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.

doi:10.1016/j.ydbio.2009.05.403

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.

doi:10.1016/j.ydbio.2009.05.404

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.

doi:10.1016/j.ydbio.2009.05.405

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.

doi:10.1016/j.ydbio.2009.05.406

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