Signaling Pathways Controlling Second Heart Field Development

Francesca Rochais, Karim Mesbah, Robert G. Kelly

Abstract—Insight into the mechanisms underlying congenital heart defects and the use of stem cells for cardiac repair are major research goals in cardiovascular biology. In the early embryo, progenitor cells in pharyngeal mesoderm contribute to the rapid growth of the heart tube during looping morphogenesis. These progenitor cells constitute the second heart field (SHF) and were first identified in 2001. Direct or indirect perturbation of SHF addition to the heart results in congenital heart defects, including arterial pole alignment defects. Over the last 3 years, a number of studies have identified key intercellular signaling pathways that control the proliferation and deployment of SHF progenitor cells. Here, we review data concerning Wnt, fibroblast growth factor, bone morphogenetic protein, Hedgehog, and retinoic acid signaling that have begun to identify the ligand sources and responding cell types controlling SHF development. These studies have revealed the importance of signals from pharyngeal mesoderm itself, as well as critical inputs from adjacent pharyngeal epithelia and neural crest cells. Proliferation is emerging as a central checkpoint in the regulation of SHF development. Together, these studies contribute to defining the niche of cardiac progenitor cells in the early embryo, and we discuss the implications of these findings for the regulation of resident stem cell populations in the fetal and postnatal heart. Characterization of signals that maintain, expand, and regulate the differentiation of cardiac progenitor cells is essential for understanding both the etiology of congenital heart defects and the biomedical application of stem cell populations for cardiac repair. (Circ Res. 2009;104:933-942.)

Key Words: heart development ■ intercellular signaling ■ progenitor cells

Congenital heart defects occur in almost 1% of live births, reflecting the complex cellular processes underlying heart development.1 In the early embryo, the heart tube elongates by addition of myocardium from progenitor cells lying outside the heart itself. The source of the new myocardium is a recently identified progenitor cell population situated in pharyngeal mesoderm termed the second heart field (SHF).2–4 Direct or indirect perturbation of addition of SHF-derived cells to the heart tube results in congenital heart defects. Defining properties of the SHF include elevated proliferation and differentiation delay with respect to cells that contribute to the linear heart tube. In this review, we briefly introduce the SHF and then focus on recent data dissecting the intercellular signaling pathways that control SHF deployment. A central element in this process concerns the regulation of proliferation in pharyngeal mesoderm dorsal to the elongating heart tube. Detailed understanding of the signals that control SHF development in the early embryo will provide insights into the regulation of resident progenitor cells in the later heart and the directed differentiation of stem cells toward a cardiac fate.

Properties of the SHF

Three articles published in 2001 highlighted the previously known but poorly appreciated observation that the heart tube elongates by addition of cells to the arterial pole and furthermore identified the origin of these cells in mesoderm contiguous and dorsal to the heart tube.5–7 Gene expression, Cre lineage and retrospective clonal analysis studies revealed that these pharyngeal mesodermal progenitor cells, now termed the SHF, are situated medially to the primary or first heart field (FHF) that gives rise to the linear heart tube and are distinguished by the expression of genes encoding the transcription factors Isl1 and Tbx1 and the growth factors Fgf8 and Fgf10.5,8–11 The SHF contains multipotent cardiac progenitor cells that can give rise to myocardial, endocardial and smooth muscle cells12,13 and is characterized by elevated proliferation and differentiation delay with respect to the FHF.14 Whereas the FHF contributes to the left ventricle, right ventricle, and inflow region of the heart, the SHF contributes to the outflow tract (OFT), right ventricle, and inflow region; the left ventricle and OFT are therefore exclusive derivatives of the FHF and SHF, respectively.2,4
Despite these differences, the FHF and SHF are contiguous in the early embryo, and recent studies suggest that FHF cells transiently express genes that continue to be expressed in the SHF, including Isl1 and Fgf8. The term SHF is thus a working definition of those cardiac progenitor cells in pharyngeal mesoderm that contribute progressively to the growth of the heart after the linear heart tube stage, reflecting both the position of progenitor cells in the early embryo and that of their descendants in the definitive heart. Here, we focus on the contribution of SHF cells to the cardiac OFT. Maximal extension of the OFT is essential for correct alignment of the base of the aorta with the left ventricle during cardiac septation. Direct or indirect perturbation of SHF deployment therefore leads to a spectrum of conotruncal congenital heart defects, ranging from failure of heart tube extension to arterial pole alignment defects observed in human patients, including double outlet right ventricle, overriding aorta, and tetralogy of Fallot. Anomalies in OFT alignment to arterial pole alignment defects observed in congenital heart defects, ranging from failure of heart tube deployment therefore leads to a spectrum of conotruncal congenital heart defects, ranging from failure of heart tube extension to arterial pole alignment defects observed in human patients, including double outlet right ventricle, overriding aorta, and tetralogy of Fallot. Anomalies in OFT alignment to arterial pole alignment defects observed in congenital heart defects, ranging from failure of heart tube extension to arterial pole alignment defects observed in human patients, including double outlet right ventricle, overriding aorta, and tetralogy of Fallot.

Figure 1. The topography of the SHF. A and B, Lateral whole-mount view and transverse section of an embryo carrying an Fgf10 lacZ transgene at the linear heart tube stage (mouse embryonic day 8.5). SHF cells (blue) are located in pharyngeal mesoderm in the dorsal pericardial wall, adjacent to pharyngeal endoderm. C and D, Lateral whole-mount and transverse section after cardiac looping (mouse embryonic day 9.5). Fgf10 transgene expression is now evident in the OFT and dorsal pericardial wall (arrowheads). E and F, Neural crest cells, visualized with a Wnt1-Cre R26R transgene (E, blue) or anti-Tbx3 antibodies (F, green), invade the pharyngeal region and become positioned between SHF cells in the dorsal pericardial wall and pharyngeal endoderm, where they are closely apposed with Fgf10 transgene expressing mesodermal cells (F, red). E is reproduced from Jiang et al with permission. Scale bars: 100 μm (B, D, and E); 50 μm (F).

Wnt/β-Catenin Signaling Regulates SHF Proliferation
Control of cardiac progenitor cell proliferation is emerging as a central feature of SHF development. A series of recent articles have demonstrated a critical upstream role for β-catenin, intracellular mediator of the canonical Wnt pathway, in this process. Signaling by secreted Wnt glycoproteins plays a pivotal role in the regulation of diverse stem cell populations and has been previously shown to have stage-dependent positive and negative effects on early mesoderm development and cardiac specification and differentiation (reviewed elsewhere). Studies in embryonic stem cells have revealed a requirement for Wnt signals in early mesoderm development, yet, subsequently, Wnt signaling restricts cardiac differentiation to lateral splanchnic mesoderm. Active Wnt/β-catenin signaling is observed in the SHF and OFT. By deleting β-catenin specifically in cardiac mesoderm using a series of Cre lines it has now been shown that canonical Wnt signaling plays an important upstream role in positively regulating SHF proliferation, without affecting development of the linear heart tube.
regulated kinase (p-ERK), an intracellular target of FGF signaling, were observed in the SHF and OFT.30 Consistent with this upstream role in SHF development, activation of β-catenin by LiCl treatment or conditional expression of a gain-of-function mutation using the same Cre lines results in expansion of SHF progenitor cells and a greater number of Isl1-positive cells in the OFT, revealing that downregulation of Wnt/β-catenin signaling is required for differentiation (Figure 3C and 3D).29–34 Wnt3a has been shown to promote SHF proliferation in explants and Isl1-expressing cells in culture, yet the Wnt ligands required for normal SHF proliferation in the embryo remain unidentified.30,34 The expression profiles of Wnt2a, Wnt2b, and Wnt8 suggest that these genes are candidates for autocrine signaling during SHF and OFT development.23,25,29 It is important to note that β-catenin plays additional roles in cell–cell adhesion, complicating
interpretation of the precise functions of β-catenin in SHF development.

**Autocrine Fgf Signaling Drives SHF Proliferation and Deployment**

Fgf10, a target of Wnt signaling in the SHF, was the first identified endogenous marker of the SHF. However, embryos lacking Fgf10 do not have OFT alignment or septation defects despite abnormal ventricular morphogenesis and malpositioning of the heart in the thoracic cavity. Analysis of Fgf8 hypomorphic alleles and conditional ablation of Fgf8 using a series of Cre lines revealed that Fgf8 is likely to be the major Fgf ligand driving SHF development. Fgf8 is known to play an important role in specifying early cardiac mesoderm, and zebrafish fgf8 is expressed in ventricular myocardium and progenitor cells and is required for ventricle formation. Early loss of Fgf8 in cardiac mesoderm in the mouse results in survival and proliferative defects in the SHF and a failure to elongate the heart tube. The hearts of surviving mutant embryos display right ventricular and OFT hypoplasia and severe alignment defects. Ablation of Fgf8 in pharyngeal mesoderm at later stages results in failure of OFT alignment; decreased Fgf signaling in pharyngeal mesoderm and OFT myocardium was associated with reduced expression of Isl1, Bmp4, and Wnt11. These experiments suggested that mesodermal Fgf8 is required for SHF development and impacts on additional signaling pathways; Fgf8 in pharyngeal endoderm may also be required for OFT septation.

The major candidate gene for del22q11.2 (DiGeorge) syndrome, encoding the T-box transcriptional activator Tbx1, has been shown to regulate Fgf ligand expression in the caudal pharynx. del22q11.2 patients have craniofacial and cardiovascular defects including arterial pole alignment defects such as tetralogy of Fallot, and Tbx1-null mice have a short OFT at midgestation and a common ventricular outlet at later stages of development (Figure 3E through 3H; reviewed elsewhere). Conditional mutagenesis has shown that these defects are attributable to a requirement for Tbx1 in pharyngeal mesoderm during cardiac looping and that Tbx1 positively regulates proliferation in the SHF, likely through transcriptional regulation of Fgf10, Fgf8, and Fgfr1 and Fgfr2 in the Isl1 expression domain, or expressing a Sprouty2 gain-of-function transgene. Mesenchymal Fgf8 activity is required for normal heart looping and proliferation in the SHF, and ablation of Fgf8 in the pharynx results in looping defects and reduced expression of Bmp4, Isl1, and Sprouty2.

Two recent articles have now shown that the critical target cell type for Fgf signaling during OFT formation is the SHF itself. Conditional ablation of both Fgfr1 and Fgfr2 in the

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**Figure 3.** The impact of genetic and experimental modulation of signaling in the SHF on OFT development. A through D, Reduction of Isl1-positive cells in the OFT of a conditional β-catenin loss-of-function mutant (B) compared to control (A). Activation of Wnt/β-catenin signaling using LiCl results in increased OFT length (D) compared to control (C). Reproduced from Cohen et al with permission. E through F, Expression of an Fgf10 enhancer trap transgene in the OFT and right ventricle (E) highlights the short narrow OFT of Tbx1 mutant (F) embryos. Expression of the same transgene in mesoderm dorsal to the heart tube (G) reveals hypoplasia of the SHF in the absence of Tbx1 (H). Through L, In situ hybridization showing Bmp4 transcripts in the wild-type SHF and OFT (I) compared to embryos lacking Fgf8 (J) or Fgfr1 and Fgfr2 (K) in the Isl1 expression domain, or expressing a Sprouty2 gain-of-function transgene (L). Reproduced from Park et al with permission. M through P, Neural crest ablation results in OFT shortening and abnormal heart looping (N) compared to a sham-operated embryo (M). Treatment with anti-Fgf8 antibody also results in looping defects (O); heart looping appears normal after neural crest ablation and anti-Fgf8 treatment (P). Reproduced from Hutson et al with permission. Q through T, Fgf10 enhancer trap expression in the SHF at the linear heart tube stage (Q and S) is extended abnormally in a posterior direction in Raldh2 mutant embryos (R and T). Reproduced from Ryckebusch et al with permission (© 2008 National Academy of Sciences U.S.A.). Scale bars: 125 μm (A and B); 250 μm (C and D).
SHF, but not in endothelial or neural crest cells, results in a reduction in proliferation within the SHF (Figure 3I through 3L). Similar observations were made using a gain-of-function Sprouty2 transgene, a known feedback inhibitor of Fgf signaling, and in mice lacking factor receptor substrate (FRS)2α, an adaptor protein mediating Fgf receptor function, in the SHF. This primary defect of the SHF results in a shorter OFT and is compounded by subsequent failure of normal myocardial differentiation and neural crest cell development, leading to defects in cushion morphogenesis in addition to alignment defects.46,49 Because loss of Fgf signaling in neural crest and endothelial cells does not affect OFT development, secondary loss of signals from the SHF and SHF-derived myocardium are likely to contribute to this progressive impairment of OFT development. Bmp4, Wnt11, and Sema3c were among the genes observed to be downregulated in conditional Fgfr1/Fgfr2 mutant embryos, and vascular endothelial growth factor A was found to be upregulated in the OFT of FRS2α conditional mutant embryos. Successive steps of SHF and OFT development are therefore controlled by a signaling cascade downstream of autocrine Fgf signaling. In Drosophila, the Fgf receptor Heartless is required for heart formation and controls directed migration of mesodermal cells inside the ectoderm to the dorsal position, where Bmp signaling activates the cardiomyogenic program.50 Whether Fgf signaling plays a role in progenitor cell migration during SHF development remains to be seen.

Bmp Signaling and the Switch From Progenitor Expansion to Differentiation

In 2001, Mjaatvedt et al showed that myocardial differentiation in SHF explants could be induced by coculture with right but not left ventricular myocardial explants, suggesting a recruitment model for SHF deployment.6 Bmp signaling was known to be required for cardiac mesoderm specification and differentiation,51–53 and Waldo et al demonstrated that blocking Bmp signaling inhibited SHF differentiation, identifying Bmp2 as a candidate molecule for recruiting SHF cells to the arterial pole of the heart tube.7 These experiments led to the early conclusion that SHF development involves Bmp driven differentiation at the distal end of the heart tube and Fgf driven proliferation in pharyngeal mesoderm. Consistent with such a model, Bmp4 overexpression promotes a cardiac cell fate in cranial mesoderm.54 Mouse genetics has shown that Bmp4, a target of Wnt/β-catenin and Fgf signaling,33,48 is required for OFT septation, including smooth muscle and endocardial cushion development.55 OFT elongation is reduced in embryos lacking both Bmp4 and Bmp7,55 suggesting complexity of Bmp ligand involvement in SHF development.

Bmp signal reception is required in the SHF and OFT, as shown by conditional loss of the Bmp type 1 receptor Bmpr1a in Isl1-expressing cells, resulting in abnormal right ventricular and OFT morphology and septation defects.56 Proliferative defects in ventricular myocardium were associated with a decrease in expression of the T-box gene Tbx20 and an increase in Isl1 expression in the OFT.56 These results are consistent with a model by which Bmp signaling downregulates Isl1 expression as SHF-derived cells enter the OFT. Earlier deletion of Bmpr1a in cardiac mesoderm reveals that Bmp signaling is essential for differentiation of the linear heart tube, but is not required for SHF specification.31 In addition to promoting differentiation, Bmp ligands negatively impact progenitor cell proliferation. Increase in Bmp2 expression in embryos lacking the homeobox gene Nkx2.5 leads to cardiac overspecification and decreased proliferation of SHF cells.55 The intracellular signaling molecule Smad1 mediates the effect of Bmp2 on proliferation because elevated proliferation in the SHF and increased OFT length are observed in embryos lacking Smad1 in cardiac mesoderm; furthermore, loss of Smad1 alleles alleviates the Nkx2.5 phenotype.15 By negatively regulating Bmp2, Nkx2.5 may therefore act to brake cardiac specification in the SHF during normal heart tube elongation. Importantly, Bmp signaling is required for Nkx2.5 expression, revealing a negative feedback loop controlling the balance between proliferative modes during progenitor expansion and differentiation.

Hh Signaling From Pharyngeal Endoderm Controls SHF Survival and Deployment

In addition to Wnt, Fgf, and Bmp signaling, Hh signaling also impacts on SHF development. Loss of the Hh receptor Smoothened results in severe defects in heart tube formation that appear to reflect compound roles of the ligands Shh and Ihh during early heart development, including the regulation of Nkx2.5 expression.58 Shh mutant embryos display defects in SHF deployment and survival; defective right ventricle and OFT development lead to alignment and septation defects, resulting in a common ventricular outlet.59 Conditional deletion of Shh has revealed that pharyngeal endoderm is the source of ligand required for SHF and OFT development; extensive cell death is observed in the SHF of conditional mutant embryos, resulting in defective OFT elongation.60 Intriguingly, Shh expression is regulated by Isl1 in pharyngeal endoderm.45 Conditional deletion of the Smoothened receptor in different pharyngeal cell types has revealed complex roles of Shh in controlling arterial pole development.45,60 Hh signal reception is required in neural crest cells for survival in the pharyngeal region, in the SHF for later OFT septation, and in pharyngeal endoderm itself to regulate a secondary signal that controls SHF survival and deployment.60 What this signal is and how Hh impacts on later OFT septation through an early role in the SHF remain unknown. Bmp4 and Fgf8 expression are not affected, although loss of Smoothened in the Isl1 expression domain perturbs expression of Neurophilin2, suggesting a potential role for Semaphorin signaling, known to be required for neural crest influx and OFT septation.45,61 These experiments demonstrate that the Shh phenotype is a composite of multiple roles of Hh signaling in different cell types, each leading to failure to separte the OFT, making the important point that different upstream etiologies can converge on common end phenotypes. Gain- and loss-of-function experiments in zebrafish embryos have revealed an early requirement for Hh signaling in controlling myocardial progenitor cell numbers through a cell autonomous role in promoting progenitor cell specification.62 This role appears to be distinct from that in the SHF. A recent study has demonstrated that Shh signaling in pharyngeal mesoderm is required not only for OFT development but...
also for atrioventricular septation.63 Isl1 expression analysis and Cre lineage experiments have shown that the dorsal mesenchymal protrusion, giving rise to part of the atrial septum extending toward the atrioventricular cushions, derives from the SHF.63,64 Furthermore, Smoothened activity is required in pharyngeal mesoderm but not differentiated endocardium or myocardium for normal atrioventricular septation.65 By implicating the SHF in development of this region of the heart, this study provides a new paradigm for understanding the etiology of atrioventricular septal defects.

**Neural Crest Cells Control SHF Development by Modulating Intercellular Signaling**

SHF development is intricately linked with that of the cardiac neural crest. Cardiac neural crest cells have long been known to be critical for OFT morphogenesis and septation.65,66 These cells, derived from the dorsal neural tube, migrate through the caudal pharyngeal arches into the distal OFT, where they drive OFT septation and contribute to valve and smooth muscle tissue. As neural crest cells move through the pharyngeal region, they are closely apposed to cells of the SHF (Figure 1D through 1F). Neural crest cells do not give rise to the myocardial walls of the OFT in birds and mammals, although they have been reported to give rise to myocardium in the developing zebrafish heart.67,68

The conditional mutagenesis experiments described above have shown that Shh signaling impacts independently on SHF and neural crest development during OFT morphogenesis and may thus coordinate the contribution of these 2 cell types to the heart tube.60 However, deployment of these 2 cell types is also interdependent. An elegant series of studies in the chick have shown that, before arriving in the heart tube, cardiac neural crest cells play a critical role in controlling addition of SHF cells to the heart tube.69–72 Consequent reduction in OFT elongation leads to overriding aorta and double outlet right ventricle and is associated with impaired ventricular function and disrupted calcium handling. In neural crest ablated embryos, failure to divide the OFT (resulting in septation defects) is therefore compounded with failure of SHF development (resulting in alignment defects). Interestingly, addition of the terminal part of the SHF, which contributes to smooth muscle at the base of the great arteries, is not compromised by neural crest ablation.72,73

Cardiac neural crest ablation results in overproliferation of SHF cells, perturbing the balance between proliferation and differentiation and resulting in abnormal myocardial bulges at the junction between the myocardium and aortic sac.72 Overproliferation appears to be a result of excessive Fgf8 signaling in the caudal pharynx, including elevated expression of Fgf target genes and Fgf8 itself, that is normally buffered by the presence of neural crest cells.74 Pharmacological inhibition of Fgf signaling (or treatment with anti-Fgf8 antibodies) after neural crest ablation leads to a recovery of this phenotype in a time and dose-dependent manner and rescue of the alignment (but not septation) defects associated with neural crest ablation (Figure 3M through 3P).74 Inhibiting Fgf signaling in the absence of neural crest ablation causes underproliferation of SHF cells, consistent with the mouse experiments discussed above, and results in a shortened OFT.74 Precise levels of Fgf signaling are therefore critical for normal OFT development. The phenotypes observed after Fgf inhibition in the chick are similar to those observed after laser ablation of the SHF and include alignment defects such as overriding aorta and pulmonary atresia.74,75

The neural crest–mediated brake in SHF proliferation appears essential for distal OFT morphogenesis. Dialogue between these 2 cell types, derived from different regions of the embryo, yet moving together through the pharyngeal region to contribute to the heart, is thus critical in coordinating arterial pole development. However, how neural crest cells buffer the effect of Fgf signaling on the SHF is currently unclear, and the identification of signals exchanged between these 2 cell types is a major research goal. A complex and dynamic series of signal exchanges is likely. As noted above, Fgf signal reception by Fgrf1 and Fgrf2 is not required in neural crest cells for OFT morphogenesis,78 although elevated neural crest cell death in Fgf8 hypomorphic mutant embryos suggests an indirect role for Fgf signaling in neural crest survival in the pharyngeal region.36,37 Recent data have shown that cardiac neural crest development is critically dependent on neural crest autonomous ERK signaling.76 Conditional deletion of the Bmpr1a receptor in neural crest cells also leads to a shortened OFT, suggesting that a Bmp-regulated gene participates in the dialogue with SHF cells.77 In support of this hypothesis, deletion of Smad4 in neural crest cells leads to abnormal SHF development and a shorter OFT.78 Loss of Tbx3, expressed in neural crest cells and pharyngeal endoderm, results in elevated proliferation in the SHF and defective OFT elongation and alignment; Tbx3 target genes in neural crest cells may thus participate in signaling to the SHF.79

**Noncanonical Wnt Signaling in OFT Development**

An alternative Wnt signaling pathway operates not through β-catenin but JNK and protein kinase C to effect intracellular cytoskeletal changes associated with the establishment of planar cell polarity.23 Wnt11 is required for cardiac development in Xenopus through a noncanonical Wnt pathway.80 The phenotypes of mouse mutants lacking the noncanonical Wnt ligands Wnt5a and Wnt11 have recently been described and reveal a role for this pathway in OFT development.81,82 Previous analysis of Vangl2 mutant mice had implicated planar cell polarity in later stages of OFT development, in particular in myocardialization of OFT cushions during septation.83 Wnt5a is expressed in the SHF and mutant embryos have a common ventricular outlet and abnormal neural crest invasion, although the expression domains of SHF genes Fgf8, Fgf10 and Tbx1 are unaltered, suggesting a role for noncanonical Wnt signaling from the SHF to neural crest cells.81 In agreement with such a hypothesis, the Fr2 receptor is transiently expressed in cardiac neural crest cells.84 Wnt11 expression in the OFT is regulated by Wnt/β-catenin and Fgf signaling pathways, consistent with hierarchies of signaling pathways controlling OFT development.16,32 Wnt11 is also a direct transcriptional target of the transcription factor Ptx2, a gene that transmits embryonic laterality signals to the developing heart tube. Failure to correctly establish laterality through
Nodal signaling in the early embryo results in complex arterial pole defects, and loss of Ptx2 in the SHF and OFT leads to arterial pole alignment defects and transposition of the great arteries. Wnt11 mutant embryos have a short OFT and develop alignment and septation defects likely mediated by loss of transforming growth factor β2 signaling, known to be a critical regulator of neural crest and endocardial cushion development during OFT septation. It remains to be seen whether noncanonical Wnt signaling plays earlier roles in SHF development.

Retinoic Acid Signaling Defines the Posterior Limit of the SHF

The above pathways predominantly affect survival, proliferation, and deployment of SHF progenitor cells, in addition to subsequent differentiation of OFT myocardium and OFT remodeling. Less is known about the signals that define the limits of the SHF in pharyngeal mesoderm. Recent evidence suggests that retinoic acid signaling may participate in this process. Excess or reduction of retinoic acid is known to result in cardiac defects; for example, altered retinoic acid levels in avian embryos support a role for retinoic acid signaling in development of the venous pole of the heart. Analysis of mouse embryos lacking the retinaldehyde dehydrogenase gene Raldh2 reveals that a number of SHF genes are abnormally expanded in a posterior direction, including Isl1, Tbx1, Fgf10, and Fgf8 (Figure 3Q through 3T). Similar effects on Tbx1 expression are observed in vitamin A–deficient avian embryos and addition of retinoic acid soaked beads locally downregulates Tbx1 expression. Early derepression of Fgf signaling in the posterior domain of Raldh2 mutant embryos may contribute to the upregulation of other SHF markers. Explant analysis has shown that despite expanded expression of SHF genes, cardiac differentiation is blocked and the heart tube of Raldh2 mutant embryos fails to extend. Retinoic acid signaling is in turn controlled by signals from the SHF: in Tbx1 mutant embryos, the Raldh2 expression domain is shifted anteriorly and retinoic acid catabolizing enzymes are downregulated. In the zebrafish, retinoic acid has been shown to regulate the size of the cardiac field, intriguingly, recent evidence suggests that this effect is mediated indirectly by adjacent cell types and that Hox expression in the forelimb field may control signals restricting atrial progenitor cell numbers.

A Potential Role for Notch Signaling in Controlling Differentiation Delay in the SHF

Proliferation and differentiation delay are the defining properties of the SHF yet little is known about how this second attribute is controlled. Notch signaling regulates diverse progenitor cell populations in the embryo and plays multiple roles in heart development (reviewed elsewhere) yet, to date, has not been implicated directly in SHF development. However, mutant embryos in which the canonical Notch pathway is inactive develop up to midgestation suggesting that this signaling pathway is not required for initial heart tube formation. In Xenopus embryos, Notch signaling represses cardiomyocyte differentiation in the dorsal pericardial wall, functioning downstream of Nkx2.5 activation. The Notch ligand Serrate is expressed in this region, whereas the Notch1 receptor is expressed throughout the heart; active Notch signaling is thus restricted to the dorsal pericardial wall. Recent evidence that Xenopus Isl1 is expressed in the dorsal pericardial wall supports the possibility that this is an analogous structural to the SHF and may contribute to the frog heart after metamorphosis. Whether Notch signaling plays a role in SHF development is an area of active research.

Biomedical Perspectives: Isl1 and Resident Cardiac Progenitor Cells

Understanding the signals that control development of the SHF in the early embryo can provide insight into the pathways that maintain and direct the differentiation of cardiac progenitor cells in the later heart. A small number of Isl1-positive cells are present in the fetal and early postnatal heart and have been proposed to represent residual SHF cells that identify a resident progenitor cell population potentially contributing to growth of the heart. Is1 plays a central role in the SHF transcriptional hierarchy, regulating multiple signaling pathways required for SHF survival, proliferation, and differentiation, including Fgf, Bmp, and Shh ligand expression. Is1 expression is also a target of different signaling pathways revealing feedback regulation in the control of SHF progenitor cells. Lineage tracing studies and purification of Isl1-positive progenitor cells from the early embryo or later heart have shown their self-renewal capacities and ability to differentiate into fully mature cardiac myocytes, endothelial cells and smooth muscle cells. In addition to Isl1-positive cells, other resident progenitor cells, extracardiac stem cells, and pluripotent embryonic stem cells are the focus of intense research efforts to harness cardiac progenitor cells for myocardial repair. The signals regulating progenitor cell development in the early embryo may also regulate Isl1 and other progenitor cell populations in the later heart and can potentially be used to direct embryonic stem and induced pluripotent stem cells toward a cardiac fate. For example, Wnt/β-catenin signaling stimulates proliferation of purified Isl1-positive cells, and a recent report in zebrafish shows that Wnt signaling is upregulated during cardiac regeneration, highlighting the potential importance of this signaling pathway for cardiac repair. Similarly, Notch signaling appears to block differentiation and allow expansion of activated cardiac precursor cells in failing hearts. These and other studies will contribute to optimizing techniques to maintain, expand, and trigger differentiation of cardiac progenitor cells for therapeutic applications.

A number of new questions and perspectives concerning the etiology of congenital heart defects emerge from these studies. We have seen that SHF development depends on a complex cascade of upstream signals (Figure 4). A current challenge is to understand how these multiple signal inputs are integrated as the topography of progenitor cells changes during OFT extension. Cross-regulation between different pathways, as seen above, is a recurrent theme, and common intracellular signaling intermediates suggest that activation of one pathway may enhance or block responsiveness to another pathway. Inter cellular signaling pathways provide a potential link between cardiac function and form during heart devel-
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**Conclusions**

Multiple signaling pathways converge to regulate addition of SHF cells to the poles of the heart tube. The signaling molecules identified to date constitute part of the signaling environment or niche that maintains SHF cells in a progenitor cell state and controls their progressive differentiation. Many of the signaling pathways converge on the regulation of SHF proliferation and myocardial differentiation; the control of differentiation delay and SHF movement into the OFT remain more obscure. Continued elucidation of the gene regulatory networks and signaling pathways that control SHF development will undoubtedly identify mechanisms underlying these processes and define the extent to which they differ from those controlling differentiation of the linear heart tube. Insights gained into the etiology of congenital heart defects through the modulation of intercellular signaling during heart tube extension.

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**Disclosures**

None.

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