Program/Abstract # 115  
Axon branching in spiral ganglion neurons  
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Proper morphogenesis of neurons during development is important for the assembly of neural circuits, since it regulates what target cells neurons come into contact with. Spiral ganglion neurons are responsible for receiving sound information from hair cells in the cochlea and sending it to the cochlear nucleus. Upon entering the hindbrain, their axons bifurcate to distribute information to different divisions of the cochlear nucleus, where different features of sound are processed. The molecular mechanisms of axon branching are poorly understood, but both intrinsic and extrinsic mechanisms are known to sculpt the final morphology of neurons. Transmembrane proteins are likely to play an important role in intrinsic processes such as branch segregation, but also in extrinsic processes such as response to environmental branching cues. Spiral and vestibular ganglion neurons develop from a common precursor pool in the otic vesicle, and both innervate hair cells in the inner ear and project axons out the eighth nerve that bifurcate in the hindbrain. However, their precise targets are distinct, and vestibular ganglion neurons are born and undergo axon bifurcation first. To identify the molecular mechanisms of axon branching, the expression profiles of spiral ganglion neurons in the process of axon branching were compared to those of spiral and vestibular ganglion neurons that had finished axon branching. Embryonic spiral and vestibular ganglia were isolated by microdissection or FACS sorting of GFP+ neurons. 193 putative transmembrane proteins were identified to be enriched in the spiral ganglion during axon branching. Validation and functional testing of these candidates are under way.

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Program/Abstract # 116  
Polarization of retinal amacrine cells by the atypical cadherin Fat3  
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Fat cadherin is required in Drosophila for developmental events including planar cell polarity (PCP), tissue growth, and dendritic tiling. Comparatively little is known about fat cadherin function during mammalian development. One mouse ortholog, fat3, is expressed by amacrine cells (AC) and ganglion cells in the developing retina. ACs modulate synaptic function within the inner plexiform layer (IPL) and function as feed forward elements within the rod circuitry. Although each AC type has a distinct dendritic morphology, one common feature is the unipolar extension of dendrites into the inner limiting membrane (INL). We have generated fat3 mutant mice lacking an exon encoding the Fat3 transmembrane domain. Although fat3 is expressed in the inner ear, mutants lack gross PCP deficits in the organization of hair cell stereocilia bundles (a classic model of vertebrate PCP). In contrast fat3 mutants have a retina phenotype characterized by the emergence of an ectopic synaptic layer located within the inner nuclear layer (INL). Formation of this layer results from a failure of ACs to develop unipolar dendritic morphologies. Instead fat3 mutant ACs extend two primary dendrites and develop a bipolar morphology with one dendrite projecting into the IPL and the second towards the INL where it contributes to the ectopic synaptic layer. In addition we see an increase in displaced ACs and a decrease in INL ACs suggesting that fat3 also directs nuclear layer localization. Finally, the expression of dachsous and four-jointed orthologs raises the possibility that a conserved Fat/Dachsous/Four-Jointed signaling complex functions during AC development.

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Program/Abstract # 117  
Morphogenesis of the mouse node depends on the FERM domain protein Ebp4.115  
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Vertebrate embryos establish asymmetric patterns of gene expression across the left–right (LR) axis, leading to the asymmetric development or placement of internal organs such as the heart and lungs. In the mouse, cilia-driven leftward flow of extracellular fluid across the node is a central event in LR patterning; mutations disrupting the shape of the node or the shape or motility of nodal cilia affect LR patterning. The node also produces the trunk notochord and floor plate, which are required for the formation of a midline barrier to maintain asymmetric gene expression in the lateral plate mesoderm (LPM). Despite this pivotal role for the node in patterning an embryonic body axis, node morphogenesis is poorly understood. We performed detailed imaging of wild type node formation using electron and confocal microscopy. We show that the node and notochordal plate form a contiguous group of ciliated, apically constricted cells that appear to emerge gradually through the endoderm germ layer on the ventral surface of the embryo. Establishment of a single node field is disrupted by the limulus (lulu) mutation; lulu disrupts the Erythrocyte protein band 4.1-like 5 (Ebp4.115) gene, which encodes a FERM domain protein required for reorganization of the actin cytoskeleton during embryogenesis. Markers of the node and notochord are expressed in lulu mutants, but multiple node-like regions form in lulu embryos, and the notochord becomes discontinuous. Consequently, most lulu embryos display bilateral expression of left-specific LPM markers. We propose that Ebp4.115 coordinates the cytoskeletal rearrangements required for node morphogenesis.

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Program/Abstract # 118  
Bmp2 in the visceral endoderm directs anterior morphogenesis during gastrulation  
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Elaboration of the vertebrate body plan requires both specification and morphogenesis as the tissues and organs take shape. Proper development and patterning in the early mouse gastrula depend on reciprocal interactions between the epiblast and two surrounding extra-embryonic tissue layers: extra-embryonic ectoderm and visceral endoderm (VE). While much has been learned about the role of the anterior visceral endoderm (AVE), little is known about the function of the posterior visceral endoderm (PVE). To further investigate the role of the PVE, we undertook studies on BMP2, a signaling molecule that is expressed in VE overlying the primitive streak at E6.5. The targeted knockout of
Bmp2 caused embryonic lethality at midgestation with defects in head, heart and extra-embryonic mesoderm. We identify and describe here a previously uncharacterized anterior morphogenesis defect of Bmp2−/− embryos. The head, heart and foregut tissues are specified properly, but their spatial relationships and thus the morphogenesis of the embryo are perturbed. Using tissue specific ablation we show that Bmp2 in the epiblast lineage is necessary and sufficient to rescue the extra-embryonic mesoderm defect, and Bmp2 in the visceral endoderm is necessary and sufficient to rescue the anterior morphogenesis defect. Together with the Bmprp1a phenotype, these findings potentially identify a signaling cascade wherein Bmp2 in the visceral endoderm signals to its receptor in the epiblast to direct anterior morphogenesis during mouse gastrulation.

Program/Abstract # 119
Cell behaviors during endoderm morphogenesis in the mouse gastrula
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We have used live imaging to investigate the cell behaviors underlying endoderm morphogenesis during mouse gastrulation. Our observations lead us to propose a model of egression-mediated cell dispersal whereby the emerging epiblast-derived definitive endoderm (DE) intercalates with the pre-existing visceral endoderm (VE) epithelium and causes its dispersal in the distal region of the embryo overlying the epiblast. Mechanistically, this phenomenon can be explained by elevated rates of cell proliferation in the epiblast compared to the VE. A rapidly proliferating epiblast could promote an egression of epiblast-derived cells into the overlying VE on the surface of the embryo. Once egressed, rapid division of these cells leads to further dispersal and dilution of VE cells and an increase in the surface area required to accommodate the rapid growth of the embryo. We have used digital quantification to corroborate the sequential steps of our model. Furthermore, we have investigated the molecular identity and cell behavior of both VE-derived and epiblast-derived DE cells overlying the epiblast in wild type embryos and in mutants.

Otoconia are complex calcium carbonate biominerals that are required for the sensation of gravity. Degeneration, displacement or ectopic formation of otoconia results in benign paroxysmal vertigo (BPV). In addition, aminoglycoside antibiotics can disrupt otocional structure and function. Despite the prevalence of balance disorders, little is known about the mechanisms regulating the development and pathology of the vestibular mechanosensory apparatus. Tilted mice have a severe balance disorder due to the congenital absence of otoconia. By positional cloning we identified missense mutations in Otopetrin 1 (Otop1) as the genetic etiology of the tilted phenotype. Otop1 encodes a multi-transmembrane domain protein required for the initiation of extracellular mineralization. To establish a null allele and to mark cells expressing Otop1, we have generated a LacZ knockin allele that inactivates all splice forms of Otop1. Using Otop1lacZ mice we demonstrate specific expression of Otop1 in the macular epithelium and altered protein subcellular localization in tilted mice. Biochemical analysis demonstrates that Otop1 can modulate purinergic mediated Ca2+ homeostasis and defines a unique set of biochemical activities including specific inhibition of the purinergic receptor, P2Y, and regulation of the influx of extracellular Ca2+ in response to ATP, ADP and UDP. Together, these studies demonstrate a direct role for Otop1 in the formation and growth of otoconia and the potential to regulate other mineralization processes.

Program/Abstract # 121
The Ig superfamily protein Lrig3 controls inner ear morphogenesis by regulating Netrin-1 expression
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Proper morphogenesis of the three inner ear vestibular canals is essential for proper balance. Canal development begins when defined regions of otic vesicle epithelium meet to form the fusion plate. In the fusion plate, the basal lamina breaks down as epithelial cells intercalate to form a single layer of cells. Fusion plate cells eventually disappear and the region is filled with mesenchyme, forming a canal out of an initial pouch-like structure. Netrin-1 is secreted from fusion plate cells and promotes basal lamina breakdown, permitting epithelial–mesenchymal interactions known to drive fusion plate formation for each of the three canals. Therefore, when and where Netrin-1 is produced fundamentally affects the complex structure of the vestibular apparatus. We report that novel protein Lrig3 controls the timing and extent of lateral canal fusion by regulating the expression of Netrin-1. Lrig3 is present in non-fusing epithelium of the otic vesicle, complementary to Netrin-1 in the fusion plate. In Lrig3 mutant mice fusion is premature and expanded, resulting in lateral canal truncation and circling behavior in adult mice. The fusion defect is accompanied by ectopic breakdown of the basal lamina and expansion of Netrin-1 expression. The canal truncation is rescued when one copy of the Netrin-1 gene is removed, confirming that the Lrig3 mutant phenotype is a result of increased levels of Netrin-1. Lrig3 is a transmembrane protein with leucine-rich repeats and Ig-motifs in the extracellular region and a short cytoplasmic tail. Current studies are aimed at uncovering the molecular functions of Lrig3.

Program/Abstract # 120
From the tilted mouse to the ototpetrin gene family: Molecular insights into the development of the vestibular mechanosensory system
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Proper morphogenesis of the three inner ear vestibular canals is essential for proper balance. Canal development begins when defined regions of otic vesicle epithelium meet to form the fusion plate. In the fusion plate, the basal lamina breaks down as epithelial cells intercalate to form a single layer of cells. Fusion plate cells eventually disappear and the region is filled with mesenchyme, forming a canal out of an initial pouch-like structure. Netrin-1 is secreted from fusion plate cells and promotes basal lamina breakdown, permitting epithelial–mesenchymal interactions known to drive fusion plate formation for each of the three canals. Therefore, when and where Netrin-1 is produced fundamentally affects the complex structure of the vestibular apparatus. We report that novel protein Lrig3 controls the timing and extent of lateral canal fusion by regulating the expression of Netrin-1. Lrig3 is present in non-fusing epithelium of the otic vesicle, complementary to Netrin-1 in the fusion plate. In Lrig3 mutant mice fusion is premature and expanded, resulting in lateral canal truncation and circling behavior in adult mice. The fusion defect is accompanied by ectopic breakdown of the basal lamina and expansion of Netrin-1 expression. The canal truncation is rescued when one copy of the Netrin-1 gene is removed, confirming that the Lrig3 mutant phenotype is a result of increased levels of Netrin-1. Lrig3 is a transmembrane protein with leucine-rich repeats and Ig-motifs in the extracellular region and a short cytoplasmic tail. Current studies are aimed at uncovering the molecular functions of Lrig3.