Introduction
Solving the mysteries of how stem and progenitor cells adopt specific cell fates and subsequently assemble into functional organs remains a fundamental challenge in biology. Research in the last decade has revealed that intricate signaling, transcriptional, and translational networks regulating key cellular decisions are at the heart of developmental biology. Insights into cell-fate specification, differentiation, and organogenesis also provide hope for the development of new therapies for human disease, particularly stem cell therapies.

The heart is among the most studied of all organs and the one most susceptible to disease. A molecular framework has emerged to explain the commitment of unique lineages to distinct regions of the heart, and the underlying decisions of cell migration, proliferation, and death that guide the three-dimensional events of cardiac development (Buckingham et al., 2005). The complexity of the molecular cascades in cardiogenesis may explain the sensitivity of the heart to perturbations before birth and into old age.

Congenital heart malformations, the most common of human birth defects, occur in nearly 1% of the population worldwide (Hoffman and Kaplan, 2002). Another 1%–2% harbor more subtle cardiac developmental anomalies that become apparent with age. With more than 1 million survivors of congenital heart disease in the United States, it is becoming clear that genetic disruptions that predispose an individual to developmental defects can have ongoing consequences over decades (Srivastava, 2004). A more precise understanding of the causes of congenital heart disease is imperative for recognition and potential intervention of progressive degenerative conditions that plague congenital heart disease survivors.

In the United States, heart disease is the number one killer of adult men and women. Another 5 million survive with insufficient cardiac function (Thom et al., 2006). Deciphering nature's secrets of heart formation might lead to new approaches to repair or regenerate damaged heart muscle. Recent evidence has led to heightened interest in the early events involved in cardiac cell-fate decisions and cardiomyocyte differentiation, migration, and survival. Stem cells have enormous potential in regenerative medicine, and insights into cardiogenesis from progenitor cells during embryogenesis will form the basis of reprogramming cells for therapeutic use (Srivastava and Ivey, 2006).

This review summarizes the conceptual advances in cardiac morphogenesis in the context of congenital heart disease and adult onset heart disease. In addition, these advances in our understanding of developmental and stem cell biology of the heart could be applied to cardiac regenerative medicine.

Origin of Cardiomyocyte Precursors
Despite decades of tracing cell lineages and descriptive embryology of the heart’s origins, a more complete and accurate picture of cardiogenesis has only recently emerged (reviewed in Buckingham et al., 2005). Two distinct mesodermal heart fields with a common origin appear to contribute cells to the developing heart in a temporally and spatially specific manner. The well-studied “primary” or “first” heart field (FHF) derives from cells in the anterior lateral plate mesoderm that form a crescent shape at approximately embryonic (E) day 7.5 in the mouse embryo, corresponding roughly to week 2 of human gestation (Figure 1). By mouse E8.0, or 3 weeks in humans, these cells coalesce along the ventral midline to form a primitive heart tube, consisting of an interior layer of endocardial cells and an exterior layer of myocardial cells, separated by extracellular matrix for reciprocal signaling between the two layers.
Previous lineage tracings using dye-labeling techniques suggested that cells along the anterior-posterior axis of the heart tube would contribute to specific chambers of the future heart (reviewed in Srivastava and Olson, 2000). However, such studies could not determine the clonal contributions of individual cells (Meilhac et al., 2004). More recent studies using Cre-lox lineage analysis and retrospective clonal lineage analysis indicate that the heart tube derived from the FHF may predominantly provide a scaffold upon which cells from a second heart field (SHF) migrate and build the requisite cardiac chambers (Buckingham et al., 2005). The SHF is marked by the LIM-homeodomain transcription factor Islet1 (Isl1) and comprises the dorsal-medial aspect of the cardiogenic plate, whereas the FHF comprises the ventral aspect of the cardiogenic plate (Cai et al., 2003) (Figure 1). The FHF differentiates at the cardiac crescent stage, whereas differentiation of the SHF is relatively delayed, occurring as the second lineage migrates in to join the differentiated cells of the first lineage. Both lineages appear to be regulated by complex positive and negative signaling networks involving members of the bone morphogenetic protein (Bmp), sonic hedgehog (Shh), fibroblast growth factor (Fgf), Wnt, and Notch proteins. Such signals often arise from adjacent endoderm, although their precise nature and role remain unknown (reviewed in Zaffran and Frasch, 2002). SHF cells may remain in an undifferentiated progenitor state until incorporation into the heart because they are close to inhibitory Wnt signals that emanate from the midline.

As the heart tube forms, SHF cells migrate into the midline and position themselves dorsal to the heart tube in the pharyngeal mesoderm (Figure 1). Upon rightward looping of the heart tube, SHF cells cross the pharyngeal mesoderm into the anterior and posterior portions, populating a large portion of the outflow tract, future right ventricle, and atria (Cai et al., 2003). Precursors of the left ventricle are sparsely populated by the SHF and appear to largely be derived from the FHF. Once within the heart, FHF and SHF cells appear to proliferate in response to endocardial-derived signals, such as neuregulin, and epicardial signals dependent on retinoic acid, although these mechanisms remain poorly understood (reviewed in Olson, 2004). The number of cardiac myocytes during development may also be regulated by the homeodomain-only protein, Hop, which functions downstream of the Nkx2-5 (Shin et al., 2002; Chen et al., 2002).

**Transcriptional Regulation of Cardiac Precursors**

FHF and SHF regulation involves numerous signaling and transcriptional cascades (Figure 2). Factors secreted from the anterior portion of the heart tube may serve as chemoattractant signals that induce migration of SHF...
cells, although the nature of such molecules remains unknown. Expression of Isl1 is extolished as progenitor cells express markers of cardiac differentiation, but Isl1 is necessary for SHF-derived cells to populate the heart (Cai et al., 2003). How Isl1 regulates SHF progenitor cells is being explored. Interestingly, Isl1-positive cells mark niches of undifferentiated cardiac progenitor cells in the postnatal heart (Laugwitz et al., 2005), suggesting that understanding the regulation of SHF-derived progenitor pools may be useful in developing approaches for cardiac repair (discussed below).

Discovery of the SHF led to the reinterpretation of findings in mice lacking critical regulatory proteins and in transgenic mice harboring enhancers of genes expressed in the heart. As molecular aspects of cardiogenesis were first being defined a decade ago, right ventricle-specific enhancers were found for several genes widely expressed in the developing heart (reviewed in Firulli and Olson, 1997). Subsequently, the right ventricle was found to be enriched in a transcription factor, Hand2 (also known as dHAND), required for expansion of the right ventricle (Srivastava et al., 1995, 1997). Given that Hand2 is highly expressed in the SHF, the right ventricular hypoplasia observed on disruption of Hand2 in mice likely represents failure of SHF cells to expand into the right ventricle (Srivastava et al., 1997; Yamagishi et al., 2001). Similarly, right ventricular hypoplasia in mice lacking Mef2c (Lin et al., 1997), a target of Isl1, Gata4, Foxh1, and Tbx20 in the SHF (Dodou et al., 2004; von Both et al., 2004), may also be a defect of SHF development. Indeed, many of the central transcriptional regulators of cardiac development in the FHF, including Nkx2-5 and Gata4, are found in the SHF and may target SHF development (Zeisberg et al., 2005) (Figure 2). Pathways regulating differentiation of SHF cells may provide the basis to induce cardiac cells from progenitor cells.

The significance of SHF transcriptional regulation is highlighted by the cardiac defects in DiGeorge syndrome, the most common human deletion syndrome. In this syndrome, which typically involves a deletion on chromosome 22q11, the transcription factor TBX1 is the cause of cardiac and craniofacial disorders. TBX1, a central SHF transcriptional regulator, is necessary for proper development of cardiac outflow tract myocardium (Hu et al., 2004; Xu et al., 2004). Shh is required to maintain Tbx1 expression in the SHF through forkhead-containing transcription factors that directly regulate Tbx1 (Garg et al., 2001; Yamagishi et al., 2003). Correspondingly, mice lacking Shh, Foxc1 and Foxc2, and Tbx1 share similar defects of the cardiac outflow tract (Kume et al., 2001; Yamagishi et al., 2003). Tbx1 regulates outflow tract myocardium and production of growth factors, such as fibroblast growth factor 8 (Fgf8), which are secreted and activate receptors on adjacent neural crest-derived cells to affect their differentiation (Abu-Issa et al., 2002; Frank et al., 2002; Hu et al., 2004).

Hand2 and the closely related Hand1 are expressed in the SHF; however, they are also expressed in the FHF, with Hand1 most enriched in the left ventricle. Hand1 expression depends on Nkx2-5 in the left ventricle, suggesting that Nkx2-5 is critical for the FHF as well (Biben and Harvey, 1997). Although disruption of tinman, the Nkx2-5 ortholog in flies, results in complete loss of cardiac cells (Bodmer, 1993), deletion of Nkx2-5 in mice is less severe with lethality at E9.5 after initial formation of the heart tube (Lyons et al., 1995; Tanaka et al., 1999). However, loss of Nkx2-5 and Hand2 causes complete failure of ventricular expansion in mice (Yamagishi et al., 2001). Conditionally disrupting Hand1 and Hand2 causes slightly less severe defects (McFadden et al., 2005). Zebrafish and fruitflies lacking the single Hand ortholog fail to expand the pool of comparable ventricular precursors, consistent with mouse studies (Yelon et al., 2000; Han et al., 2006).

Preservation of atrial precursors in mouse and fish Hand mutants suggested that a separate progenitor population may contribute to the atria. Indeed, mice lacking the nuclear receptor CoupTFII lack atrial myocytes (Pereira et al., 1999), as do mice lacking Tbx5 (Bruneau et al., 2001). Distinct aspects of atrial versus ventricular gene expression appear to be regulated by Irx4 of the Iroquois family of transcription factors (Bao et al., 1999).

Figure 2. Pathways Regulating Region-Specific Cardiac Morphogenesis
A partial list of transcription factors, signaling proteins, and miRNAs that can be placed in pathways that influence the formation of regions of the heart is shown. Positive influences are indicated by arrowheads, and negative effects by bars. Physical interactions are indicated by dashed lines between factors. In some cases relationships of proteins are unknown. Pathways regulating neural crest cells have been reviewed elsewhere (Stoller and Epstein, 2005). FHF, first heart field; SHF, second heart field.
Epigenetic factors may also contribute to cardiomyocyte differentiation and chamber morphogenesis. Disruption of the chromatin remodeling protein Smyd1 (or Bop) results in a phenotype reminiscent of Hand2 mutants: a small right ventricular segment and poor development of the left ventricular myocardium (Gottlieb et al., 2002). Smyd1 contains a SET domain that harbors methyltransferase activity and also recruits histone deacetylase (HDAC) activity that together repress genes (Gottlieb et al., 2002). Smyd1 activity is necessary for Hand2 expression in cardiac precursors, likely through an unknown intermediate. Interestingly, Smyd1 is a direct target of Mef2c (Phan et al., 2005), suggesting that Isl1, Mef2c, Smyd1, and Hand coordinate regulation of development of ventricular cardiomyocytes (Figure 2). A direct role for HDACs was also demonstrated by a failure of ventricular growth in mutant mice lacking HDAC5 and HDAC9 (Chang et al., 2004b). These and other pathways appear to be regulated by a muscle-specific member of the SWI/SNF complex, Baf60c (Lickert et al., 2004), suggesting that transcriptional activity of cardiac DNA binding proteins is highly regulated through epigenetic events.

MicroRNA Regulation of Cardiomyocyte Differentiation

Although transcriptional and epigenetic events regulate many critical cardiac genes, translational control by small noncoding RNAs, such as microRNAs (miRNAs), has recently emerged as another mechanism to “fine-tune” dosages of key proteins during cardiogenesis (Zhao et al., 2005; Kwon et al., 2005). miRNAs are genomically encoded 20–22 nucleotide RNAs that target mRNAs for translational inhibition or degradation by many of the same pathways as small interfering (si)RNAs (He and Hannon, 2004; Ambros, 2004). The function and mRNA targets are known for few of over 400 identified human miRNAs.

miR-1-1 and miR-1-2 are highly conserved and specifically expressed in the developing cardiac and skeletal muscle progenitor cells (Zhao et al., 2005). miR-1-1 is initially enriched in the atrial precursors before becoming ubiquitous in the heart; miR-1-2 is specific for the ventricle, suggesting chamber-specific effects. Interestingly, their expression is controlled by well-studied transcriptional regulatory networks that promote muscle differentiation (Figure 3). Cardiac expression depends on serum response factor (SRF), and skeletal muscle expression requires the myogenic transcription factors MyoD and Mef2. SRF recruits a potent coactivator, myocardin, to cardiac and smooth muscle-specific genes that control differentiation (Wang et al., 2001). Consistent with a role in differentiation, overexpression of miR-1 in the developing mouse heart decreases ventricular myocyte expansion, with fewer proliferating cardiomyocytes remaining in the cell cycle. miR-1 and miR-133a are transcribed in a polycistronic message and coexpressed in heart and skeletal muscle, sharing common transcriptional regulation (Figure 3). As in cardiac muscle, miR-1 promotes the differentiation of skeletal myoblasts in culture, but interestingly, miR-133a inhibits differentiation and promotes proliferation of myoblasts (Chen et al., 2006).

A full understanding of how miRNAs regulate biological events has been limited by the inability to reliably identify mRNA targets. This inability reflects the limited degree of sequence matching necessary for target recognition, typically at nucleotides 2–7 in the 5’ end of the miRNA. In silico searches based on sequence matching often yield several hundred potential targets, making validation difficult. miRNA targets identified through genetic screens in flies and worms are consistently located in accessible domains defined by RNA secondary structure features and free energy of binding (Zhao et al., 2005). Incorporation of these features into bioinformatic approaches significantly improves the specificity of target prediction and was used to identify Hand2 mRNA as a target.
of miR-1. This result suggests that precise regulation of Hand2 protein levels may be involved in controlling the balance between cardiomyocyte proliferation and differentiation (Figure 3). HDAC4, an inhibitor of Mei2, may also be a target of miR-1 in skeletal muscle, further promoting effects of Mei2 during differentiation (Chen et al., 2006).

The single fly ortholog of miR-1 is involved early in triggering cardiac progenitor cell differentiation and later in maintaining cardiac gene expression (Kwon et al., 2005). A milder miR-1 phenotypic also suggested a variable phenotype (Sokol and Ambros, 2005), although knocking down miR-1 activity by O-methyl-modified antisense oligonucleotides proved uniformly lethal (Leaman et al., 2005). miR-1 in flies regulates the Notch signaling pathway by directly targeting mRNA of the Notch ligand, Delta (Kwon et al., 2005), potentially explaining the involvement of miR-1 in deriving differentiated cardiac cells from an equivalent group of progenitor cells (Figure 3). Thus, miR-1 may be a muscle-specific miRNA that directs progenitor cells toward a cardiac cell fate by regulating mediators of differentiation.

**Complex Regulation of Cardiac Morphogenesis**

Although the contribution of cell lineages to heart development is reasonably well understood, subsequent events, such as the integration of multiple cell types, chamber formation, and the patterning of distinct regions of the heart, are being elucidated. Some aspects are described below and others have been reviewed elsewhere (Olson, 2004; Parmacek and Epstein, 2005). Notably, development of the cardiac electrical conduction system, derived from specialized cardiomyocytes, is not discussed here but is essential to normal cardiac function (reviewed in Mikawa et al., 2003). Other recent findings highlight the importance of integration between specialized conduction cells and the myocardium (Costantini et al., 2005).

A discrete dorsal-ventral (DV) polarity occurs in the primitive heart tube. As the heart tube loops to the right, the ventral surface of the tube rotates to become the outer curvature of the looped heart, and the dorsal surface forms the inner curvature. The outer curvature becomes the site of active growth, whereas remodeling of the inner curvature controls the alignment of inflow and outflow tracts of the heart. A model in which individual chambers “balloon” from the outer curvature in a segmental fashion has been proposed (Christoffels et al., 2004). Consistent with this model, numerous genes, including Hand1 and the sarcomeric protein Serca2, are expressed specifically on the outer curvature of the heart (Biben and Harvey, 1997; Thomas et al., 1998). Also, through a complex transcriptional network, the unique identity of inner curvature cells is determined by Tbx2-mediated repression of genes typically found on the outer curvature (Harrelson et al., 2004). Another Tbox transcription factor, Tbx20, represses Tbx2 activity in the outer curvature as it expands into the cardiac chambers to establish regional patterning of expanding or remodeling myocardium (Sternard et al., 2005; Takeuchi et al., 2005; Cai et al., 2005; Singh et al., 2005). Remodeling of the inner curvature allows migration of the inflow tract to the right and the outflow tract to the left, facilitating proper alignment and separation of right- and left-sided circulations. In addition to its role in repressing Tbx2, Tbx20 affects expansion of FHF- and SHF-derived cells and is necessary for outflow tract development, possibly by regulating Nkx2-5 and Me2f2c (Takeuchi et al., 2005).

Defects of inner curvature remodeling may underlie human congenital heart malformations that improperly align the atria, ventricles, and outflow tract. These include situations where both atrio-ventricular valves communicate with the left ventricle (double-inlet left ventricle), or when the aorta and pulmonary artery both exit from the right ventricle (double-outlet right ventricle), similar to those observed in many mutant mouse models (reviewed in Franco and Campione, 2003). Such defects are often observed in conjunction with abnormalities in the determination of left-right asymmetry. Thus, left-right decisions may affect the direction of cardiac looping and proper alignment of chambers, likely by regulating gene expression along the inner versus outer curvature and ventral versus dorsal surface of the developing heart.

Given that the elegant molecular network regulating left-right asymmetry of the body plan has been well reviewed (Palmer, 2004), we will note only the relationship between left-right asymmetry and proper alignment of the cardiac chambers. The cascade of left-right signals, including Shh and Nodal, converge on the transcription factor Pitx2. Pitx2 is initially expressed asymmetrically along the left-right axis in the linear heart tube, but this asymmetry translates into a dorsal-ventral polarity in the looped heart tube. Because Pitx2 regulates cell proliferation via cyclin D2 and also controls cell migration (Kioussi et al., 2002), it may link signals regulating the direction and process of cardiac looping. Within certain subdomains, regulation of Pitx2 by Tbx1 integrates transcriptional pathways controlling morphogenesis and left-right asymmetry (Nowotschin et al., 2006).

Congenital cardiac defects involving the cardiac outflow tract, aortic arch, ductus arteriosus, and proximal pulmonary arteries account for 20%–30% of all congenital heart disease. This region of the heart undergoes extensive and complex morphogenetic changes with contributions from neural crest cells and the SHF. Mesenchyme cells originating from the crest of the neural folds are essential for proper septation and remodeling of the outflow tract and aortic arch (reviewed in Hutsin and Kirby, 2003). Such neural crest-derived cells migrate from the neural folds and retain the potential to differentiate into multiple cell types. The migratory path and ultimate fate of these cells depend on their relative
origins along the anterior-posterior axis and are partly regulated by the Hox genes (Le Douarin et al., 2004). Neural crest cells from the otic primordia to the third somite migrate through the developing pharyngeal arches to the mesenchyme of the aortic arch arteries and the mesenchyme that forms the outflow tract septum (Figure 1). This segment of the neural crest is often called the cardiac neural crest.

Mutations in many signaling cascades affect neural crest migration or development in mice, including the endothelin and semaphorin pathways, and cause outflow tract defects similar to those in humans (reviewed in Stoller and Epstein, 2005). Apoptosis of some neural crest-derived cells seems essential for proper morphogenesis, whereas others differentiate into vascular smooth muscle in distinct regions of the aortic arch. For example, human mutations of the neural crest-enriched transcription factor Tafap2β result in abnormal persistence of the ductus arteriosus, a specialized aortic arch vessel essential for fetal cardiac physiology, possibly reflecting abnormal smooth muscle differentiation (Satoda et al., 2000) (Figure 1). Other genetic mutations may affect specific regions of the aortic arch.

Disruption of SHF development by mutating genes, such as Tbx1 and Fgf8, results in defects similar to those with neural crest disruption (Figure 2), including persistent truncus arteriosus (failure of outflow tract septation), misalignment of the outflow tract of the heart with the ventricular chambers, and ventricular septal defects (reviewed in Baldini, 2005). Reciprocal interactions between the SHF and neural crest-derived cells in the outflow tract are likely essential because SHF-derived myocardial cells neighbor neural crest-derived cells and secrete in a Tbx1-dependent manner growth factors, such as Fgf8, that influence neural crest cells (Hu et al., 2004). It will be interesting to determine if cardiac outflow tract defects result from alterations in SHF migration, differentiation, or proliferation.

Appropriate positioning and function of cardiac valves is essential for chamber septation and unidirectional flow of blood through the heart. A molecular network involving Bmp2 and Tbx2 defines the position of the valves relative to the chambers (Harrelson et al., 2004; Ma et al., 2005). During early heart tube formation, “cushions” of extracellular matrix between the endocardium and myocardium contribute to valve formation, which occurs at each end of the heart tube. Reciprocal signaling, mediated in part by TGF-β family members, between the myocardium and endocardium in the cushion region induces a transformation of endocardial cells into mesenchymal cells that migrate into the extracellular matrix cushion (reviewed in Armstrong and Bischoff, 2004). These mesenchymal cells differentiate into the fibrous tissue of the valves and are involved in septation of the common atrioventricular canal into right- and left-sided orifices.

Smad proteins are intracellular transcriptional mediators of signaling initiated by TGF-β ligands. Smad6 is specifically expressed in the atrioventricular cushions and outflow tract during cardiogenesis and is a negative regulator of TGF-β signaling. Targeted disruption of Smad6 in mice results in thickened and gelatinous atrioventricular and semilunar valves, comparable to those in human aortic and pulmonary valve disease (Galvin et al., 2000). Similarly, the absence of Ptpn11, which encodes the protein tyrosine phosphatase Shp-2, results in dysplastic outflow valves through a pathway involving epidermal growth factor receptor (Chen et al., 2000). The importance of PTPN11 in congenital heart disease was shown by the discovery of point mutations in PTPN11 that result in activation of the Shp-2 phosphatase in patients with Noonan syndrome, who commonly have pulmonic valve stenosis (Tartaglia et al., 2001).

Disruption of signaling pathways involving the transcription factor Nfatc revealed a requirement of this calcium-activated regulator. Nfatc is expressed specifically in the valve mesenchyme precursors, and mice without Nfatc lack cardiac valve formation (de la Pompa et al., 1998; Ranger et al., 1998). Signaling via the phosphatase, calcineurin, results in nuclear translocation of Nfatc and is similarly involved in cardiac valve formation, in part by regulating vascular endothelial growth factor (Vegf) expression in the endocardium (Chang et al., 2004a). Interestingly, mutations of Ptpn11 that activate Shp-2 phosphatase increase calcium oscillations and disrupt Nfatc nuclear localization, providing a possible mechanism for the valve defects observed in Noonan syndrome (Uhlen et al., 2006).

The Notch signaling pathway is also implicated in cardiac valve development. In fish and frogs, Notch is required for development of the endocardial cushions that contribute to valve tissue (Timmerman et al., 2004). In humans, heterozygous NOTCH1 mutations disrupt normal development of the aortic valve and occasionally the mitral valve (Garg et al., 2005) (Table 1). The severity of valve disease associated with NOTCH1 mutations in humans varies widely from mild disease in which the aortic valve has two rather than three leaflets (bicuspid aortic valve) to severe defects in valve opening in utero, resulting in left ventricular growth failure. About 15% of “normal” relatives of children with hypoplasia of the left ventricle (hypoplastic left heart syndrome) have subclinical bicuspid aortic valves (Cripe et al., 2004), suggesting that disruption of the NOTCH signaling cascade may underlie a spectrum of aortic valve disease. Mutations in Jagged1, a Notch ligand, also cause outflow tract defects associated with the autosomal-dominant disease, Aalalille syndrome (reviewed in Krantz et al., 1999). The hairy-related family of transcriptional repressors (Hrt1, Hrt2, and Hrt3) may mediate the Notch signal during valve and myocardial development; however, their targets are unknown (Nakagawa et al., 1999; reviewed in Kokubo et al., 2005).
Table 1. Genetic Causes of Congenital Heart Disease

<table>
<thead>
<tr>
<th>Genetic Mutation</th>
<th>Syndrome Name</th>
<th>Cardiac Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNX2-5</td>
<td>—</td>
<td>Atrial septal defect, ventricular septal defect, electrical conduction defect</td>
</tr>
<tr>
<td>GATA4</td>
<td>—</td>
<td>Atrial septal defect, ventricular septal defect</td>
</tr>
<tr>
<td>MYH6</td>
<td>—</td>
<td>Atrial septal defect</td>
</tr>
<tr>
<td>NOTCH1</td>
<td>—</td>
<td>Aortic valve disease</td>
</tr>
<tr>
<td>NNX2-5</td>
<td>Holt-Oram</td>
<td>Atrial septal defect, ventricular septal defect, electrical conduction defect</td>
</tr>
<tr>
<td>TBX1</td>
<td>DiGeorge</td>
<td>Cardiac outflow tract defect</td>
</tr>
<tr>
<td>TFAP2b</td>
<td>Char</td>
<td>Patent ductus arteriosus</td>
</tr>
<tr>
<td>JAG1</td>
<td>Alagille</td>
<td>Pulmonary artery stenosis, tetralogy of Fallot</td>
</tr>
<tr>
<td>PTPN11</td>
<td>Noonan</td>
<td>Pulmonary valve stenosis</td>
</tr>
<tr>
<td>Elastin</td>
<td>William</td>
<td>Supravalvar aortic stenosis</td>
</tr>
<tr>
<td>Fibrillin</td>
<td>Marfan</td>
<td>Aortic aneurysm</td>
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Narrowing of vessels or valves is indicated as stenosis; patent ductus arteriosus represents persistence of a fetal vessel connecting the aorta and pulmonary artery that normally closes after birth; tetralogy of Fallot describes a misalignment of the aorta and pulmonary artery with the ventricular chambers such that the aorta communicates with both chambers.

Genetics of Human Septal Defects

Recent findings with the cardiac transcription factors NNX2-5, TBX5, and GATA4 exemplify the synergy between human genetics and studies of model organisms for understanding the etiology of congenital heart disease (Table 1). Numerous point mutations in NNX2-5 occur in families with atrial septal defects and progressive cardiac electrical conduction abnormalities (Schott et al., 1998). Retrospective analysis of mice heterozygous for Nkx2-5 disruption revealed a similar phenotype and progressive apoptotic loss of conduction cells, suggesting a likely mechanism for the human phenotype (Biben et al., 2000; Jay et al., 2004).

Humans with Holt-Oram syndrome, caused by mutations in TBX5, have cardiac anomalies similar to those with NNX2-5 mutations (atrial and ventricular septal defects) as well as limb abnormalities (Mori and Bruneau, 2004). Intriguingly, mutations causing defects in the heart and limbs are clustered in different regions of the protein, suggesting that TBX5 engages different downstream genes or cofactors, depending on unique structural motifs in the protein. One potential cofactor, NNX2-5, may cooperate to activate common target genes (Hiroi et al., 2001).

Mutations in the zinc-finger-containing protein GATA4 cause similar atrial and ventricular septal defects in autosomal-dominant nonsyndromic human pedigrees (Garg et al., 2003). GATA4 or related proteins are essential for cardiogenesis in flies, fish, and mice (reviewed in Epstein and Parmacek, 2005). Like NNX2-5, GATA4 and TBX5 also form a complex to regulate downstream genes, such as myosin heavy chain. Consistent with a role in combinatorial interactions, a familial GATA4 point mutation disrupts GATA4’s ability to interact with TBX5 (Garg et al., 2003). Conversely, several human TBX5 mutations disrupt the GATA4-TBX5 interaction, suggesting that they cooperate in cardiac septation events (Garg et al., 2003). GATA4, TBX5, and NNX2-5 may form a complex needed for proper cardiac septation (Figure 2). Disruption of any of the proteins or their interactions can result in atrial or ventricular septal defects. The septal genes regulated by these factors are unknown, but intriguingly, mutations in human α-myosin heavy chain (MYH6), a direct target of GATA4, TBX5, and NNX2-5, also cause atrial septal defects (Ching et al., 2005). This observation suggests a mechanism by which these genes cause septation defects.

Adult Consequences of Cardiac Malformations

As human survivors of cardiac malformations enter their third and fourth decades of life, new cardiac disease processes are becoming apparent, including abnormal electrical conduction and diminished contractile function of the heart. Earlier, these “secondary” defects were ascribed to abnormal blood flow due to structural anomalies, but recent evidence suggests that the same genes that cause defects in heart development might be directly involved in cardiac dysfunction and cell-lineage disturbances in adulthood (reviewed in Srivastava, 2004).

For example, human and mouse mutations in NNX2-5 cause a developmental atrial septal defect and also progressively disrupt electrical conduction through the cardiac chambers and can result in sudden death later in life (Schott et al., 1998; Pashmforoush et al., 2004). The atrioventricular (AV) node, the essential site of electrical communication between the atria and ventricles, is smaller than normal in adult Nkx2-5 mouse mutants. Over time, specialized muscle-derived conduction cells are lost and replaced by fibrotic tissue, resulting in progressive defects in electrical conduction (Jay et al., 2004).
Another example involves the aortic valve. World-wide, 1%–2% of people are born with a bicuspid aortic valve, typically silent in childhood (Hoffman and Kaplan, 2002). However, one-third of bicuspid aortic valves develop premature age-dependent calcification, resulting in poorly mobile, nonfunctioning valves later in life (Rajamannan et al., 2003). As a result, calcification of the aortic valve is the third leading cause of heart disease in adults and requires over 50,000 valve replacements per year in the US. The recent discovery that NOTCH1 mutations in humans cause bicuspid aortic valves and later calcification indicates that early developmental and later degenerative disease can share a common genetic cause (Garg et al., 2005).

Calcified human valves are characterized by ectopic osteoblast-specific gene expression, suggesting a cell-fate change of mesenchymal or inflammatory cells (Rajamannan et al., 2003). Notch1 can repress a central transcriptional regulator of osteoblast cell fate, Runx2/Cbfa1 (Ducy et al., 1997), indicating a potential mechanism for NOTCH1 to suppress calcification in the valve tissue (Garg et al., 2005). It will be interesting to determine if polymorphisms in NOTCH1 are associated with altered risk of aortic valve or even vascular calcification, given the similar osteoblast gene expression in atherosclerotic vascular smooth muscle (Steitz et al., 2001). If so, effective preventive interventions over decades may be possible with pharmaceuticals, such as statins, to lower levels of cholesterol, a well-known risk factor for calcification.

Cardiac Stem Cell and Regenerative Approaches

The notion that genes involved in early cardiogenesis may be re-deployed to help protect, repair, or regenerate heart muscle has motivated efforts to understand early developmental pathways (Parmacek and Epstein, 2005). Two recent examples highlight the potential utility of this approach.

Reports that niches of small, noncardiomyocyte populations in the postnatal heart can differentiate into cardiac muscle and endothelial cells have generated considerable excitement that the heart, like other organs, may have a resident pool of progenitor cells. Some controversy exists regarding the markers of these cells with partially nonoverlapping reports of Sca-1-, c-kit-, or Abcg2-positive progenitors that can differentiate into cardiomyocytes (reviewed in Leri et al., 2005). A fourth population of progenitors expressing Isl1 suggests an important connection between postnatal progenitor cells and an early developmental pathway regulating cardiomyocyte precursors (Laugwitz et al., 2005). As described earlier, Isl1 is expressed in SHF cells before they differentiate into myocytes and is down-regulated upon expression of sarcomeric proteins. In mice and humans, niches of Isl1-positive cells in early postnatal heart may be remnants of developmental progenitor cells among terminally differentiated myocytes (Laugwitz et al., 2005). It will be interesting to determine if these cells can differentiate into cardiomyocytes, endothelial cells, and conduction system cells.

Deeper understanding of the networks governing SHF proliferation and differentiation may allow expansion of the postnatal cardiac progenitors for therapeutic purposes. Interestingly, Shh, a regulator of pluripotency in some settings (Kusano et al., 2005), is an early regulator of the SHF and controls expression of Tbx1 in the cardiac progenitors (Yamagishi et al., 2003). Whether Shh regulates the postnatal progenitors is unknown, but attempts to expand endogenous cardiac progenitors hold promise.

In addition, bone marrow-derived and circulating stem cells introduced into mice and humans after an acute myocardial infarction may result in some improvement in cardiac function; however, this remains controversial (reviewed in Leri et al., 2005; Srivastava and Ivey, 2006). Although it is unlikely that bone marrow-derived stem cells differentiate into myocytes, they may fulfill a non-cell-autonomous function by secreting paracrine factors that promote survival or neoangiogenesis to protect and rescue the ischemic myocardium (Mangi et al., 2003). The reparative capacity of bone marrow-derived stem cells in rodents can be increased by overexpressing the survival and angiogenic kinase Akt (protein kinase B) in the cells before introduction, resulting in elevation of secreted factors with potential benefit for at-risk myocardium (Gnecchi et al., 2006).

Identifying critical secreted factors may obviate the need for cell-based therapy in favor of specific survival and angiogenic factors during the acute ischemic period (Gnecchi et al., 2006). Here again, recent studies of a developmental protein suggest a mechanism to achieve this goal. Thymosin β4, a 43-amino-acid secreted peptide, contains an actin binding domain that is abundantly expressed in the developing heart, where it can regulate cell migration events during morphogenesis (Bock-Marquette et al., 2004). Thymosin β4 functions in a complex with integrin-linked kinase to activate downstream events, including Akt phosphorylation. Systemic or local treatment of mice with thymosin β4 immediately after coronary occlusion significantly protected at-risk hypoxic myocardium (Bock-Marquette et al., 2004), likely through promotion of survival and neo-angiogenesis (Grant et al., 1999). Interestingly, thymosin β4 is abundantly secreted by bone marrow-derived stem cells, and its secretion is increased many fold in bone marrow-derived stem cells overexpressing Akt, suggesting a positive feedback loop reinforcing thymosin β4 production (Gnecchi et al., 2006). The role of thymosin β4 and other secreted proteins in transducing any potential effects of bone marrow-derived stem cells is unknown, but they hold significant therapeutic potential.

Summary

Cardiac developmental biology has progressed rapidly over the last decade, and the heart is now one of the better-understood organs at the molecular, physiologic, and anatomic levels. Advances in human genetic tools have also increased understanding of the importance of
developmental pathways in human disease. Congenital heart disease can be seen as a defect of morphogenesis and, in some cases, a failure of differentiation among subsets of lineages that contribute to the heart. We are now embarking on a phase in which knowledge of developmental pathways and high-throughput methods of genotyping rare and common gene variants should allow rigorous investigation into the causes of human heart disease. With increasing recognition that congenital heart disease has a significant genetic contribution, we can imagine that genetic variation underlies both the morphogenetic defect and the predisposition to long-term consequences that will affect clinical outcomes for the millions of congenital heart disease survivors. Thus, vigorous efforts to identify genetic variation associated with congenital heart disease and outcome will be essential as therapeutic or preventive measures to alter the disease course may be possible throughout childhood and in the adult. We may even eventually predict genetic risk among parents and focus preventive strategies on those at greatest risk to genetically transmit disease. A deeper understanding of the interactions between various signaling and transcriptional networks and their ultimate downstream targets will be necessary to identify potential approaches in at-risk parents. The efficacy of folic acid in prevention of neural-tube defects provides hope for similar prevention of congenital heart disease (Mitchell et al., 2004).

As disease-related biology evolves, parallel advances in stem cell biology should usher in an era of new approaches. Exciting future technologies may generate disease-specific embryonic stem cell lines for mechanistic studies of disease etiology and development of patient-specific stem cells as therapeutics. Although it may become possible to guide stem or progenitor cells into a cardiac lineage based on our knowledge of early developmental pathways, many hurdles must be overcome for therapeutic use. Issues such as stem cell expansion, delivery, incorporation, electrical coupling, and safety remain to be addressed. Despite these significant challenges, there is reason for optimism as we continue to unravel the mysteries surrounding the lineage determination, differentiation, and morphogenesis of cardiac cells.

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