complete morphogenesis of a functional conduit for excretory waste. Our studies of axolotl embryos showed that GDNF signaling through the Ret/GFRalpha-1 receptor plays a role in posterior PD extension; we are extending our studies to investigate whether GDNF plays a similar role in Xenopus. Here, we show that Xenopus laevis expresses two GDNF paralogs similar to the long form of mammalian GDNF, and one alternatively-spliced form. We also show that GFRalpha-1 is necessary for the second phase of PD elongation. In addition, the expression patterns of Xenopus GDNF, GFRalpha-1 and Ret indicate that this signaling system could play a role in both PD and RD morphogeneses. Our strategy for testing whether GDNF is a PD or RD chemoattractant will also be discussed.

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Program/Abstract # 223
Isomorphic and domain dependence of nonmuscle myosin II in vivo and in vitro
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Nonmuscle myosins (NMs) II-A and II-B are essential for embryonic mouse development, but their specific roles are not completely defined. Here we examine the isoforms and their domain specificities in vivo and in vitro by studying mice and cells in which nonmuscle myosin heavy chain (NMHC) II-A is genetically replaced by NMHC II-B or chimeric NMHC IIls that exchange the rod and head domains of NMII-A and II-B. In contrast to the failure of visceral endoderm formation resulting in embryonic day (E)6.5 lethality of A−/− mice, replacement with NM II-B or chimeric NM IIls restores a normal visceral endoderm. This is consistent with NM IIls role in cell adhesion and also confirms an essential, isoform-independent requirement for NM II in visceral endoderm function. The knock-in II-B and chimeric mice die between E9.5 and 12.5 due to defects in placenta formation associated with abnormal angiogenesis and cell migration, revealing a unique function for NM II-B in placenta development. In vitro results further support a requirement for NM II-A in directed cell migration and focal adhesion formation. These findings demonstrate an isoform-specific role for NM II during these processes, making replacement by another isoform, or chimeric NM IIls less successful. The failure of these substitutions is not only related to the different kinetic properties of NMII-A and II-B, but also to their subcellular localization determined by the C-terminal rod domain. These results highlight the functions of the N-terminal motor and C-terminal rod domains of NM II.

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Program/Abstract # 224
Characterization of an Ankyrin Repeat Socs Box gene in the early heart development of the basal chordate, Ciona intestinalis
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FGF signaling drives the specification of heart precursor cells through the activation of an Ets family transcription factor (Ets1/2) in the basal chordate, Ciona intestinals. Microarray analysis of gene expression from sorted heart lineage cells has led to the identification of candidate FGF/Ets target genes. I am currently studying the function and regulation of the FGF/Ets target gene Ankyrin Repeat Socs Box (ASB). In situ data shows ASB mRNA uniquely expressed in the heart precursor cells shortly after the start of neurulation. Functional analysis of ASB through misexpression suggests that ASB plays a significant role in heart development. Under conditions in which the signaling pathways controlling heart specification are blocked, the expression of ASB in heart precursor cells is sufficient for the partial rescue of heart cell migration. Analysis of a minimal enhancer region of ASB indicates that ASB is a direct target of transcription factors Ets1/2 and FoxF. Functional analysis of ASB will help determine the specific role of ASB in Ciona heart development. Detailed analysis of the regulatory elements driving the expression of ASB will help define the precise impact of FGF/Ets on the heart gene regulatory network, allowing us to identify and isolate the regulatory regions of other predicted FGF/Ets target genes utilizing a bioinformatics approach.

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Program/Abstract # 225
Critical functions of myocardial Mycn in the developing mouse heart
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The focus of this study is to define the role of the transcription factor Mycn in the developing mouse myocardium. Haploinsufficiency for MYCN causes Feingold syndrome, a developmental disease characterized in part by congenital heart defects (CHD). Mice lacking Mycn also display complex and variable CHD. Furthermore, Mycn is a target of Bone Morphogenetic Protein (BMP) signaling in the developing mouse heart. Disruption of BMP pathways results in abnormal development of the heart wall, valves and septa. Thus, Mycn is implicated in key cardiogenic processes but its exact functions remain to be established. I am testing the hypothesis that Mycn is an essential regulator of mouse cardiomyogenesis. Using conditional gene inactivation, Mycn is removed from the myocardium of mutant embryos. Mycn deletion was confirmed with semi quantitative PCR, western blot, and immunohistochemistry assays. In addition, western blot analyses reveal decreased expression of Mycn targets, CcnD1 and Id2. Loss of myocardial Mycn results in underdeveloped ventricles and embryonic lethality by E12.5, likely due to cardiovascular insufficiency caused by decreased ventricle contractility. Normally, ventricle chambers grow via cardiomyocyte proliferation and differentiation into muscular projections called trabeculae. The defective ventricle morphogenesis in mutants suggests a crucial role for Mycn in cardiomyocyte proliferation, survival and/or differentiation. Indeed, loss of Mycn causes a significant decrease in proliferation at E9.5. To discover the other effects of Mycn depletion, I am examining apoptosis and the expression of genes involved in ventricle morphogenesis.

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Program/Abstract # 226
Mice null for Crim1 display altered BMP/TGFβ signaling, defects in multiple organ systems and die in utero with severe cardiovascular defects
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The purpose of this study was to determine what role the developmentally expressed molecule Crim1 plays in organogenesis. Crim1 is a trans-membrane protein capable of binding a range of
cystine-knot growth factors (including VEGF, BMPs, TGFβs, and PDGFs), and is dynamically expressed during embryogenesis. We have previously shown that a Crim1 hypomorphic mouse mutant (Crim1<sup>KST264</sup>) displays perinatal lethality with defects in multiple organ systems. Here we report data from a conditional mutant mouse line to produce embryos null for Crim1. Like Crim1<sup>KST264</sup> mice, mice null for Crim1 displayed digit syndactyly, eye and renal defects, and exencephaly at variable penetrance. However, Crim1 null mice die by E17 dpc with severe cardiac defects, including ventricular septal defects and coronary and epicardial malformations. Moreover, some of the phenotypes resemble those of mice with defects in cystine-knot growth factors, including BMP4. Thus, we hypothesized that the phenotype of Crim1 mutants is due to aberrant TGFβ superfamily signaling, resulting in early patterning defects. Immunohistochemistry revealed reduced levels of phosphorylated p38 MAPK in Crim1 mutant embryos. Furthermore, qRT-PCR showed changes in Id3 and Runx3, indicative of alterations in BMP (and TGFβ) signaling pathways. We conclude that Crim1 is essential for normal development, which may occur through modulation of BMP/TGFβ superfamily of growth factors.

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Program/Abstract # 227
The activity of cerberus-like 2 during cardiogenesis, morphological and morphogenetics studies
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Mouse cerberus-like 2 (cerl-2) is a Cerberus/Dan family member that is asymmetrically expressed on the right side of the mouse node. cerl-2 encodes for a secreted protein that binds directly to nodal thus inhibiting its signaling pathway. cerl-2 KO mice display multiple laterality defects including randomization of the L/R axis. However, we have found cerl-2 associated cardiac defects that cannot be explained by laterality abnormalities (incomplete atrial and ventricular septation). We observe a consistent increase of ventricular muscle and to access whether this singular phenotype is independent of LR establishment we have used the transgenic mouse line m1c1v-nlacZ24 as a correct right ventricle/OFT orientation. Based in our observations, we propose that in addition to the previously described laterality-related defects, another distinct mechanism may contribute to the spectrum of complex cardiac defects in cerl-2 KO mice. The molecular basis of vertebrate cardiogenesis is increasingly becoming unraveled. Research in this area will be an essential step, as the targets will be the most amenable sites of intervention, both in a therapeutic sense and for the purpose of prevention. Considering the high conservation of genetic pathways regulating cardiac development in species, the study of the mouse/human orthologue genes involved in the nodal signaling pathway should bring us new data on Congenital Heart Disease (CHD) and on laterality defects.

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Program/Abstract # 228
Bves and NDRG4 modulate epicardial cell differentiation
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The epicardium differentiates to contribute cells to the coronary vasculature. In development a portion of the epicardial cells undergo EMT, delaminate, and differentiate into vascular smooth muscle (SM). We have discovered a pair of proteins, Bves and NDRG4, which biochemically interact in epicardial cells and colocalize in cells that have mesenchymal morphology. The cellular functions attributed to Bves and NDRG4 are complementary: they both affect cellular morphology, migration rate, or proliferation; all components of the overall differentiation state. Bves and NDRG4 may synergistically regulate differentiation in the epicardium. To test this, we are using an epicardial cell line that retains the ability to differentiate in culture to modify Bves and NDRG4 expression and assay for presence of differentiation markers via QRT-PCR and IF. Initial data show that overexpression of Bves or NDRG4 enhances SM marker expression. We are currently testing differentiation after co-overexpression and knockdown in the epicardial cells. To complement these studies, crossections of an NDRG4<sup>−/−</sup> mouse are being investigated using IF to determine if the epicardium is intact and if the coronary vessels develop properly. Additionally, we are using a technique that involves culturing an embryonic heart to facilitate migration of the epicardium into a culture dish to investigate differentiation in the NDRG4 knockout line via QRT-PCR and IF. We expect to see impaired expression of differentiation markers. Together these data will determine if Bves and NDRG4 synergistically affect differentiation in the epicardium.

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Program/Abstract # 229
Fgf3 and Fgf10 are required redundantly for neural crest migration and cardiovascular development
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Heart development requires contributions from, and interactions between, discrete cell populations including primary and secondary heart fields (SHF), cardiac neural crest (CNC), and the proepicardial organ (PEO). Fgf3 and Fgf10 are expressed in sites relevant to early heart development, including the hindbrain, pharyngeal endoderm, SHF and PEO, but single null mutants do not have significant heart defects. Fgf3; Fgf10 double mutants, however, die by E11.5. These embryos lack NC-derived proximal 9th cranial ganglia and 4th pharyngeal arch arteries and arch segmentation, and exhibit pericardial edema and dilated atria suggestive of heart failure. Heart tube looping and chamber morphogenesis proceed normally, but hypoplastic ventricles and outflow tract cushions are observed with variable penetrance. To test the hypothesis that Fgf3 and Fgf10 are coordinately required for correct migration and/or survival of CNC cells, and for normal heart development, we assessed expression of relevant markers. Specification and early migration of NC are normal, but migration is reduced by E9.5–10.5. Expression of Islet1 is markedly reduced in the SHF, whereas Fgf8 and Fgf15 are unaffected. Double mutants also show posterior pole defects, including reduced investment of epicardial cells from the PEO. Studies are underway to determine the spatiotemporal relationships between Fgf3, Fgf10 and their receptors, and to determine the expression sites required for normal CNC and cardiovascular development.

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Program/Abstract # 230
Wnt signaling promotes proliferation to pattern the zebrafish craniofacial skeleton
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Formation of the craniofacial skeleton from the neural crest (NC) requires the coordinated action of multiple tissues and signaling