**Program/Abstract # 370**
**A mutagenesis genetic screen to identify zebrafish embryos with defects in vasculature development**
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The zebrafish *Danio reiro* is a powerful vertebrate model system to study developmentally regulated processes, such as the formation of the vascular tree. The vascular system of the zebrafish embryo forms by a combination of vasculogenic andangiogenic processes, mirroring the early vascular development of humans. Moreover, most of the genes currently known to act during embryonic vascular development are highly conserved invertebrates. Zebrafish embryos develop externally, are optically transparent, and are produced in large clutches. All these advantages can be exploited to carry out genetic screens to look for mutants with defects in early stages of vascular development. We conducted an ENU mutagenesis to identify vascular-specific mutants in an F3 genetic screen. To facilitate the screening process, we used a stable germline transgenic, *fli1-EGFP*$^{y1}$, that expresses enhanced green fluorescent protein in endothelial cells *in vivo* under the control of the *fli1* promoter. The robust expression of EGFP in the vascular endothelium enabled us to screen visually for blood vessel patterning defects and changes in vasculogenesis at 4days post-fertilization. We have found many new mutants with phenotypes that include vascular patterning defects, ectopic vessels, missing vessels, and vascular shunts. Genetic mapping and identification of the genes responsible for the mutant phenotypes will lead to increased understanding of the formation of the human vasculature as well as on the pathogenesis of numerous cardiovascular diseases.

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**Program/Abstract # 371**
**Heart field reduction in embryos depleted for voltage-gated calcium channel beta subunit CACNB2**
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Voltage-gated calcium channels (VGCCs) are oligomeric complexes composed of pore-forming CACNA subunits and several auxiliary proteins. Auxiliary CACNB subunits regulate VGCC electrophysiology and chaperone CACNA subunits to the cell membrane. In humans, mutations in CACNA subunits are associated with congenital cardiac arrhythmia, but the developmental functions of the CACNB subunits are poorly understood. To determine the contributions of CACNB2 to cardiac development, we depleted zebrafish embryos of CACNB2 transcripts using morpholinos. CACNB2 morphant heart fields contained fewer cells expressing cardiac markers, suggesting that fewer cardiomyocytes were specified, or diminished survival. Cell proliferation at later stages did not compensate for this deficiency; heart tubes in morphants contained 30 fewer cardiomyocytes at 48hpf, as looping and chamber morphogenesis progressed. Moreover, morphant heart tubes fragmented easily when placed under pressure, suggesting that cardiomyocyte adhesion was weakened. Previous work showed that mutations in CACNA that inactivate cardiac VGCCs lead to atrial fibrillation. In contrast, heart rhythm was normal in CACNB2 morphants, suggesting that other CACNB proteins may compensate for the depletion, providing intact VGCC activity. We are currently assaying whether CACNB2 phenotypes are mediated by loss of VGCC function, or by loss of other CACNB2:partner interactions. The latter possibility is intriguing in light of recent data suggesting that CACNBs, as MAGUK-family proteins, may interact with multiple protein partners via their SH3 or guanylate kinase domains.

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**Program/Abstract # 372**
**Investigating the role of TGFbeta signaling in asymmetric morphogenesis of the zebrafish heart**
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The Nodal signaling pathway plays a conserved role in establishing differences between the left and right sides of all vertebrate embryos. This initial patterning along the leftright axis is required for the subsequent asymmetric positioning and morphogenesis of the visceral organs. Recent work has increased our understanding of these events; however, the mechanisms by which asymmetric expression of Nodals and other TGFbeta signals instruct the movements of cells and tissues remain largely unidentified. We are using zebrafish cardiac development as a model to investigate the requirements for TGFbeta signaling in directing asymmetric morphogenesis. We have found that the laterality of Nodal signaling influences the first asymmetry evident in the heart; a left-directed migration of myocardial cells within the cardiac cone. These cell movements subsequently determine the direction of cardiac jog. Interestingly, in studying embryos of different genotypes with the common phenotype of absent Nodal signaling, we have found that jogging laterality defects are significantly different from one genetic class of embryos to another. These results suggest that specific components of the Nodal signaling pathway may play distinct and different roles in cardiac laterality determination. Further investigation of the specific requirements for these pathway components will hopefully provide new insights into how TGFbeta signaling is regulated and integrated within the heart to promote consistent asymmetries in myocardial cell migration and overall cardiac laterality determination.

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**Program/Abstract # 373**
**Determining the role of the Tbx5 transcription factor in zebrafish cardiac development**
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Tbx5, a T-box transcription factor, is required for cardiac development. Mutations of Tbx5 lead to HoltOram Syndrome (HOS) in humans. As in HOS, mutation of zebrafish *tbx5* affects both heart and forelimb structures. Homozygous tbx5/*heartstrings* (*hst*) exhibit bradycardia, failure of the heart tube to loop, cardiac edema and absence of pectoral fins. Here, we investigate the effects of *tbx5* mutation on 1) cell proliferation in the developing heart tube, 2) volumetric growth of cardiomyocytes, and 3) chamber morphology, especially during heart tube stages. Previous *tbx5* overexpression studies in chick and mouse demonstrated that Tbx5 provides a growth arrest signal that limits cardiomyocyte proliferation during chamber morphogenesis stages. In the converse experiment we find that loss-of-function mutation of zebrafish *tbx5* did not lead to increased cardiomyocyte number, with no net effect on cell proliferation of heart tube cardiomyocytes at 48 or 72h post-fertilization. We hypothesize that inability of the *hst* heart tube to

loop may result from deficiencies in cardiomyocyte shape, size or differentiation. We provide an update on ongoing work to determine the earliest developmental timepoints at which *tbx5* is necessary for normal cardiac function, as well as the functional relevancy of graded *tbx5* expression, by using the *Tg(hsp70:tbx5-GFP)* and *Tg(cmlc2:tbx5-GFP)* lines of zebrafish.

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**Program/Abstract # 374**
**FGF signaling regulates a secondary phase of cell addition to the initial heart tube in zebrafish**
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Developing organs are assembled from multiple populations of progenitor cells that originate from distinct locations at different developmental stages. During heart development, the initial heart tube forms from cardiomyocytes arising from a portion of the anterior lateral plate mesoderm referred to as the first heart field (FHF). Prior studies in amniotes have shown that new cardiomyocytes originating from a second heart field (SHF) are later added to the poles of the heart tube. Many congenital heart diseases affect portions of the heart derived from the SHF; however, we still do not understand the mechanisms that regulate the specification, migration, and differentiation of SHF cells. Recent studies from our laboratory have provided the first evidence that there are two phases of cardiomyocyte differentiation in zebrafish, strongly suggesting the existence of a zebrafish SHF. Treatment of zebrafish embryos with SU5402 inhibitor from 24 to 48hpf, covering the window when cells are added to the arterial pole, significantly reduces the number of cells added to the arterial pole. Thus, after differentiation of the FHF is complete, FGF signaling is still important for the addition of new cardiomyocytes from the SHF. Continuous observation of a transgenic reporter of FGF signaling indicates FGF-responsive cells scattered in a region adjacent to the arterial pole, followed by congregation of FGF-responsive cells at the arterial pole. These findings, together with the expression of *fgf8* in the ventricle, suggest a model in which Fgf8 functions as an attractive cue regulating the migration of new cardiomyocytes to the arterial pole.

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**Program/Abstract # 375**
**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). Birth defects caused by abnormal CNC and SHF development include DiGeorge and CHARGE syndromes. Aspects of these syndromes are phenocopied in fibroblast growth factor (Fgf)8 or Fgf15 null mutant mice. Fgf3 and Fgf10 are expressed in sites relevant to early heart development, but single null mutants do not have heart defects. Fgf3$^{-/-}$;Fgf10$^{-/-}$ double mutants, however, die at E11.011.5. They lack NC-derived proximal 9th cranial ganglia, exhibit pericardial edema, hypoplastic ventricles and outflow tract cushions, and lack 4th pharyngeal arch arteries, showing that Fgf3 and Fgf10 are required redundantly for normal CNC and cardiovascular development. To test the hypothesis that Fgf3 and Fgf10 are required for correct migration and/or survival of CNC, and for development or morphogenesis of the heart, we assessed expression of NC and cardiac markers. We find that specification and early migration of NC are normal, but NC migration is reduced by E9.510.5. Expression of Nkx2.5 and Islet1 is markedly reduced in the double mutant; whereas Fgf8 and Fgf15 are unaffected. In contrast to the exclusively anterior pole defects of Fgf8 or Fgf15 mutants, Fgf3$^{-/-}$;Fgf10$^{-/-}$ embryos also show posterior pole defects, including reduced investment of epicardial cells from the PEO. Studies are underway to define the expression sites of Fgf3 and Fgf10 required for normal CNC and cardiovascular development.

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**Program/Abstract # 376**
**BMP signaling regulates progenitors of the mammalian heart**
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Development of the right ventricle and outflow tract of the mammalian heart involves cell populations within the primary heart tube, as well as extracardiac contributions, as cells from outside the primary heart tube progressively add to it. Proper morphogenesis of these tissues is critical for cardiac function. The anterior heart field (AHF) is a secondary cell lineage of the myocardium that contributes substantially to the outflow tract and right ventricle. Here we present evidence that extracardiac BMP signaling is essential for the addition of progenitor cells to the heart. Several tissue-specific genetic ablations and explant culture experiments demonstrate a direct requirement for BMP signaling in regulating myocardial differentiation and proliferation in the AHF. Embryos lacking BMP receptor 1A (BMPR1A) in the AHF invariably display severely hypomorphic outflow tract and right ventricle structures. In contrast, Bmpr1a in the primary heart tube is dispensable for development of these tissues, but is necessary for later cardiac gene expression and cardiomyocyte proliferation. We further find that BMP antagonism by Noggin is necessary to keep myocardial proliferation in check. Surprisingly, although BMPR1A signal transduction requires the canonical signal transducer Smad4 in the primary heart tube, BMPR1A signaling in the developing AHF is independent of Smad4. Thus, BMP signaling and its antagonism balance myocardial proliferation in the ventricles. Earlier, BMP signaling acts via a Smad4-independent pathway to regulate addition of myocardial progenitors to the outflow tract and right ventricle.

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**Program/Abstract # 377**
**Manta ray a novel ENU mutant with brain and craniofacial defects**
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In a forward genetic screen in mice we identified a novel mutant line with a multitude of severe abnormalities and fetal lethality. We named this line manta ray (mray) in reference to its craniofacial abnormalities, which include orofacial clefting. In addition to craniofacial defects, homozygous mutants are defective in brain, heart, skin and vascular development. The brain defects in particular, include a smaller sized forebrain partly resulting from cortical thinning. The craniofacial phenotype points to an abnormal neural