Program/Abstract # 122
The kinome of lung branching morphogenesis — A systems approach to identify phosphoregulators of mouse lung development
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A major goal in understanding organ formation is determining the link between developmental signals and the fundamental molecular pathways responsible for guiding and controlling organogenesis. Although much is known about the molecules that initiate morphogenesis, including Wnts, Hedgehogs, BMPs and growth factors, there is a considerable gap in understanding the downstream targets of these regulators, the links between them, and the mechanisms by which these regulators generate three-dimensional tissues with complex features such as the intricate branching pattern of the lung. In order to fill this gap we have developed a systems approach to obtain a comprehensive understanding of the functional relationship between kinase/phosphatase-regulated molecular pathways and their roles in organizing the formation of the mouse embryonic lung. To this end, we have applied a combination of functional genomics (loss of function screen) with morphological and live imaging analysis in lung explant cultures. Notably, our analysis from the ongoing screen has already revealed a high degree of distinct morphological phenotypes including change in tubule size, branch numbers and position. Moreover, our image analysis does suggest new mechanistic and dynamic properties of epithelial and mesenchymal cells in molding emerging branches during lung development. Thus, the combination of functional genomics and live imaging provides a powerful strategy to further decipher the molecular networks and principles underlying branching morphogenesis.

doi:10.1016/j.ydbio.2008.05.134

Program/Abstract # 123
Cleftin: A novel fibronectin-induced gene that promotes branching morphogenesis
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Branching morphogenesis is a fundamental process shared by many organs. In this process, the epithelium of the mouse submandibular gland forms a cleft that deepens to generate lobules. Cleft formation is an initiation process that is associated with the conversion of cell–cell to cell–matrix adhesions. Fibronectin is known to appear transiently and focally in forming cleft regions, accompanied by an adjacent loss of E-cadherin, but the mechanism is still unknown. Our hypothesis is that cleft epithelia may express functional genes that regulate cleft formation. To test this hypothesis, we identified and characterized novel genes that are expressed in cleft but not bud epithelia by laser microdissection and T7-SAGE libraries. A BTB/POZ domain-containing protein showed differential expression in the cleft epithelium. Expression in developing salivary glands was maximal at embryonic day 13, a period of extensive salivary gland branching. We have termed this protein “cleftin.” Highly localized expression of cleftin mRNA was identified around the tip of the cleft by in situ hybridization. To determine whether cleftin can be induced, we tested several matrix molecules. We found that fibronectin can rapidly induce cleftin. Functional analysis of branching morphogenesis using stably transfected MDCK cells showed that cleftin expression decreased E-cadherin. Furthermore, cleftin-expressing cells showed increased branching in 3D collagen gels. Knocking down cleftin using siRNAs inhibited branching in developing mouse salivary glands and lungs. These results indicate that cleftin has an important role in branching morphogenesis.

doi:10.1016/j.ydbio.2008.05.135

Program/Abstract # 124
A novel region in the murine allantois may prevent branching morphogenesis
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The murine allantois is the precursor to the fetal umbilical cord, which channels fetal blood to the chorionic disk for exchange with the mother. During elongation, the distal allantois develops a highly branched vasculature that provides a wide vascular surface area for fusion with the chorion. By contrast, the proximal region develops a thick, unbranched vasculature that amalgamates with the embryonic dorsal aorta and yolk sac blood vessels. Failure of the allantois to properly vascularize and/or fuse with the embryonic and chorion blood vessels has been linked to birth defects such as low birth weight, pre-natal death and cerebral palsy. Here, we tested the hypothesis that the outer covering of the allantois, called the mesothelium, may play a role in vascular branching of the umbilical cord. Our results reveal the presence of a unique feature of the allantoic mesothelium whose properties suggest that it may prevent vascular branching in the proximal allantois whilst facilitating union with embryonic and yolk sac vasculature.

Program/Abstract # 125
Notch signaling acts at multiple stages to regulate bile duct morphogenesis
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The mammalian biliary system transports bile from the liver to the intestine. Dysfunction of bile ducts is a significant cause of liver disease. The development of bile ducts requires the proper differentiation and morphogenesis of bile-duct precursor cells into a complex three-dimensional structure. Studies of human disease and mouse models have implicated Notch signaling in this process, but the mechanism is still poorly understood. Here, we established a modular transgenic system to heritably activate Notch signaling in hepatoblasts and differentiated liver cells. We find that Notch does not specify bile-duct precursor cell fate during the period of embryonic development. However, Notch acts in a dose-dependent manner to regulate bile duct morphogenesis postnatally. Overexpression of Notch signaling promotes the formation of biliary tubules and the persistence of postnatal bile-duct cells. Taken together, our results suggest that Notch signaling regulates bile duct
Development through the regulation of morphogenesis, instead of cell fate specification.

doi:10.1016/j.ydbio.2008.05.137

Program/Abstract # 126
Visualizing morphogen distribution in lumen
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Diffusion dynamics of extracellular signalling molecules have been extensively studied recently. In several developing systems such as neural tube, it has been suggested that morphogen in lumen i.e. inside neural tube, may play an important role during development. However, the detection of diffusing molecule is technically difficult because the molecule must be immobilized by fixation to be visualized by immunohistochemistry. In the case of lumen, extracellular matrix is lacking which can trap diffusing signalling molecule, so distribution of diffusing molecule cannot be detected with conventional immunohistochemistry. In the present study, we developed a simple method to visualize the distribution of diffusive signalling molecule in lumen. We validated the result by collecting the liquid in lumen and detecting the molecule by Western blot. Then we applied the method to several developing organs to examine the role of the signalling molecule in lumen during development.

doi:10.1016/j.ydbio.2008.05.138

Program/Abstract # 127
Shh signaling regulates reciprocal epithelial–mesenchymal interactions controlling palate development
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The mammalian secondary palate arises by outgrowth from the oral sides of the paired maxillary processes flanking the primitive oral cavity. The outgrowth of the bilateral palatal shelves depends on reciprocal interactions between the oral ectoderm and the underlying neural crest derived mesenchyme. Previous studies have implicated Sonic hedgehog (Shh) as an important epithelial signal for regulating palatal growth. However, the cellular and molecular mechanisms through which Shh regulates palatal development in vivo have not been directly analyzed, due in part to early embryonic lethality of mice lacking Shh or other essential components of the Shh signaling pathway. Using Cre/loxP-mediated tissue-specific inactivation, in either the developing palatal epithelium or palatal mesenchyme, of the Smoothened (Smo) gene, we show that the epithelially expressed Shh signals to the palatal mesenchyme to regulate cyclin-D1 expression and palatal mesenchyme cell proliferation. In addition, Shh signaling maintains Fgf10 mRNA expression in the palatal mesenchyme and secondarily affects palatal epithelial cell proliferation. Together with previous reports that the mesenchymally expressed Fgf10 signals to the palatal epithelium to positively regulate Shh mRNA expression, these data demonstrate that Shh and Fgf10 function in a positive feedback loop mediating the reciprocal epithelial–mesenchymal interactions that regulate palatal outgrowth.

doi:10.1016/j.ydbio.2008.05.139

Program/Abstract # 128
Wnt2 signaling regulates morphogenesis of the inflow tract and atrioventricular canal during cardiac development
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The central role of Wnt ligands in early cardiac commitment, expansion, and differentiation is poorly understood. Wnt2a is expressed in precardiac mesoderm and later in the inflow portion of the heart including the atria. Our Wnt2a mutants have more than 80% embryonic and perinatal lethality. Morphological defects are observed in the development of the atrioventricular canal, endocardial valves and in the atrial and ventricular myocardium. Loss of Wnt2a signaling leads to decreased sarcocmere development in the myocardium of Wnt2a mutant heart. Wnt2b is expressed in a similar pattern as Wnt2a in cardiac mesoderm. Of note, Wnt2b null mice are viable and do not display obvious cardiac phenotypes. Remarkably, Wnt2a–2b DKO mice die by E14.5 and display more severe defects in AV canal, endocardial cushion, and atrial myocardium development than Wnt2a single mutants. Microarray studies have indicated that endocardial marker genes are significantly upregulated, whereas myocardial marker genes are significantly downregulated in Wnt2a mutant hearts. Two cardiac-specific transcription factors, GATA6 and Sall3, are markedly reduced in Wnt2a mutant hearts, providing insight into possible mechanisms underlying the phenotype in Wnt2a mutants. The expression of Isl1 is significantly downregulated in the atrium of Wnt2a mutant hearts. These data implicate that Wnt2 regulates differentiation of cardiac precursors into myocardial and endocardial lineages, which is required for proper AV canal morphogenesis, endocardial valve formation, and atrium development.

doi:10.1016/j.ydbio.2008.05.140

Program/Abstract # 129
Daam1 is required for mouse heart morphogenesis
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Daam1 is a formin-homology protein implicated in b-catenin independent Wnt signaling. Daam1 complexes with the Wnt effector protein Disheveled and Rho-family GTPases in the presence of Wnt receptor binding and is required for Wnt proteins to activate RhoA. Daam1 is highly expressed in cardiac myocytes during mouse heart development. We therefore deleted Daam1 specifically in the heart to determine the role that Daam1 plays during heart morphogenesis. Daam1 mutant hearts have greatly enlarged right atria relative to the hearts of wild type siblings at all ages examined. Furthermore, histological examination reveals the presence of atrial septal defects in Daam1 mutant hearts and an expansion of atrial tissue around proximal portions of the incoming vena cava. Finally, the myocardium of Daam1 mutants has a disrupted cellular architecture in both the atrial and ventricular compartments and transmission electron microscopy reveals defects in the intercalated discs of Daam1 mutants. To further examine the role that Daam1 plays in cell–cell adhesion, we transfected primary cultures of neonatal ventricular myocytes with either Daam1 or control siRNA. In control treated cultures, beating foci of cardiac myocytes are highly interconnected to one another by cellular protrusions but these protrusions are often thin or incomplete in cultures treated with Daam1 siRNA.

doi:10.1016/j.ydbio.2008.05.141