putative phosphatase, paladin, that is expressed by premigratory neural crest cells and upregulated at the initiation of neural crest migration in both chick and mouse embryos. Paladin knockdown in chick embryos inhibits neural crest migration and delays expression of the neural crest transcription factors snail-2 and sox10, but does not affect the expression of other markers of neural crest specification. Additionally, we have begun to characterize neural crest migration in a mouse knockout, analyze the importance of the phosphatase activity of paladin during neural crest development and identify potential targets of paladin activity. Together, these data indicate that paladin is an important regulator of neural crest migration and support the notion that phosphorylation plays an important role in this process. Funded by NIH F32DE019973 and the Minnesota Medical Foundation.

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Program/Abstract # 304
Kctd15 inhibits neural crest formation by modulating Wnt signaling
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In vertebrates, Neural Crest (NC) cells and pre-placodal cells originate from the neural plate border. Induction, maintenance, migration and differentiation of these cells depend on Wnt, Bmp, Fgf, Notch and retinoic acid signaling pathways. NC cells eventually delaminate and migrate to different locations where they differentiate into other derivatives. Wnt signaling is necessary to induce NC, whereas placodal fate is suppressed by Wnt, but it remains unclear how progenitor cells at the neural plate border, exposed to similar levels of Wnt signaling, are specified to form NC or placode. Here we show that potassium channel tetramerization domain containing15 (Kctd15) can suppress NC induction and differentiation. Kctd15 is expressed at the border of neural and non-neural ectoderm in zebrafish and Xenopus. Overexpression of Kctd15 inhibits NC induction and expansion of pituitary placode while loss of Kctd15 leads to expansion of the NC domain. The loss of NC induction observed after knock-down of Wnt8.1 in zebrafish embryos was rescued by coinjection of Wnt8.1 and Kctd15 morpholinos, linking Kctd15 function to the Wnt pathway. As in whole embryos, NC induction in Xenopus animal caps elicited by Wnt and chordin was suppressed by Kctd15. However, Kctd15 could not suppress NC induction by activated β-catenin (ca β-cat) in embryos or in animal caps. Our results indicate that Kctd15 is a novel, highly effective and specific regulatory component in NC induction that may function to restrict the NC domain in the developing embryo by attenuating the output of the canonical Wnt pathway.

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Program/Abstract # 305
Investigating mesothelial cell potential in gut development
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In the developing heart, a population of mesothelial cells known as the proepicardium (PE) migrates to and over the heart to form the epicardium, undergo epithelial–mesenchymal transition (EMT), and gives rise to the cells of the coronary vasculature. The serosal mesothelium (SM), located in the peritoneal cavity, is involved in development of the gut vascular system. Collectively, these data demonstrate that mesothelial cells are paramount to blood vessel formation in both gut and heart, suggesting that there may be a conserved mechanism in mesothelial development. We hypothesize that there is an interchangeable potential between PE and SM cells in a developing embryo. To test this hypothesis, quail PE or SM cells are transplanted into either the peritoneal or pericardial cavity of a chick embryo. These experiments will determine if PE and SM cells have the ability to form a mesothelium, undergo EMT, and contribute to vascular cells in any organ housed in the coelomic cavity. In addition, we are characterizing the development of SM using epithelial markers in the early gut tube. Our data indicate two apposing basement membranes are present before the SM develops—one supporting the endoderm and one supporting a mesodermal epithelium on the outermost part of the gut—with a tissue space in between. Over time, we observe that the outer epithelial cells undergo a series of morphological changes. Taken together, these studies will demonstrate the interchangeability of the PE and SM as well as elucidate the origins of SM cells.

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Program/Abstract # 307
Transcription factor heterogeneity in proepicardial and epicardium-derived cell populations during heart development
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During embryonic development, the proepicardium (PE) migrates over the myocardium to form the epicardium. Epicardial cells undergo epithelial-to-mesenchymal transition to form epicardium-derived cells (EPDCs), which invade the myocardium and differentiate into fibroblasts, smooth muscle and endothelial cells. The PE and EPDCs are comprised of heterogeneous cell populations, which differentially
Program/Abstract # 308
Agtr1lb acts non-cell-autonomously for proper cell migration during myocardial progenitor development
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During vertebrate embryogenesis the heart is the first organ to form and function. Fate-mapping studies in vertebrate embryos have demonstrated that prior to gastrulation myocardial cells originate from a fixed position in the embryo. During gastrulation these cells migrate to form bilateral stripes in the anterior lateral plate mesoderm (ALPM), where they first express nkn2.5, the earliest marker of myocardial progenitors. How cardiac cell fate is influenced during gastrulation remains unclear. A zebrafish mutant, grinch, in which there is a significant reduction (or complete absence) in the number of cardiomyocytes formed has previously been described by our lab. The grinch phenotype is due to a mutation in the gene encoding the G protein-coupled receptor Agtr1lb. Here we investigate the mechanism through which Agtr1lb regulates the formation of myocardial precursors. RNA in situ hybridization analyses of grinch mutants and morphants show that there is a decrease in specific domains of the ALPM. Lineage tracing studies demonstrate that this is likely due to aberrant cell migration during gastrulation. We find that in morphant embryo cells of the presumed heart field fail to reach the ALPM. Additionally, transplant studies reveal a non-cell-autonomous role for the function of Agtr1lb in myocardial progenitor development. Present studies are centered on imaging migrating progenitors in real time to examine specific defects in grinch mutants. Our work provides novel insight into the earliest mechanisms that influence cardiac progenitor development.

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Program/Abstract # 309
FGF/Ets target genes in Ciona intestinalis heart cell specification
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Activation of the transcription factor Ets1/2 through FGF signaling is known to specify heart precursor fate in Ciona intestinalis. In previous research, we identified candidate target genes of Ets1/2 through microarray analysis. Through in situ hybridization assays we have identified a subset of these candidate genes that are expressed specifically in the heart precursor cells immediately following their specification. To find the enhancers for the regulation of these presumed Ets target genes, we are employing bioinformatics to find conserved areas of DNA in the upstream non-coding DNA between C. intestinalis and Ciona savignyi. This analysis will be used to guide ongoing efforts to clone and test predicted enhancer regions using reporter constructs. In depth analysis of identified enhancers will be used to find transcription binding sites for Ets and identify co-transcription factors presumed to act in concert with Ets to drive heart precursor cell specification.

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Program/Abstract # 310
FGF signaling regulates spindle dynamics in Ciona heart precursor cells
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Asymmetric cell division is a fundamental mechanism in developmental biology by which a single fertilized cell can develop into a multi-cellular organism. Previous studies have shown that asymmetric divisions are typically caused by a shift in spindle orientation and position. In Ciona intestinalis, one such division is required to establish the heart precursor cells. Each of the four B7.5 lineage cells (founder cells) divides asymmetrically to produce a large tail muscle cell and a smaller heart precursor cell. Previous research has shown that this asymmetric division requires a non-polarized FGF signal from the adjacent mesenchyme, which results in uniform FGF receptor occupancy on the B7.5 cells. This causes a localized change in cytoskeletal dynamics resulting in asymmetric division of the B7.5 lineage. Embryos treated with a dominant negative form of the FGF receptor undergo symmetric founder cell division. Also, polarity gene manipulation (constitutively active Cdc42) and inhibition of MAPK signaling result in loss of founder cell asymmetry. To better understand how these molecules interact and their direct effect on division symmetry, we are using live fluorescent microscopy to investigate spindle dynamics within the founder cell. In addition, we are using molecular cloning techniques to identify target candidate molecules that might also be involved in this pathway. While the effect of spindle dynamics on asymmetry has been well studied, no previous study has linked FGF signaling to asymmetric division and the mechanism of cell division symmetry in vertebrate heart precursor cells has yet to be understood.

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Program/Abstract # 311
How heart cells embrace their fate in the chordate Ciona intestinalis
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The establishment of polarity and subsequent asymmetric cell division is required for differentiation throughout development. In Ciona intestinalis, such a division occurs in the heart founder cells, with each of four founders giving rise to a small heart progenitor cell and a larger tail muscle cell. Although FGF signaling occurs prior to division, ERK is activated only in the smaller daughter and results in heart cell-specific behaviors such as migration and proliferation. The mechanism by which FGF signaling is propagated only in the heart lineage is not yet understood. Our data implicates polarity of the actin cytoskeleton in