Program/Abstract # 245
The C. elegans flamingo homologue FMI-1 is involved in pioneer-mediated axon guidance in the ventral nerve cord
Andreas Steimel a, Irene Wacker b, Harald Hutter ab
a Department of Biological Sciences, Simon Fraser University, Burnaby BC, Canada
b MPI for Medical Research, Heidelberg, Germany

The ventral cord of C. elegans consists of two ventral cord axon tracks, which are established through sequential outgrowth of pioneer and follower axons. The PVPR axon pioneers the left axon track closely followed by the PVQL axon. In a genetic screen for animals with defects in ventral cord axon guidance we isolated alleles of the non-classical cadherin Flamingo and vertebrate CELSR1, 2 and 3. Fmi-1 mutant animals display highly penetrant PVP and PVQ axon guidance defects and disrupt the pioneer–follower relationship between the PVP and PVQ axons. The PVQ axons cross the ventral midline, leave the ventral cord or stop prematurely in almost all fmi-1 mutant animals. Other ventral cord axons are also affected. HSN axons circle around the vulva in 68% of fmi-1 mutant animals and almost invariably stop before reaching the head region. Interneuron axons in the right axon track are affected in a smaller fraction of fmi-1 mutant animals. A 2.6kb fmi-1-promoter GFP reporter construct is predominantly localized to axons, suggesting that FMI-1 could mediate adhesion between pioneer and follower axons.

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Program/Abstract # 246
The role of PDGF-AA-fibronectin interactions in the directed migration of mesendoderm cells during gastrulation
Erin M. Smith, Maria Mitsi, Matthew A. Nugent, Karen Symes
Department of Biochemistry, Boston University School of Medicine, Boston, MA, USA

Platelet-derived growth factor- AA (PDGF-AA) plays vital roles in cell motility in cultured cells, whole organisms, and tumors. Our previous work suggests that during gastrulation in Xenopus laevis embryos PDGF-AA signaling provides guidance cues that direct mesendoderm cells as they migrate across the fibronectin-rich extracellular matrix lining the blastocoel roof. We have also shown that the PDGF family member, vascular endothelial growth factor (VEGF) binds to fibronectin and that this interaction is modulated by heparin-mediated structural rearrangements within fibronectin. Thus, we hypothesized that a similar heparin-mediated change in fibronectin organization exposes binding sites for PDGF-AA and that this PDGF-AA-fibronectin interaction is important for directed cell migration during gastrulation. In support of this, we observed that 125I-PDGF-AA binds specifically to fibronectin, but only after the fibronectin has been treated with heparin. Using proteolytic fragments of fibronectin we localized the heparin-dependent PDGF-AA binding site to a 40kDa carboxy-terminal region of fibronectin. To demonstrate that these interactions are involved in directing cell migration during gastrulation, we utilized an established ex vivo assay in which we observed that heparitinase III treatment randomizes the direction of mesendoderm cell movement. Taken together these data indicate that heparan sulfate mediates PDGF-AA-fibronectin interactions necessary for the directed migration of the mesendoderm cells during gastrulation.

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Program/Abstract # 247
Regulation of twist function in developmental and pathological epithelial-mesenchymal transitions
Rachel M. Lander, Carole LaBonne
IBIS, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL, USA

Investigating the role neural crest factors play in embryogenesis is key to understanding vertebrate development and also tumorigenesis. Neural crest cells are multipotent, proliferative stem cells that undergo an epithelial–mesenchymal transition (EMT) and migrate to distant regions of the embryo where they give rise to a variety of derivatives. Key transcription factors controlling EMTs during neural crest development, such as Twist, also appear to regulate tumor metastasis. Twist is a bHLH (basic helix–loop–helix) transcription factor expressed in premigratory, migratory, and differentiating neural crest. Depletion of Twist in Xenopus has demonstrated the necessity of Twist throughout neural crest development, including EMTs. In order to understand how Twist regulates the neural crest and metastatic progression of tumor cells, we are investigating the post-translational mechanisms controlling its activity. We show that Twist interacts with known regulators of the neural crest and pathological EMTs including Slug, Sox9, and FoxD3. These interactions are dependent on the WR domain of Twist. We map the amino acids within this domain mediating these interactions and identify key residues mutation of which abrogates
binding. Using Chick Chorioallantoic Membrane assays, we are investigating the invasive potential of non-metastatic breast cancer cells expressing either wildtype Twist or Twist carrying mutation in the WR domain that prevents binding to other EMT regulatory factors. Our studies lend important insights into the function of a key regulator of developmental and pathological EMTs.

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Program/Abstract # 248
Myosin-X is required for proper behavior of neural crest cells in Xenopus laevis
Yoo-Seok Hwang, Ting Luo, Yanhua Xu, Thomas D. Sargent
Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD, USA

Non-muscle myosins consist of a N-terminal actin-binding motor domain and multiple C-terminal domains responsible for protein–protein and protein–membrane interactions. One such myosin, Myosin-X (MyoX) has been shown to play important roles in the formation of filopodia and in meiotic spindle organization. Xenopus MyoX was identified in a screen for genes expressed in neural crest (NC) under the control of the transcription factor TFAP2. At neurula stage MyoX has the highest level of expression in the NC, but is also abundant in paraxial mesoderm and forebrain. MyoX knockdown using splice-inhibitory morpholinos resulted in retarded migration of cranial NC cells into branchial arches and subsequent reduction of head and jaw cartilage and reduction in the NC-derived trigeminal nerve. These effects could be partially rescued by co-injection of full-length MyoX mRNA. In vitro migration assays on fibronectin using explanted cranial neural crest cells showed significant inhibition of migration resulting from MyoX knockdown. NC cells migrate most efficiently on fibronectin, utilizing integrin alpha5-beta1 as the receptor. It has been reported that the FERM domain of MyoX physically interacts with a conserved NPLY motif present in the cytoplasmic domain of most beta integrins, including beta1. This interaction with integrin beta5 is critical for colocalization of integrin and MyoX at filopodial tips and for cell adhesion, suggesting the hypothesis that MyoX functions in NC cells to regulate pi1integrin distribution or activation. This is currently being tested by immunohistochemical and live imaging techniques.

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Program/Abstract # 249
Xenopus sonic hedgehog is involved in retinal axon guidance
Andrea R. Morris a, Laura Carlson a,b, Matthew Marsh c, Helen Kinsman a
a Department of Biology, Haverford College, Haverford, PA, USA
b Neuroscience Program, University of Pennsylvania, Philadelphia, PA, USA

The role of classic morphogens such as Sonic hedgehog as axon guidance cues has recently been examined in a variety of vertebrate organisms. In this work, we have tested whether Xenopus sonic hedgehog (Xshh) signaling is involved in guiding retinal axons along the optic tract in the developing brain. Xshh is expressed in the brain during retinal axon extension, in close association with these axons, which themselves express the shh co-receptors patched and smoohened. Blocking Xshh appears to disrupt retinal ganglion cell axon guidance — causing abnormal pathfinding along the optic tract and abnormal targeting at the optic tectum. Misexpression of a Shh signal peptide in vivo also causes retinal axon guidance errors. These data suggest that Xshh signaling is required for normal retinal axonpathfinding and target recognition in the developing visual system.

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Program/Abstract # 250
The GSK-3β and α-catenin binding sites of β-catenin exert opposing effects on directional persistence and filopodial protrusions of optic axons in situ
Anar Shah a, Alissa Peizer a, Melanie Southard a, Tamira Elula a,b
a College of Osteopathic Medicine, Touro University-California, USA
b Department of Basic Sciences, Touro University-California, USA

We overexpressed two deletion mutants of the N-terminal domain of β-catenin in individual optic axons in Xenopus laevis tadpoles. One deletion mutant contained both the α-catenin and GSK-3β binding sites of β-catenin (NTERM) whereas the second mutant contained only the GSK-3β binding site (β-cat 107). Epi-fluorescence images showed that NTERM expression in optic axons caused them to follow a more curvy path to the tectum than that of control optic axons and make guidance errors. In contrast, expression of β-cat 107 induced optic axons to follow a straighter path into the tectum. Higher magnification images showed that NTERM also collapsed filopodia in growth cones whereas β-cat107 increased the number of filopodia in growth cones. These data suggest that the GSK-3β and α-catenin binding sites of β-catenin exert opposing effects on the directional persistence and filopodial protrusions of optic axons.

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Program/Abstract # 251
Interactions between [beta]-Catenin and Fgf signaling coordinate directional migration of groups of cells
Andy Aman, Tatjana Piotrowski
University of Utah, Department of Neurobiology and Anatomy, Salt Lake City, UT 84132, USA

The zebrafish lateral line is an excellent model for studying fundamental developmental mechanisms, such as cell migration in vivo. The lateral line consists of sensory organs derived from a cephalic placode/primordium, which undergoes posterior migration towards the tail tip. During migration the primordium deposits sensory organs every 3–5 somites until it reaches the tail tip. The directionality of this collective cell migration is not controlled by a gradient of an extrinsic guidance molecule but is controlled by the polarized expression of two chemokine receptors within the group of cells. Here we report that a feedback mechanism between [beta]-Catenin and Fgf signaling polarizes these clusters of collectively migrating cells by differential regulation of gene expression in the leading versus the trailing edge of the primordium. We also find that these signaling pathways regulate the asymmetric, anteroposterior expression of the two chemokine receptors in the primordium, which is crucial for its directed migration. These findings are important because they uncover an undescribed molecular mechanism capable of locally establishing polarity and directionality in a group of migrating cells. Although [beta]-Catenin, Fgf and chemokine signaling pathways are well known to be involved in cancer progression, we provide with these studies the first in vivo evidence that these pathways are functionally linked.

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