We are interested in examining the viability of Xenopus laevis as a model organism in which to study the earliest events of lung development. Previously we sequenced and characterized the expression of the lung specific genes Surfactant protein C (Spc) and Surfactant protein B (Sbp). These genes are exclusively expressed in the embryonic and adult lung and share significant homology with their mammalian homologs. Here we report the sequence of xFgf10, the Xenopus homolog to the mouse Fgf10 gene necessary for lung development. In addition, we report the effect of overexpressing the transcription factor Nkx2.1 in endodermal cells fated to develop lung. Nkx2.1 is expressed at the earliest stages of lung development. Previously we sequenced and characterized the expression of the lung specific genes Surfactant protein C (Spc) and Surfactant protein B (Sbp). These genes are exclusively expressed in the embryonic and adult lung and share significant homology with their mammalian homologs. Here we report the sequence of xFgf10, the Xenopus homolog to the mouse Fgf10 gene necessary for lung development. In addition, we report the effect of overexpressing the transcription factor Nkx2.1 in endodermal cells fated to develop lung. Nkx2.1 is expressed at the earliest stages of lung development in mice and frogs, is necessary for proper lung formation and is essential for the expression of the lung specific genes Spc and Sbp in cultured mouse cells. Overexpression of Xenopus Nkx2.1 resulted in increased expression of Spc as measured by real-time PCR. The effect of Nkx2.1 on Sbp expression is currently under investigation.

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It is well-established that Ngn3 is both necessary and sufficient to induce endocrine islet cell differentiation during embryogenesis. Because detectable Ngn3 is only detectable in progenitor cells that will eventually become hormone-expressing islet cells, but not in hormone-positive cells, it has been proposed as an endocrine progenitor cell marker. Here we present several pieces of evidence to support the presence of sustained Ngn3 expression and its functional involvement in the adult islet cells. (1), our RT-PCR and western blot-based studies show that Ngn3 mRNA and protein are present in wild type adult islet cells and this expression is enhanced by partial pancreatectomy. (2), immunofluorescence reveals the presence of discrete nuclear Ngn3 signals in differentiated islet cells at several stages. (3), a Ngn3-CreERT knockin mouse specifically activates Cre reporter in adult islet cells. (4), Ngn3 inactivation specifically in differentiated islet cells compromises endocrine function. Finally, inactivation of Ngn3 in post-differentiated islet cells compromises the expression of several genes that are known to be regulated by Ngn3 at embryonic stages. Specifically loss of Ngn3 function in islet cells reduced the expression of Glut2, Mafa, Nkx6.1, and Pdx1, but not insulin and Mafa. These findings suggest that Ngn3 expression exist in the adult islet cells and this expression contributes to endocrine functional maintenance.

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High levels of Ngn3 expression in pancreatic progenitor cells are both necessary and sufficient to initiate endocrine differentiation. While it is clear that the Notch-Hes1-mediated signals control the number of Ngn3-expressing cells in the developing pancreas, it is not known what factors control the level of Ngn3 expression in individual pancreatic cells. Here we report that Myt1b and Ngn3 form a feed-forward expression loop that regulates endocrine differentiation. Myt1 expression largely, but not totally, relies on Ngn3 activity. Surprisingly, a portion of Myt1 expressing pancreatic cells express glucagon and other α cell markers in Ngn3 nullizygous mutant animals. These results demonstrate that Myt1b and Ngn3 positively regulate each other's expression to promote endocrine differentiation. In addition, the data uncover an unexpected Ngn3 expression-independent endocrine cell production pathway, which further bolsters the notion that the seemingly equivalent endocrine cells of each type, as judged by hormone and transcription factor expression, are heterogeneous in their origin.

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At metamorphosis the Xenopus laevis tadpole exocrine pancreas remodels in two stages. At climax thyroid hormone (TH) induces dedifferentiation of the entire exocrine pancreas to a progenitor state. The mRNAs that encode exocrine specific proteins undergo almost complete extinction at climax while PDX-1, Notch-1 and Hes-1, genes implicated in differentiation of the progenitor cells, are activated. At the end of spontaneous metamorphosis the pancreas begins to redifferentiate. A major difference between a tadpole and frog pancreas is the paucity of ducts in the tadpole. The redifferentiated frog pancreas has typical ducts found in other vertebrate pancreases. Exogenous TH induces the dedifferentiation phase not the redifferentiation phase. Pancreases of transgenic tadpoles expressing a dominant negative form of the thyroid hormone receptor (TRDN) controlled by the elastase promoter are resistant to TH. Their acinar cells do not down regulate exocrine specific genes or activate Notch-1 and Hes-1. These transgenic frogs do not form a normal ductal system. The dedifferentiation of the exocrine pancreas at climax also controls maturation of the endocrine pancreas by allowing the preexisting beta cell (insulin) to cluster and form islets. Exogenous TH induces the clustering of beta cell and expression of the TRDN transgene or exposure of premetamorphic tadpoles to the antithyroid compound, methimazole inhibit the clustering. Therefore, the TH-dependent dedifferentiation of the exocrine pancreas at climax is

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a necessary step in the formation of a mature typically vertebrate frog pancreas.

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Program/Abstract # 477
Roles of Bmp, Fgf and Wnt signaling in liver formation and recovery in zebrafish embryos
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Bmp, Fgf and Wnt have been implicated in liver specification, differentiation, and proliferation in several systems including zebrafish. Liver specification and subsequent differentiation were blocked in embryos following a block in Bmp or Fgf signaling and in wnt2bb mutant embryos. However and surprisingly, the liver eventually recovered in most of these embryos, suggesting that endodermal cells remain competent to give rise to the liver. To understand the process of liver recovery, we blocked Bmp, Fgf or Wnt signaling in the wnt2bb mutant background. The inhibition of Wnt signaling using a transgenic line overexpressing Dkk1, an inhibitor of the canonical Wnt signaling pathway, under the heat-shock promoter, completely blocked liver recovery in wnt2bb mutant embryos. In addition, the inhibition of Bmp signaling using a transgenic line overexpressing a dominant-negative Bmp receptor also blocked liver recovery. In contrast, the inhibition of Fgf signaling using SU5402, an Fgf inhibitor, promoted liver recovery in wnt2bb mutant embryos. Furthermore, this inhibition also promoted liver recovery in wnt2bb mutant embryos with Bmp or Wnt signaling repressed. The knockdown of fgf10 using morpholinos also promoted liver recovery in these embryos. Since it was recently reported that fgf10 mutant embryos have defects in the extrahepatic duct and contain ectopic hepatocytes, we hypothesize that extrahepatic duct progenitor cells can give rise to duct and liver, and that Fgf signaling mediated by Fgf10 represses these cells to become liver, thereby allowing them to become duct.

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Program/Abstract # 478
Zebrafish homologue of FKBP65 plays a role in intestinal smooth muscle differentiation
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Zebrafish intestinal smooth muscle differentiates from a thin layer of mesenchymal cells surrounding the developing epithelial layer. As with other vertebrates, circular is first to differentiate early on the third day of embryogenesis followed by longitudinal smooth muscle late on the third day. Few regulatory pathways have been identified which control intestinal smooth muscle differentiation. FKBP65 has previously been shown to regulate differentiation of avian intestinal smooth muscle. We have begun to characterize the role of the zebrafish homologue of fkbp65 gene in intestinal smooth muscle development. We find the zebrafish fkbp65 is expressed in the intestinal mesenchyme during the third day of embryogenesis at a time when smooth muscle is developing. We find that zebrafish fkbp65 expression is present at the correct time and place to play a role in intestinal smooth muscle development. To address the role of FKB65 in intestinal smooth muscle differentiation we used both FK506, a general inhibitor of the class of FKBP5s, and a 5′ morpholino to the gene. We find that injection of either the FK506 or morpholino inhibits differentiation of smooth muscle primarily in the anterior intestine. These results suggest that the zebrafish homologue of FKBP65 plays a similar role in smooth muscle differentiation to the avian system. Inhibition of smooth muscle differentiation in only the anterior intestine suggests that there may be another homologue that plays a role in posterior smooth muscle differentiation.

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Program/Abstract # 479
Effect of thyroid hormone on gut development in a direct developing frog
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During metamorphosis of Xenopus laevis, thyroid hormone (TH) induces apoptosis of the tadpole gut and development of the adult gut. Eleutherodactylus coqui, a direct developing frog, lacks a tadpole. Its embryonic gut is a miniature adult form with a mass of large yolk-rich cells attached to the intestine. The yolk-rich cells provide nutrition but do not contribute to the adult gut (Buchholz et al., 2007 Dev Dyn 236:1259–1272). We asked whether TH is involved in E. coqui gut development as it is in X. laevis. The expression of TH receptors, EcTRα and EcTRβ, was detected in the developing gut by RT-PCR, indicating a TH role. To test a TH requirement, endogenous TH synthesis was inhibited with methimazole. The diameter of the methimazole treated gut was reduced, and the submucosa and muscularis layers were significantly smaller than those of the untreated embryos. Despite these gross histological differences, RT-PCR indicated no obvious differences in expression of EcSox17, EcShh, EcBMP4 and EcCad between methimazole treated and untreated embryos. Embryos treated with methimazole failed to utilize their yolkly tissue, but survived for weeks without any further development. When T3, the active form of TH was added along with methimazole, the gut resembled that of controls. There were, however, many more cells in guts from T3-treated embryos compared to untreated ones and from untreated embryos compared to methimazole treated ones. These results suggest that a major role of TH in the development of the E. coqui gut is to stimulate cell proliferation of gut tissue and utilization of yolk.

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Program/Abstract # 480
Alpha 2-macroglobulin regulation of axial and gut morphogenesis in Xenopus laevis
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α2-macroglobulin is a major serum protein which inhibits protease activity. In Xenopus laevis, two α2M genes, Endodermin (Edd) and Panza, have been isolated. Edd is expressed in endoderm and dorsal mesoderm cells and with the onset of gut coiling expression is restricted to the liver. In contrast, Panza is expressed in the dorsal domain of the gut endoderm. During gut coiling Panza expression is initiated and maintained in the liver. The overlapping expression of Edd and