cells continue to interact with hepatoblasts directly or indirectly to promote differentiation and formation of the intrahepatic bile duct (IHBD) system which follows the earlier-established portal venous system. It is also unknown whether the bud-surrounding endothelial cells incorporate into mature liver vasculature, contribute to the sinusoidal endothelial population or both. One signaling pathway that could be involved in the endothelial compartment is Notch. We have genetic tools to lineage-trace and disrupt Notch signaling in the endothelia to investigate its cell-autonomous and non-autonomous roles, thereby expanding our knowledge regarding vascular formation and communication with the liver epithelium during development.

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Program/Abstract # 204
Characterization of zeppelin, a novel zebrafish kidney mutant
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Kidneys remove metabolic waste using functional units known as nephrons, which are composed of a blood filter (or renal corpuscle), tubule, and duct. The renal corpuscle contains epithelial cells known as podocytes, which form a filtration barrier and allow collection of substances from the blood. While several genes are known to be essential for podocyte development, the signaling pathways that specify the podocyte lineage are poorly understood. The zebrafish provides a powerful genetic system to study kidney development in vertebrates as the genes that pattern organs are highly conserved with humans. Zeppelin (zep) was isolated in a forward genetic screen for kidney mutants based on the appearance of edema at 7 days post fertilization (dpf). To explore kidney development in zep, we performed whole mount in situ hybridization to evaluate formation of specific nephron cell types. Based on expression of the transcription factors wt1a and wt1b, zep mutants have reduced podocyte numbers at 24 hours post fertilization (hpf). In contrast, expression of tubule markers such as cdh17 and slc9a3 was unaltered in zep. To examine whether podocyte formation was delayed in zep, we performed a timecourse study and found that podocytes were reduced throughout embryogenesis. These data suggest that zep is a podocyte-specific genetic defect, and may be explained by the inability to specify the podocyte lineage. In future studies, we plan to isolate the zep genetic lesion using positional and/or candidate cloning strategies. The identification of zep will help to define the genetic pathways of podocyte formation, and may provide insights into human podocyte development and kidney disease.

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Program/Abstract # 205
Genetic analysis of nephron patterning in zebrafish
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Kidneys provide several vital functions to an organism, including the excretion of waste and the maintenance of water and electrolyte balance. Vertebrate kidneys are comprised of nephrons, functional units that filter the blood then modify the filtrate to retain essential metabolites and accomplish osmoregulation. Nephrons have several main parts: (1) a blood filter, (2) an epithelial tubule that is regionally patterned into proximal and distal segments that reabsorb and secrete particular solutes, and (3) a duct that drains the unit. Relatively little is understood about how different nephron cell types arise from renal progenitors during kidney development, largely due to the complex nature of mammalian kidney formation. However, recent studies have shown that nephron composition is conserved between zebrafish and mammals. We are using the advantages of the zebrafish model to perform genetic studies of nephron patterning. Zebrafish embryos form a pair of nephrons from bilateral stripes of intermediate mesoderm precursors, and nephrons show a segmentation pattern as early as 24 hours post fertilization. To investigate the development of discrete nephron cell types, we are conducting a haploid screen. Adult male zebrafish were mutagenized with ethylnitrosurea, and mated to wildtype females to generate F1 fish. Haploid clutches from F1 females were collected and renal progenitor populations were assessed by whole mount in situ hybridization using markers of specific nephron segments. This ongoing approach will enable us to identify genes that are essential for nephron patterning, and will likely provide new insights into kidney development and disease in humans.

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Program/Abstract # 206
The fate of Ret-expressing cells in the kidney and their role in maintaining renal branching morphogenesis
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The GDNF/Ret signaling pathway is required for normal development of the metanephric kidney. Ret activity in the Wolffian duct is necessary for formation of the primary ureteric bud. In subsequent growth of the renal collecting system, Ret-expression is confined to the branching “tip” domain. It remains unclear, however, what the fates of these Ret-expressing tip cells are and to what extent Ret activity is required to maintain branching morphogenesis once the primary ureteric bud has been established. Here we use an inducible Cre knocked into the Ret locus to perform lineage tracing of Ret-expressing cells in the mouse kidney. This system was also used to mosaically remove Ret from a portion of the cells in its normal expression domain using a conditional Ret allele, or to ablate these cells altogether using an R26R-DTA allele. Ret-expressing tip cells are a self-renewing population that contributes to both the “tip” and “trunk” domains of the growing renal collecting system. Furthermore, this bipotential fate of Ret-expressing tip cells is maintained throughout kidney development. The mosaic loss of Ret from the tip domain impairs normal branching, yielding a smaller kidney. Ret activity is required for both the formation of the primary ureteric bud and subsequent branching events. Surprisingly, initial results suggest ablation of tip cells does not impair normal branching. If, indeed, it is more disruptive for a tip cell to lose Ret than for that cell to die altogether, this suggests the renal branching program utilizes a mechanism to measure cell number in the tip and compensate for cell loss.

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Program/Abstract # 207
Identification and characterization of Etv4/5 target genes during ureteric bud branching morphogenesis
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During development, GDNF signalling through the Ret receptor tyrosine kinase is critical for the initial evagination of the ureteric bud (UB) from the Wolffian duct and for its subsequent branching morphogenesis to give rise to the collecting system of the kidney. Downstream of the GDNF/Ret signalling cascade, the two ETS transcription factors Etv4 and Etv5 are activated in the tip cells of the UB. Both Etv4 and Etv5 are jointly required for kidney development. However the mechanisms by which Etv4 and Etv5 regulate the cellular responses that lead to UB branching remain to be fully elucidated. By carrying out microarray screens comparing Etv4−/−;Etv5−/+ mutant kidneys with wild-type kidneys, several putative target genes of these transcription factors were identified. Many of these genes, including Slco4c1, Vsnl1, Krt23, Lamc2 and the metalloproteinase pair Adamts18 and Adamts16, showed UB-specific or UB-tip specific expression in wild-type kidneys and were either absent or down-regulated in the mutant kidneys. Furthermore many of these genes have evolutionarily conserved ETS-binding sites in their promoter and enhancer elements, enabling chromatin immunoprecipitation studies to identify whether these candidate genes are direct targets of Etv4 and/or Etv5. Overall identification of novel genes downstream of Etv4 and Etv5 with previously unknown roles in kidney organogenesis could potentially help explain certain human renal birth defects at a molecular/genetic level.

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Program/Abstract # 208
Role of Etv4 and Etv5 in pancreatic development
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FGF10 is a key signaling factor involved in the mesenchymal–epithelial interactions of many developing organs and tissues. In the embryonic pancreas, FGF10 is secreted from the mesenchyme to expand the progenitor population in the neighboring pancreatic epithelium. While Fgf10 is crucial for active proliferation of the pancreatic progenitors, few of its downstream genes have been identified. In an FGF10 overexpression model, we found that FGF10 upregulated expression of two novel ETS-family transcription factors, Etv4 and Etv5, in the pancreatic progenitors. In wild type embryos, both factors are expressed almost exclusively within the pancreatic epithelial progenitors. We examined the pancreas of Etv4 and Etv5 null embryos to ascertain which effects of FGF10 are mediated through these two factors. Etv5 null embryos showed a reduction in total pancreatic mass. The mature cell types were also diminished in the Etv5 null embryos. The pancreas of Etv4 null embryos appeared normal. However, genomics-based profiling of the Etv4 null pancreas revealed reduced expression of factors necessary for endocrine and exocrine cell development, although this effect was not as severe as observed in the Etv5 null embryos. Considering that the DNA binding domains of these factors are almost identical, we conclude that genetic redundancy between these factors likely exists. This is currently being tested using compound breeding of Etv4 and Etv5 knockout mice.

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Program/Abstract # 209
Hox6 genes are important niche factors that play critical roles in the proper formation and maintenance of the pancreas
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Despite significant advances in our understanding of endocrine pancreatic development, the function of the pancreatic mesodermal niche in this process is less well understood. However, niche factors are necessary for proper endocrine development and are likely to be of critical importance in designing successful regenerative therapies aimed at replacing lost islet cells in diabetic patients. Preliminary data generated in our laboratory demonstrates a critical role for Hox6 genes in pancreatic organogenesis. Hox6 genes are expressed exclusively in the pancreatic mesoderm (and not endoderm) and suggest a primary role for Hox6 genes in proper development of the pancreatic niche. The pancreatic phenotypic abnormalities observed in our Hox6 triple mutants confirm this, as total pancreatic volume in mutants is reduced compared to littermate controls and there is a greater than 90% reduction in insulin-expressing cells. In addition, insulin- and glucagon-expressing cells do not form proper islets and are abnormally positioned within the pancreas. Finally, while triple mutants die shortly after birth, surviving compound mutants exhibit hyperglycemia and impaired responses in glucose tolerance tests. Moreover, these defects are exacerbated with age and, as Hox6 genes remain expressed in the pancreas through post-natal and adult stages, suggest that Hox6 genes may contribute to post-natal endocrine maintenance as well. Overall, these data suggest that Hox6 genes are critical pancreatic niche factors, necessary for the proper development and maintenance of pancreatic organogenesis.

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Program/Abstract # 210
Notch mediated patterning and cell fate allocation of pancreatic progenitor cells
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Early pancreatic morphogenesis is characterized by the transformation of an uncommitted pool of pancreatic progenitor cells into a branched pancreatic epithelium that consists of “tip” and “trunk” domains. These domains have distinct molecular signatures and differentiate into different pancreatic cell lineages. Cells at the branched tips of the epithelium develop into acinar cells, while cells in the trunk subcompartment differentiate into endocrine and duct cells. Recent genetic analyses have highlighted the role of key transcriptional regulators in the specification of these subcompartments. Here, we analyzed the role of Notch signaling in patterning of the pancreatic epithelium through mosaic overexpression of a Notch signaling antagonist, dominant negative mastermind-like1 (dnMAML1), resulting in a mixture of wild type and Notch-suppressed pancreatic progenitor cells. Relative to the wild type cells, the Notch suppressed cells lose “trunk” maker genes and gain expression of “tip” genes. These cells undergo a process of sorting and rearrangement, leading to positioning of the Notch suppressed cells at the tip of the branched pancreatic epithelium, while the wild type cells occupy the “trunk”. The Notch suppressed cells subsequently differentiate into acinar cells, while duct and endocrine are formed predominantly from the wild type cells.

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