Program/Abstract # 112
Rac/JNK/dub regulates intercellular adhesive dynamics during gut morphogenesis
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The endoderm cells of the primitive gut tube undergo convergent-extenson rearrangements to lengthen and concomitantly narrow the gut, while forming the epithelial lining of the mature digestive tract. Although Rho/ROCK/Myosin II signaling is required for this process, the role of other Wnt/PCP signaling components is unknown. Here we show that the activity of Jun N-terminal kinase (JNK) is required for Xenopus gut morphogenesis. When JNK signaling is reduced, the digestive organs are patterned normally along the length of the gut tube, but tissue-elongating morphogenetic processes are disrupted, yielding a shorter digestive tract with a broader diameter. The cell rearrangements that drive gut tube lengthening are perturbed in JNK-deficient embryos, leaving a core of non-adherent cells in the gut lumen. Indeed, dissociation/reaggregation assays suggest that JNK-deficient endoderm cells are less adherent. Inhibition of JNK results in a reduction of adherens junction proteins E-cadherin, and alpha and beta catenin in the developing gut, suggesting that JNK signaling is normally required to maintain adhesion between endoderm cells as they remodel their junctions during intercalary rearrangements. Moreover, loss of JNK function is phenocopied by ectopic expression of a dominant negative version of Xenopus duboraya (dub), an actin-modulating protein previously shown to be phosphorylated by JNK. The effects of JNK are also phenocopied by expression of dominant-negative Rac, a known upstream mediator of JNK in Wnt/PCP pathways. Taken together, our data suggest that a Rac/JNK/dub pathway orchestrates tissue-elongating endoderm cell rearrangements during gut morphogenesis by regulating intercellular adhesive dynamics.

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Program/Abstract # 113
Jaw joint morphogenesis requires Fat/Dachsous signaling in zebrafish
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Little is known about the mechanisms of cell–cell communication necessary to assemble skeletal elements of appropriate size and shape. Skeletal progenitors may behave as coherent units by communicating via the planar cell polarity (PCP) pathway. In Drosophila, two sets of factors control PCP independently: the Fat and the Stan systems. While a requirement for components of the Stan system was recently demonstrated in regulating the oriented divisions of chondrocytes and cellular intercalation in long bones, a role for the Fat system in skeletal development has not been reported. We find that loss of Fat, Dachsous, Four-jointed or Atrophin-orthologues in zebrafish embryos all result in similar skeletal defects, including fused jaw joints. These results provide genetic evidence that morphogenesis of the joint region is controlled through a conserved Fat signaling pathway, a process that has not previously been associated with defects in skeletal tissue polarity. Confocal imaging of Fat-deficient joints shows that interzone cells undergo apopotic intercalation – a PCP-regulated behavior in other contexts such as gastrulation – resulting in a continuous chondrocyte stack from mandibular- to maxillary-domains. In addition, we show that pre-chondrocytes of upper- and lower-jaws display polarities that are mirrored across the joint, providing evidence that morphogenesis of the joint may require the coordinated polarities of upper- and lower-jaw domains and that Fat signaling propagates this polarity.

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Program/Abstract # 114
Spatiotemporal regulation of cortical actomyosin dynamics during convergence and extension of the Xenopus embryo
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Spatiotemporally organized mesoderm cell intercalations are required to sculpt the body axis of vertebrate embryos during gastrulation. Although the mediolaterally oriented cell movements are thought to be under control of the non-canonical Wnt-signaling pathway, we have little understanding on how this pathway directs dynamic actomyosin networks which drive cell shape changes and movements. Here we aim to investigate the intrinsic dynamics of actomyosin network and how the non-canonical Wnt-signaling regulates these actomyosin dynamics within the converging and extending mesoderm of Xenopus. Using confocal imaging of F-actin (moesin) and MyosinII (MRLC-YFP), we follow the actomyosin network in live tissues. Quantitative analyses of F-actin morphology and FRAP indicate that directed movements of multiple contractions correlate with mesoderm cell elongation during C&E. To understand how the non-canonical Wnt pathway regulates the actin contractions, we assessed the actin dynamics of mesoderm cells whose Wnt signal is perturbed by Frizzled receptors (XFz7) and mutant Dishevelled proteins (Xdd1). Induced signal with XFz7 produced the significantly long-lived and highly contractile actin network. In contrast, Xdd1 expressing cells had about two-fold less frequent and randomly moving actin contractions compared to control. Both induced or reduced non-canonical Wnt-signals produce similar round shaped mesoderm cells, however, the actin contractions within those cells were significantly different in frequency and persistency. In summary, the dynamic actomyosin complex mediates cell shape changes in parallel direction of their movements and is under permissive control of non-canonical Wnt-signaling during C&E.

Program/Abstract # 115
BMP7 directs epithelial cell fate choice and dorsal–ventral partitioning of the embryonic cloaca by collaborating with the PCP pathway
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development of the mammalian urethra, the rectum and external genitalia depends on the proper remodeling of the epithelium of the embryonic cloaca. We reported previously that loss of function of a single ligand of the Bone morphogenetic protein (BMP) family, BMP7 results in severe defects in separation of the cloaca into the urethral and rectal compartments, and in morphogenesis of the genital urethra and ventral portions of the external genitalia (Wu et al., 2009). Here, we investigated the mechanisms of BMP7 function in partitioning of the cloaca in the Bmp7::lacZ murine model (Godin, 2009).
et al., 1998). We show that BMP7 signaling in the cloacal endoderm and the urorectal mesenchyme is mediated by the non-canonical WNT/planar cell polarity (PCP) pathway. Loss of Bmp7 results in disruption of the membrane PCP complexes, containing VANGL2, and in a significant decrease in the activity of the Rho, JNK and c-Jun kinases, the downstream mediators of the PCP pathway. We further show that knockout of Bmp7 results in disruption of the apical–basal polarity in the cloacal endoderm, a delay in epithelial differentiation, decreased survival, and defects in cell adhesion in the septum area. Based on our data, we propose that BMP7 signaling from the urorectal mesenchyme directs dorsal–ventral partitioning of the cloaca by collaborating with PCP pathway, and by directing cell fate choice of the epithelial progenitors in the septum area.

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Program/Abstract # 116
A role for planar cell polarity during kidney tubule morphogenesis
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Failure of proper kidney tubule development can result in polycystic kidney disease (PKD), one of the most common genetic disorders inherited in humans. With no available cure, more than half of the patients with PKD develop kidney failure. Genetic analysis has revealed that many of the causative gene products identified from patients with PKD localize to or interact with components of the primary cilium, but how they regulate kidney tubule morphogenesis is unclear. Recent research suggests that kidney tubule morphogenesis may involve convergent extension movements and oriented cell division, two processes that require input from the planar cell polarity (PCP) pathway. However, the interconnectivity between components within the primary cilium, the PCP pathway and the developmental processes that regulate kidney tubule morphogenesis is tenuous. We present results that demonstrate a definitive role for the PCP pathway during kidney tubule morphogenesis. Specifically, we observe the asymmetric localization of core PCP components along the proximal–distal axis of kidney tubule cells. We show that these core components are required for molecular asymmetry, for the proper orientation of cells with respect to the tubule axis, and for the regulation of kidney tubule diameter. How might our observed PCP-dependent effects be reconciled with the identified gene products associated with PKD? Surprisingly, the level of Polycystin-2, a gene product that is mutated in a class of PKD, is significantly reduced within primary cilia after disruption of the PCP pathway. Together, our data suggest that the PCP pathway regulates kidney tubule morphogenesis through the trafficking of components, such as Polycystin-2, to the primary cilium.

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Program/Abstract # 117
The BMP co-receptor Dragon is required for normal renal branching morphogenesis
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cThe cellular and molecular mechanisms involved are not fully understood. Normal kidney development is dependent on mesenchymal–epithelial interactions in embryonic kidneys. Evidence suggests that bone morphogenetic protein (BMP) signaling is critically involved in the reciprocal inductive interactions between mesenchymal and epithelial cells. Kidneys of BMP4 null heterozygote mice showed slower growth and branching of ureteric buds. BMP4 is expressed in the mesenchyme adjacent to the bodies of ureteric branches. BMP4 normally utilizes BMPRII rather than ActRIIA for signaling. Interestingly, ActRIIA but not BMPRII is the predominant BMP type II receptor found in the bodies of ureteric branches, thus raising the question as to how BMP4 from the mesenchyme can efficiently signal in ureteric branch cells. Recently we have found that Dragon (also known as RGMb), a GPI-anchored protein, functions as a BMP co-receptor that allows BMP4 to signal via ActRIIA. Our results show that Dragon is highly expressed in the epithelial cells of ureteric branches in the embryonic kidney. Dragon overexpression increased tubule formation and branching in IMCD3 cells in 3D culture. Kidneys from Dragon null mouse fetuses at E13.5 showed significant reduced ureteric branching compared to the wild type. These results suggest that Dragon promotes tubulogenesis in vitro and renal branching morphogenesis in vivo.

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Program/Abstract # 118
Identifying genes involved in ureteric bud morphogenesis
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The ureteric bud (UB) emerges from a swelling of the caudal Wolffian duct (WD) at ~E10.5 of mouse development. This swelling develops as a result of Ret-dependent cell movements and is termed the “primary UB tip domain”. Correct UB outgrowth is essential for further kidney development; renal agenesis occurs when the UB fails to emerge — as seen in mice homozygous for loss of Ret. To identify genes that might mediate Ret-dependent UB outgrowth we screened for genes that are differentially expressed between rostral and caudal WD, and those whose expression depends on Ret. Microarray analysis of E10.5 wild type rostral WD, wild type caudal WD, and Ret−/− caudal WD revealed genes with differential expression. Several genes with known roles in UB development were confirmed to be upregulated in the caudal WD, as were a number of genes with known roles in cell movement and associated processes, but not known to be involved in kidney development. We examined expression of these candidate genes using in situ hybridization and transgenic reporter lines, and confirmed novel UB expression for a number of the candidates. Cxcr4, encoding a receptor for the chemokine CXCL12 which is implicated in cell migration, was upregulated in the caudal WD. Previous work in our lab suggests a role for this gene in UB morphogenesis downstream of Ret. We therefore investigated whether Cxcr4−/− cells can contribute to the UB in Cxcr4−/−;<sup>−</sup> mice of chimaeric embryos. Cxcr4-deficient cells could populate all parts of a morphologically normal UB, including the tips, indicating that Cxcr4 is not required for UB development. We are now investigating the interplay between Cxcr4, Cxcr7 (a related chemokine receptor), and Cxcl12 in kidney development.

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