cutaneous abnormalities caused by activated RAS and to understand the basic functions of RAS regulation in development, we generated a mouse model in which Kras is constitutively active in the skin. Ectodermal activation of Kras caused multiple skin abnormalities which phenocopy the spectrum and pattern of cutaneous defects in human Costello syndrome. In the epidermis, activated RAS increased the production of epidermal progenitors leading to an overall expansion of the skin and the appearance of the characteristic Costello redundant skin phenotype. In contrast, we found that Kras inhibited hair growth through defects in proliferation. Analysis of genes involved in regulating hair growth revealed a striking downregulation of Sonic hedgehog (Shh) gene expression. Likewise, we found that initiation of Shh expression during the new hair cycle was also inhibited by Kras. These findings suggest that at least two of the defining phenotypic abnormalities in Costello and other RAS-related syndromes involve insufficient expression of Shh and more generally, that RAS signals may play a role in a negative feedback inhibition of the Shh-signaling center in the hair follicle.

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Program/Abstract # 454
The LIM-domain binding protein Ldb1 is required for proper endocardial cushion formation during heart development in *Mus musculus*

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Congenital heart defects are the most common type of major human birth defect, affecting more than 30,000 births in the United States each year. There is clear evidence that *L*IM-*d*omain *b*inding protein Ldb1 is crucial for heart formation: Ldb1 knockout mice never form a heart, and die at E9.5–10.0. Additionally, our evidence suggests that Ldb1 is required for events throughout heart development. In order to elucidate these roles, we employed a conditional (floxed) knockout of Ldb1 driven by Tie2-Cre (a.k.a. Tek-Cre). Tie2-Cre is expressed from E9.5 in endothelial tissues. Tie2-Cre; Ldb1 (floxed) embryos arrest development at E12.5–13.5, and die by E15.5. Through histological and immunohistochemical analyses of the conditionally mutant hearts we have found defects in the atrioventricular (AV) endocardial cushion, the endocardium and the myocardium. The AV endocardial cushion appears hypocellular, while the endocardium is hypercellular. However, there is no increase in apoptosis apparent in the AV cushion. Together with the hypercellular endocardium, the lack of increased cell death suggests rather a failure of the epithelial-to-mesenchyme transition that leads to the observed AV cushion defects. Our results demonstrate that Ldb1-mediated transcriptional events are crucial not only during early cardiogenesis, but also for AV endocardial cushion formation and endocardial regulation.

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Program/Abstract # 455
Monocilia in the embryonic mouse heart imply a direct role for cilia in cardiac morphogenesis

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Blood flow and cardiac function are essential for cardiac morphogenesis: however, how these mechanical signals are sensed by cardiac cells during development remains unclear. Cilia function as mechanosensors in other fluid-filled organs, thus cilia could also be fluid flow sensors in heart development. They have an indirect role in heart development via the requirement for cilia at the embryonic organizer (node) in the development of global left–right asymmetry. We present evidence that cilia also have a direct role in cardiac morphogenesis after the establishment of LR asymmetry. Cilia are found in the mouse embryo heart at e8.5–e12.5. We demonstrate abnormal development of the endocardial cushions (ECCs) and compact myocardium (CM) in e9.5 mouse embryos with absent cilia due to mutation of the heterotrimeic kinesin component Kif3a or abnormal ciliary mechanosensing due to mutation in polycystin2. In contrast, hearts from embryos with abnormal LR development due mutation in left–right dynein resulting in paralyzed, but structurally normal cilia, show less penetrant ECC defects and normal CM. These observations support a role for cilia in cardiac development distinct from their early function in LR development. Cilia may function as mechanosensors in heart development, integrating flow, cardiac function and morphogenesis.

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Program/Abstract # 456
Investigating Bmp-signaling functions in second heart field

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Secondary heart field (SHF) contributes to outflow tract (OFT) and right ventricular myocardium, OFT endocardium, and vascular smooth muscle. Previous data from chick and mouse models implicated that Bmp signaling may play an important role in SHF development. To investigate functions of Bmp signaling in SHF diversification, we inactivated *Bmp2* and *Bmp4* specifically in SHF using conditional null alleles and the *Mef2c AHF cre* allele. We also used a doxycycline regulated *Bmp4* allele to induce expanded *Bmp4* specifically in SHF. We found that there are quantitative requirements for *Bmp2* and *Bmp4*-mediated signaling. The most sensitive *Bmp2,4*-responsive event is epithelial mesenchymal transition (EMT) in proximal OFT while expansion of CNC in OFT requires intermediate doses of *Bmp2,4*. Expansion and differentiation of the SHF itself is relatively resistant to loss of *Bmp2,4* signaling while pharyngeal endoderm and branchial arch artery (BAA) remodeling retain normal. *eHand* was abolished in OFT of *Bmp2,4* double loss-of-function mutants, indicating that Bmp signaling are required for CNC patterning and *eHand* could be a direct downstream target of Bmp signaling since it contains several *Smad* binding sites. *Nkx2.5* was dramatically elevated in *Bmp2, 4* double loss-of-function mutants and down-regulated in expanded *Bmp4* mutants, suggesting Bmp signaling negatively regulates *Nkx2.5*. Our findings uncover that *Bmp2* and *Bmp4*-mediated signaling play a crucial positive role in SHF diversification and potentially function by regulating *eHand* and *Nkx2.5*.

Keywords: secondary heart field (SHF), outflow tract (OFT), Bmp signaling

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Program/Abstract # 457
Nodal dependent and independent axis conversions during asymmetric morphogenesis of the zebrafish heart

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Patterning of the vertebrate embryo along the left–right (L/R) axis is required for proper positioning and asymmetric development of the visceral organs. The conserved role of the Nodal signaling pathway during this process has been well established, but how asymmetric gene expression is interpreted by tissues to result in asymmetric morphogenesis is still not well understood. To address this question, we have studied the processes of cardiac jogging and looping in zebrafish. We find that Nodal signaling influences the direction of myocardial migration within the cardiac cone, just prior to jogging, and that the direction of these cellular movements are reversed in mutants with defects in asymmetric gene expression. In addition, we find that this event results in a repositioning of the original L/R axis to the dorsal–ventral (D/V) axis of the linear heart tube. Finally, we have discovered the existence of a rotation within the heart tube just prior to cardiac looping which converts the D/V axis back to the L/R axis. While the direction of this rotation is reversed in morphants with defects in asymmetric gene expression, the reestablishment of the original L/R axis occurs properly, regardless of Nodal signaling laterality. These results suggest a role for asymmetric gene expression in directing the first axis conversion during cardiac jogging but indicate that the second axis conversion at the initiation of cardiac looping may occur in a Nodal-independent manner.

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Program/Abstract # 458

3-*O*-sulfotransferase is required for cardiac development and physiology in zebrafish

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Heart development involves precise coordination of patterning events, cell movements and cell physiology in order to generate a functional heart. Cell surface and extracellular heparan sulfate proteoglycans (HSPGs) are core proteins with modified glycosaminoglycan (GAG) chains that are thought to mediate interactions between cells and their environments. We have cloned multiple 3-*O*-sulfotransferases (3-OSTs)1 presumed to add sulfate to the 3-position carbon of GAGs in HS, and are systematically assessing their roles in development. Morpholino knockdown of one of the 3-OST family members, 3-OST-7, results in a hypoplastic cardiac ventricle that does not contract properly, resulting in poor blood circulation and pericardial edema. What is the underlying mechanism for the observed cardiac ventricular defect? Primary heart field specification and patterning, and development of the vasculature and atrioventricular valve appear to be normal. In contrast, the outflow tract fails to form properly in morphant embryos. Moreover, action potential and intracellular calcium measurements indicate that ventricular contraction is uncoupled from excitation. Together these results indicate that 3-OST-7 has multiple roles in heart development, and that 3-OST function might provide a novel mechanism for the regulation of cardiac cell physiology. 1. Cadwallader AB, Yost HJ. Combinatorial expression patterns of heparan sulfate sulfotransferases in zebrafish: I. The 3-*O*-sulfotransferase family. Dev Dyn. 2006;235:3423-31.

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Program/Abstract # 459

Channel independent functions of L-type calcium channel beta-2 subunit

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Calcium channel beta-2 (CACNB2) subunits regulate voltage-gated channel electrophysiological dynamics and chaperon newly synthesized pore-forming alpha subunits to the plasma membrane. In addition to these canonical roles as calcium channel modulators, recent studies indicate that the beta subunits may have channel-independent functions that relate to their MAGUK (Membrane Associated Guanylate Kinase) protein structure. MAGUK family proteins perform a variety of scaffolding functions in the cell. We report the discovery of two CACNB2 genes in zebrafish. We find that the cardiac cells of CACNB2 morpholino-treated embryos dissociate more easily under pressure, and are reduced in number. To determine if cardiac myocyte morphology and cell adhesion is compromised in beta-2 morphants, we assayed cardiac cellular organization with several membrane markers including N-Cadherin.

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Program/Abstract # 460

Tbx5-mediated β2 CaMK-II expression is required for heart looping and pectoral fin development

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Mutations in the gene encoding Tbx5, result in Holt–Oram syndrome (HOS), which is characterized by defective cardiac development and stunted forelimbs. The genetic targets of Tbx5 responsible for proper heart and limb development are still being identified. In zebrafish embryos, the functional suppression of type II Ca^{b+}/calmodulin dependent protein kinase (CaMK-II) results in both aberrant cardiac looping and diminished pectoral fin development similar to tbx5 morphant and mutant (*heartstrings: hst*) embryos. Morphants of just one of the seven genes encoding catalytically active CaMK-II in early zebrafish embryos (β2 CaMK-II; *camk2b2*) exhibit the *hst* phenotype. *Camk2b2* mRNAs are transiently expressed in the heart and limb buds at the time of heart looping. Cardiac abnormalities in *camk2b2* and *tbx5* morphants can be reversed by overexpression of cytosolic CaMK-II. Normal fin development can be restored by CaMK-II in *camk2b2* morphants, but not in *tbx5* morphants. Both *tbx5* morphant and *hst* embryos exhibit diminished β2 CaMK-II, while the introduction of excess Tbx5 into zebrafish embryos and mouse fibroblasts increases β CaMK-II expression. Tbx5 also promotes transcription of a *camk2b2*-reporter, most likely through direct interaction with the evolutionarily conserved Tbx5 binding elements found in β CaMK-II genes. These findings indicate that Tbx5 induces β CaMK-II expression, which is necessary for normal cardiac and limb development.

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Program/Abstract # 461

Hedgehog signaling plays a cell-autonomous role in maximizing cardiac developmental potential

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