RBP-JK1* was removed during embryonic lens formation, we observed reciprocal changes in lens progenitor cell proliferation versus fiber cell differentiation, consistent with reduced proliferation in *Hes1* mutants. Our model for *Notch* regulation of lens proliferation and differentiation will be presented. 1. Development 124: 1139–1148 (1997)2. Development 128: 491–502 (2001)3. Human Molecular Genetics 8: 723–730 (1999)4. Nature Genetics 16: 235–242 (1997).

doi:10.1016/j.ydbio.2007.03.169

Program/Abstract # 112

The Yin and Yang of Notch signalling; trans-activation and cis-inhibition fine-tune Notch signalling

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The Notch receptor is *trans*-activated by single pass transmembrane ligands present on the surface of neighbouring cells. In mammals Dll1, Dll4, Jag1 and Jag2 can activate the Notch1 receptor in trans, and inhibit Notch1 signalling when expressed in the same cell as the receptor. It is considered that Notch signalling must be tightly regulated, and we propose that this is achieved through the interplay of *trans*-activation and *cis*-inhibition. Using an in vitro co-culture system that recapitulates Notch signalling, we show that Dll3 (the most divergent ligand of Notch) cannot *trans*-activate Notch1 but can *cis*-inhibit signalling. In addition we show that Dll3, unlike the other ligands, localises to the Golgi apparatus in preference to the cell surface. Furthermore, mutant forms of Dll3 that cause the congenital vertebral malsegmentation disorder spondylocostal dysostosis (SCD) are mislocalised. In Dll3 null mouse embryos and individuals with SCD, somite formation is abnormal resulting in vertebral anomalies. Therefore, we examined Notch1 signalling in the presomitic mesoderm (PSM) of Dll3-null embryos by immunohistochemistry. Comparison of these cells in the PSM with those that express Dll3 demonstrates that in Dll3 null mouse embryos, the domain in which Notch1 is activated is expanded indicating that Dll3 is required to restrict Notch1 signalling in the PSM. This is consistent with an inhibitory role for Dll3 in Notch1 signalling.

doi:10.1016/j.ydbio.2007.03.170

Program/Abstract # 113

The role of endocytosis in activin signalling during mesoderm induction in *Xenopus*

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Work from several laboratories has shown that endocytosis plays a critical role in modulating morphogen signalling, transmission and response during embryonic development. I am investigating to what extent endocytic trafficking influences mesoderm induction by Xnr2 and activin in the *Xenopus* embryo, both in terms of the signalling ranges of these molecules and in terms of the ways that cells respond to them. I have used a dominant-negative form of dynamin and RN-tre to inhibit endocytosis, and observed signalling in real time using GFP-tagged forms of these TGF-b family members, as well as Alexa 488-labelled activin. My data show that inhibition of endocytosis by RN-tre injection decreases morphogen signalling ranges, and up-regulation of endocytosis by over-expressing Rab5 increases it. Use of quantitative RT-PCR indicates that these endocytic reagents have little effect on the ability of the cells to respond to activin. I am now further investigating to what extent endocytosis affects signal production and signal transmission.

doi:10.1016/j.ydbio.2007.03.171

Program/Abstract # 114

The role of *Endothelin-1/Endothelin Receptor A* signaling in neural crest specification and cell survival

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The neural crest (NC) is a multipotent cell population that gives rise to different tissues after extensive migration along the vertebrate embryo. In the frog *Xenopus laevis*, the NC is specified in the ectoderm from late gastrula stage by interactions between different signals emanated from the epidermis, neural plate and mesoderm. It has been proposed that neural crest induction is a multi step process, but so far these different steps are not completely understood. In this work, we show for the first time the existence of a NC maintenance step which occurs after the initial induction of NC, and that is dependent on Edn1 signal released from the mesoderm underlying the neural crest at the neurula stage. We have cloned *Xenopus Preproendothelin-1* and *ECE-1* homologues, and the analysis of the expression

patterns confirmed that mesoderm is a source of Edn1 signal. Gain- and loss-of-function approaches, the use of a specific Ednra inhibitor, and embryological experiments show that *Edn1*/*Ednra* signaling is required for neural crest development through a dual mechanism that controls neural crest specification and cell survival. The blocking of the apoptosis by a *Slug*-inducible construct in NC explants indicates that the control of NC specification by *Edn1*/*Ednra* signaling is independent from the control of cell survival. In addition, the epistatic analysis shows that *Ednra* is downstream *Msx1* and upstream *Sox9* and *Sox10* in the NC specification cascade. Our results provide insight on a new role of *Edn1*/*Ednra* cell signaling pathway during NC development.

doi:10.1016/j.ydbio.2007.03.172

Program/Abstract # 115
Substrate selectivity by proprotein convertases
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Proprotein convertases (PCs) are a family of serine proteases important for cleavage of various substrates, including growth factors and morphogens during development and viral proteins during pathogenesis. There are seven PCs that have been identified in vertebrates—PC1, PC2, furin, PC4, PACE4, PC6, and PC7. In vivo evidence suggests that each of these PCs cleaves distinct, albeit in some cases overlapping, targets. However, targets have been difficult to identify because in vitro analyses have yielded little if any specificity; and moreover, mouse models in which PCs have been knocked out are too complex due to other PCs compensating and cleavage of multiple targets being disrupted. In order to combine the directness of in vitro analysis with in vivo relevance, I have developed an assay using biochemistry in *Xenopus* oocytes to identify in vivo targets of PCs. Specifically, I have used this assay to identify the PCs responsible for cleaving three candidate TGFb family members—BMP4, Xnr2, and $Activin_{bB}$. For the first time in vivo, I have direct biochemical evidence that BMP4 is cleaved by furin and PC6, Xnr2 is cleaved by furin and PACE4, and $Activin_{bB}$ is cleaved by furin. I will next use this assay to probe the question of what determines PC selectivity, as this has not been well characterized. Preliminary evidence suggests that sequences within the substrate prodomain may contribute to selectivity, so I have swapped prodomains between these three TGFb family members and will determine which PCs can now cleave the chimeric constructs in vivo. These studies, once completed, will further our understanding of how PCs accomplish substrate selectivity.

doi:10.1016/j.ydbio.2007.03.173

Program/Abstract # 116
Role of adherens junctions in regulating neurogenesis in the vertebrate central nervous system (CNS)
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Adherens junctions (AJs) are protein complexes that localize to apico-lateral regions of epithelial cells and maintain tissue integrity and cell polarity. In the newly formed neural tube, progenitor cells are interconnected by AJs and form an epithelial layer surrounding the brain ventricles, while neurons are loosely associated mesenchymal cells found in more basal regions of the neural tube. AJs are typically thought to function as cell fate determinants that are asymmetrically inherited by the progenitor cell upon cell division. Hence the retention of these junctional complexes in progenitor cells or their loss in differentiating neurons has conventionally been interpreted as a signal that regulates cell fate. However recent data suggest that these junctional complexes might in fact be maintained in newly born neurons, raising the possibility that the latter might signal back to progenitor cells to maintain them in an undifferentiated state. Here, using the zebrafish as a model system, we begin to analyze the pattern of inheritance of AJs in dividing progenitor cells and correlate the presence of AJ components with the fate of daughter cells. In addition, we address the in vivo role of AJs in the developing neural tube by characterizing several mutants in which these complexes are disrupted. Preliminary data suggest that AJs are required for regulating neuronal differentiation or/and cell division.

doi:10.1016/j.ydbio.2007.03.174

Program/Abstract # 117
Subcellular distribution of endogenous Delta protein in the zebrafish embryo reveals a potential role for Notch in determining Delta endocytosis
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Mind bomb (Mib) mediated endocytosis of the Notch ligand, Delta, is essential for effective Notch signaling. In order to understand regulation of Delta endocytosis in zebrafish embryos, we examined the sub-cellular localization of endogenous Delta protein. In neural tissues, DeltaD protein was mainly localized in cytoplasmic puncta. In contrast, in mib mutant embryos most of the DeltaD was on the plasma membrane. This is consistent with the role of Mib in DeltaD endocytosis and suggests that Mib-mediated endocytosis normally results in most of the DeltaD retained in an intracellular compartment. However, surface expression of DeltaD could in part be due to exaggerated expression that results from loss of Notch signaling in mib mutants. To determine how exaggerated deltaD expression contributes to