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Signaling and Transcriptional Networks in Heart Development and Regeneration

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SUMMARY

The mammalian heart is the first functional organ, the first indicator of life. Its normal formation and function are essential for fetal life. Defects in heart formation lead to congenital heart defects, underscoring the finesse with which the heart is assembled. Understanding the regulatory networks controlling heart development have led to significant insights into its lineage origins and morphogenesis and illuminated important aspects of mammalian embryology, while providing insights into human congenital heart disease. The mammalian heart has very little regenerative potential, and thus, any damage to the heart is life threatening and permanent. Knowledge of the developing heart is important for effective strategies of cardiac regeneration, providing new hope for future treatments for heart disease. Although we still have an incomplete picture of the mechanisms controlling development of the mammalian heart, our current knowledge has important implications for embryology and better understanding of human heart disease.

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1 INTRODUCTION

The heart is the first organ to function in the embryo, and this function is essential for survival during fetal life. The beating heart is the most readily detectable sign of life. In humans, defects in heart formation occur in 1%–2% of the population, resulting in a broad range of congenital heart defects. Heart development is a finely tuned complicated process that requires precise differentiation and assembly of many cell types. Heart development has been well studied, and the pathways that coordinately regulate cardiac differentiation and morphogenesis are well defined (Fig. 1). The study of mouse heart development has been particularly important for understanding congenital heart disease and for developing strategies for cardiac regeneration.

In the early heart, cells expressing cardiac markers coalesce at the midline of the embryos to form a linear heart tube that soon begins to contract. The linear heart tube expands and begins a series of contortions. This cardiac looping shifts the heart tube toward the right of the embryo, the first overt sign of breaking left–right symmetry. Major morphogenetic events form the rudimentary chambers of the heart: the two atria and two ventricles. Septa form between the pairs of chambers to isolate each chamber, and valves soon connect the atria to the ventricles. The heart matures during the later stages of fetal life and is fully functional at birth. Morphogenesis of the mouse heart resembles that of the human, and thus has been critical for understanding human congenital heart disease.

2 CARDIAC PROGENITORS AND EARLY DIFFERENTIATION

The cells that make up the heart originate from mesoderm emerging from the primitive streak. The earliest known committed cardiac precursors express the T-box transcription factor *Eomesodermin* (*Eomes*) (Costello et al. 2011). *Eomes*-expressing cells contribute quite specifically to the cardiovascular system, including the heart and vasculature. *Eomes* is important for activating *Mesp1*, another transcription factor whose expression was considered to be one of the earliest markers of cardiac precursors. Identification of *Mesp1* as a marker of early cardiac mesoderm was based on Cre-loxP-based genetic tracing that suggested that *Mesp1*-expressing cells contribute predominantly to the heart (Saga et al. 1999). In fact, the *Mesp1* lineage is much broader, encompassing almost all of the trunk mesenchyme, the limbs, and the vasculature (Yoshida et al. 2008). Notably, accurate lineage contributions based on genetic tracing approaches depend on a critical combination of the fidelity of the Cre-expressing transgene and the homogeneous expression of the reporter transgene. In Mesp1- and Eomes Cre lines, a knockin approach is likely to ensure fidelity, but the reporter strain can greatly affect interpretation of the lineage contribution. In differentiated embryonic stem (ES) cells, *Mesp1* is also active during early stages of cardiac differentiation, but as in vivo, *Mesp1* expression predicts many mesodermal lineages (Bondue et al. 2008; David et al. 2008; Lindsley et al. 2008). Therefore, *Mesp1* expression characterizes cardiac progenitors, among multiple cell types.

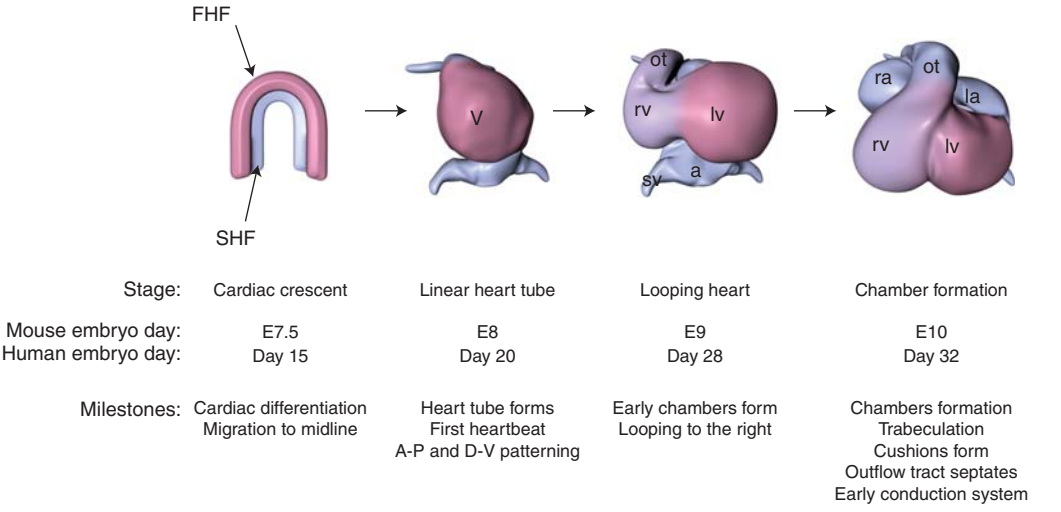

Figure 1. Major steps in heart development. Diagrams of developing hearts (ventral views) are shown. At the cardiac crescent stage, the two heart fields represent different cardiac precursors. The first heart field (FHF, pink) contributes to the left ventricle (lv), and the second heart field (SHF, blue) contributes to the right ventricle (rv) and later to the outflow tract (ot), sinus venosus (sv), and left and right atria (la and ra, respectively). Days of development for mouse and human are shown below. (From Bruneau 2008, with permission.)

Classic embryological manipulations pinpointed the origins of the heart in the gastrula. Using cell transplantation and labeling of live embryos, followed by embryo culture, precursors of the heart were identified as occupying anterior mesoderm, proximal to the embryonic–extraembryonic boundary (Tam et al. 1997; Kinder et al. 1999). An important insight from these experiments was that the populations contributing to the heart are plastic, in that cells taken from a different location or developmental time point could contribute to the heart when placed in the appropriate location. This plasticity points toward the cellular environment as more important than the initial identity of the cells. These landmark experiments broadly located the cardiac precursors, but did not identify them specifically. Therefore, the cells fated specifically to the heart have not yet been identified and characterized.

Hints about the molecular identifying marks labeling cardiac precursors have been gleaned from embryos and cultured ES cells. From the molecular cues that drive cardiac differentiation in vivo, a strategy for differentiating ES cells into cardiac myocytes was devised (Kattman et al. 2006; Moretti et al. 2006; Yang et al. 2008; Kattman et al. 2011). This led to identification of Flk1 and PDGFRα as cell-surface markers that label a population of mesoderm destined to become cardiomyocytes. Flk1 was identified in these studies, but further mouse genetic labeling experiments showed $Flk1^+$ derivatives contribute to many other mesodermal derivatives (Ema et al. 2006). PDGFRα was identified in *Nkx2-5*-expressing cardiac precursors (Prall et al. 2007) and provides added specificity with Flk1, to identify and isolate cardiac progenitors (Kattman et al. 2011). However, these do not mark cardiac precursors exclusively, but instead allow enrichment of cardiac progenitors.

2.1 Allocation of Progenitors

Cardiac progenitors originating from mesoderm are rapidly allocated to two major populations, referred to as heart fields (Buckingham et al. 2005; Vincent and Buckingham 2010). The first heart field (FHF) is thought to contribute to the left ventricle and parts of the atria. Adjacent to the FHF, the second heart field (SHF) contributes predominantly to the arterial pole of the heart (e.g., outflow tract and right ventricle) and also to the venous pole (e.g., sinus venosus and atria). Unlike the FHF, the SHF actively contributes cardiac precursors in early organogenesis, whereas the FHF is more rapidly incorporated into the differentiating heart. The SHF can be identified by the expression of *Isl1*, although its expression is much broader than just the SHF. *Isl1* was associated with the SHF from Cre-mediated genetic tracing. Although *Isl1* was not expressed to any significant degree in the differentiating heart, the descendants of the *Isl1*-expressing cells populated large segments of the heart (Cai et al. 2003). Isl1 has since been used as a marker of the SHF, but as with any genetic label, it must be interpreted carefully. Isl1 is active in cardiac neural crest (Engleka et al. 2012), which complicates analyses of mouse deletion phenotypes that use *Isl1::Cre* to manipulate genes. Other markers, such as *Fgf10* and a specific enhancer of the *Mef2c* gene, also mark a portion of the SHF, a more anterior domain referred to as the anterior heart field, which gives rise to the outflow tract and right ventricle (Kelly et al. 2001; Zaffran et al. 2004; Verzi et al. 2005).

A similar conclusion was reached, with some notable added insight, by a retrospective lineage tracing approach. Meilhac and colleagues used a genetic labeling approach that relies on the random activation of a marker, localized to the nucleus lacZ, which has an insertion that disrupts its function (Meilhac et al. 2003, 2004a). Rare recombination events activate the gene in a few cells, permanently labeling the cell lineage. The modified reporter gene was inserted at a gene expressed in all cardiac myocytes during development, the α cardiac actin (*Actc1*) gene. During embryonic development random recombination events activate the transgene, but its expression is restricted to cardiac myocytes, thus neatly labeling clones of cells in the developing heart. This strategy was very useful to retrospectively analyze hundreds of mouse embryos, and derive conclusions based on the distribution of clones. The main conclusion was that two main cardiac progenitor populations exist. One arose very early and had common progenitors for all regions of the heart except the outflow tract, and one segregated later to contribute to the outflow tract, right ventricle, and atria, but not the left ventricle. These results are comparable to those obtained from genetic tracing experiments, except for one key distinction: prediction of an early common cardiac progenitor. This retrospective approach does not allow identification or isolation of the distinct progenitors.

The SHF has been well studied using the genetic tools available. In addition to the broad contributions to the outflow tract and right ventricle, cells labeled by the *Mef2cAHF::Cre* transgene are also found in a region called the dorsal mesenchymal protrusion, or DMP (Hoffmann et al. 2009). The DMP invades the posterior wall of the atria, and DMP-derived cells contribute to the primary atrial septum, which participates in separating the left and right atria from one another.

The SHF also contributes to the posterior, venous pole of the heart. Here, expression of SHF markers overlaps with other cardiac marker genes, and it is not clear if these are true progenitors (Galli et al. 2008). The cell lineage allocation is more complex: Sinus venosus progenitors are a separate population, marked by the absence of *Nkx2-5* (and perhaps *Isl1*), and instead by the unique presence of *Tbx18*

(Christoffels et al. 2006; Mommersteeg et al. 2010). Thus, three distinct cardiac progenitor populations are evident, each with a distinct signature and contribution to the developing heart. The allocation of cardiac lineages is likely to be more complex, as more specific markers are identified.

2.2 Signals that Induce the Heart Progenitors

Cardiac differentiation is induced by signaling cues from adjacent tissues. In early mesoderm formation, graded levels of the TGFB-family member Nodal are important for specifying different types of mesoderm. Higher levels of Nodal favor cardiac mesoderm (Brennan et al. 2001). After specification of cardiac mesoderm, bone morphogenic protein (BMP) and Wnt signals are modulated in the early stages of cardiac differentiation. Wnt signaling initially promotes cardiogenesis, but later is inhibitory as progenitors begin to differentiate into various cardiac derivatives (Naito et al. 2006; Kwon et al. 2007). Wnt/β-catenin-induced expansion of cardiac precursors requires *Isl1* downregulation, which promotes cardiac differentiation (Kwon et al. 2009). BMP function is not clear in the earliest stages of cardiac commitment, although inhibiting BMP signaling seems important to promote the emergence of cardiac mesoderm (Yuasa et al. 2005). Later BMPs seem to be important for expansion of cardiac progenitors, as genetic deletion of the BMP receptor in *Mesp1*-expressing mesoderm results in major defects in heart formation (Klaus et al. 2007). In cardiac progenitors, BMPs maintain the balance between precursor expansion and differentiation (Prall et al. 2007). Notch signaling is also important in cardiac precursors: Deleting *Notch1* in the SHF leads to a greater number of cardiac precursors, in part by increasing the activity of Wnt signaling and thus promoting expansion of cardiac progenitors (Kwon et al. 2009).

2.3 Transcriptional Regulation of Cardiac Differentiation

Precise regulation of tissue-specific gene transcription is essential for the correct differentiation and patterning of the heart. Transcription factors regulate aspects of heart development, including terminal differentiation, identity of cardiac chambers, establishment of patterning boundaries, and formation of transcriptional gradients (Olson 2006; Srivastava 2006). For example, the homeodomain transcription factor Nkx2-5 is critical for terminal differentiation of the myocardium; without Nkx2-5, the heart does not progress beyond a primitive arrangement of chamber primordia (Lyons et al. 1995). Paradoxically, *Nkx2-5* is required for restricting the numbers of progenitors, by restricting the expression of BMPs, and thus maintaining the balance between progenitor proliferation and differentiation (Prall et al. 2007). The T-box transcription factor Tbx5 is required for the growth and differentiation of the posterior segment of the heart, the atrial and left ventricular precursors (Bruneau et al. 2001b). The GATA factors GATA4 and GATA6 redundantly regulate the onset of cardiac differentiation (Zhao et al. 2008). Similarly, serum response factor (SRF) modulates expression of multiple cardiac genes (Niu et al. 2008). Other transcription factors (e.g., bHLH Hand proteins) control aspects of chamber differentiation (Srivastava et al. 1997; Firulli et al. 1998). Several of these transcription factors functionally interact to regulate downstream gene expression. Although networks of interacting transcription factors are being defined, the full complement of their target genes is not clear, nor is how these factors are integrated at the level of chromatin to activate transcriptional programs. In chromatin localization studies of Nkx2-5, Gata4, Tbx5, and Mef2a in culture, cardiac transcription factors often bind together at genes that are coregulated during heart development, suggesting a high degree of cooperativity between cardiac transcription factors (Fig. 2) (He et al. 2011).

In addition to cooperative activation of gene expression, several cardiac transcription factors function to sculpt cardiac gene expression during patterning of the early developing heart. As an example, Tbx5 and Tbx2 function antagonistically in the atrioventricular (AV) canal. *Tbx5* is expressed throughout the atrial and left ventricular myocardium, including the intervening AV canal. In the AV canal, a related T-box transcription factor, *Tbx2*, is expressed. In chamber myocardium, Tbx5 activates chamber-specific genes, such as *Nppa*, in cooperation with Nkx2-5. In the AV canal, Tbx2 competes for Tbx5 binding to target DNA elements, and to Nkx2-5, and represses the same genes (Habets et al. 2002). This antagonistic activity readily patterns the developing heart.

Heterozygous mutations of several transcription factor genes that are important in early heart development cause human congenital heart disease (CHD) (Bruneau 2008). In Holt-Oram syndrome, *TBX5* mutations cause atrial septal defects (ASDs), ventricular septal defects (VSDs), and conduction system disease (Mori and Bruneau 2004). Mutations in *NKX2-5* cause similar defects and others, such as tetralogy of Fallot or Ebstein's anomaly of the tricuspid valve (Schott et al. 1998; Benson et al. 1999). *GATA4* mutations result in a subset of defects caused by *TBX5* and *NKX2-5* mutations, ASDs and VSDs (Garg et al. 2003). Deficiency in another T-box gene, *TBX1*, is at the root of 22q11.2 microdeletion syndrome (DiGeorge syndrome); this finding came from advanced mouse genome engineering, which sought to recapitulate human chromosomal microdeletions (Lindsay et al. 2001; Merscher et al.

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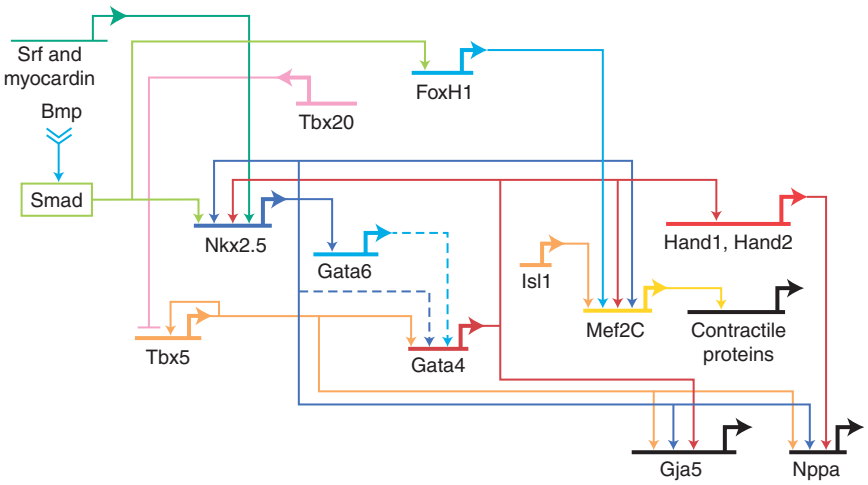

Figure 2. Transcriptional networks in heart development. A representative transcriptional network is shown. (Adapted from Davidson and Erwin 2006.)

2001). Two very important insights came from these studies. First, developmentally important transcription factors are critically important in the etiology of CHD. Second, the dosage of these factors must be precisely regulated, as most disease-causing mutations appear to result in haploinsufficiency. This latter finding hints at mechanisms of disease owing to decreased dosage. Furthermore, most of these disease-related transcription factors interact with each other, and their interactions are stoichiometrically regulated. In crosses between *Tbx5*, *Nkx2-5*, and *Gata4* mutant mice, these factors genetically interacted, and compound heterozygosity of any two genes leads to far more severe CHDs than haploinsufficiency of single genes (Moskowitz et al. 2007; Maitra et al. 2008).

2.4 Epigenetic Control of Cardiac Differentiation

Transcription factors function within the regulatory confines of chromatin, the dense arrangement of DNA around the histone octamers that forms nucleosomes (Kouzarides 2007; Li et al. 2007). With its formidable packaging, chromatin provides an important level of gene regulation, via the packing density and chemical modification of unstructured histone "tails." Regulation of the developing heart greatly relies on epigenetic regulation by chromatin remodeling and histone modifications (Bruneau 2010; Chang and Bruneau 2012). Mapping epigenetic regulation during cardiac differentiation on a global scale revealed complex but specific temporal patterns of histone modifications coordinately regulate cardiac genes (Wamstad et al. 2012). Therefore, complex coordination of epigenetic regulators on sets of cardiac genes is likely an essential component of cardiac differentiation, with important implications for heart development.

In the heart, chromatin remodelers have been uncovered as interacting with DNA-binding transcription factors to modify their target gene expression. For example, Baf60c (Lickert et al. 2004) is one isoform of three subunits that are part of the Brg1/Brm-associated factor (BAF) complexes. These important chromatin-remodeling complexes shift nucleosomes side to side to regulate access to regulatory DNA. Baf60c is expressed very early in precardiac mesoderm at late gastrulation and prenodal plate stages and later in the heart, somites, and the developing central nervous system. RNAi-mediated knockdown of Baf60c in mouse embryos yields malformed hearts and aberrant gene expression. Baf60c functions as a bridge, creating or reinforcing molecular interactions between cardiac transcription factors (GATA4, Nkx2-5, and Tbx5) and Brg1, thus presumably bringing the BAF complex to target genes bound by GATA4, Nkx2-5, or Tbx5 (Lickert et al. 2004). Baf60c is one of a trio of required cardiogenic transcription factors that include GATA4 and Tbx5, which activate the entire cardiac program de novo in noncardiac mesoderm (Takeuchi and Bruneau 2009). Baf60c allows initial binding of GATA4 to target loci, indicating that it is essential for the cardiogenic activity of GATA4 and Tbx5.

Insights into the importance of BAF complexes come from deletion of the main BAF complex ATPase, *Brg1*. Deleting *Brg1* in the developing endocardium showed that BAF complexes can be required for very specific functions in establishing endocardial–myocardial signaling: Loss of endocardial Brg1 derepresses a major gene involved in controlling the matrix between the endocardium and myocardium, disrupting cardiac morphogenesis (Stankunas et al. 2008). This finding illustrates the fine degree of regulation by BAF complexes, and shows that they can be just as important as repressors of gene expression, in addition to

their activation function. Deleting *Brg1* in the differentiating myocardium more broadly deregulates gene expression, leading to defects in chamber morphogenesis (Hang et al. 2010; Takeuchi et al. 2011). Interesting from the perspective of interactions with disease-causing transcription factors, mice heterozygous for *Brg1* deletion have significant CHDs, indicating dosage dependency of BAF complexes in the heart (Takeuchi et al. 2011). *Brg1* haploinsufficiency is considerably worsened by combined haploinsufficiency of *Tbx5*, *Nkx2-5*, or *Tbx20* (Takeuchi et al. 2011). This indicates a strong genetic interaction between DNA-binding factors and BAF complexes, which predicts a tightly regulated stoichiometric relationship between transcription factors and chromatin-remodeling complexes. These data suggest transcription factor haploinsufficiency in CHD involves impaired recruitment of BAF complexes to target genes. This important insight highlights the key interplay between DNA-binding factors and chromatin-remodeling complexes.

The potential role of histone acetylation in cardiac development is highlighted largely by work performed on the HATs p300 and CBP and their interactions with cardiac transcriptional regulators. Mice lacking p300 have multiple defects in embryogenesis, including cardiac defects such as reduced ventricular trabeculation and impaired expression of cardiac genes (Yao et al. 1998). p300 interacts with cardiac transcription factors, such as MEF2D and GATA factors (Kakita et al. 1999; Dai and Markham 2001; Slepak et al. 2001; Kawamura et al. 2005). Thus, p300-mediated histone acetylation is likely to be a widespread mechanism for coactivating cardiac genes. Tbx5 interacts with TAZ, a WW-domain-containing transcriptional regulator, which recruits the HATs p300 and PCAF, thereby enhancing Tbx5-dependent *trans*-activation of the *Nppa* promoter (Murakami et al. 2005). This cooperativity is prevented by mutations in *TBX5* in patients with Holt-Oram syndrome, in which CHDs are prevalent. Thus, defective Tbx5-mediated histone acetylation may contribute to the congenital heart defects associated with the syndrome. Tbx5 and other T-box transcription factors, as well as SRF, interact with Tip60, a MYST-family HAT, to potently activate target genes, including SRF itself (Kim et al. 2006).

Repressing transcription by epigenetic regulators is also important for normal heart development. Histone deacetylases (HDACs), which repress gene expression, have been largely characterized from mouse knockouts as important for adaptive gene regulation in the adult heart, but their roles in the developing heart are emerging. Loss of individual HDAC genes yields mice with little or no detectable embryonic cardiac defects, but mice lacking multiple HDAC genes die in utero from cardiac defects (McKinsey et al. 2001; Montgomery et al. 2007). Of potential significance to the developing heart, HDAC function is highly regulated by cellular signaling processes. Thus, in the developing heart, HDACs may have complex functions that depend on interactions with DNA-binding transcription factors and growth factor signaling pathways. Indeed, two transcription factors essential for heart development—Smyd1 (also known as mBop) and homeodomain-only protein (Hopx)—function in part by associating with HDACs (Gottlieb et al. 2002; Trivedi et al. 2010). SRF, which is important for activating several cardiac genes, interacts with HDACs through Hopx (Kook et al. 2003). The Hopx/HDAC interaction is also important for regulating the function of GATA4, by directly modulating its acetylation (Trivedi et al. 2010). Thus, through their associations with transcription factors, HDACs are important for heart development.

One of the best-studied repressive marks is the trimethylation of lysine 27 of histone H3 (H3K27me3). This mark is laid down by the polycomb repressive complex 2 (PRC2), which comprises three core subunits: Suz12, Eed, and the catalytic histone methyltransferase, Ezh2 (Surface et al. 2010). *Ezh2* deletion in cardiac precursors leads to defective cardiac morphogenesis, including thinned myocardial walls and ventricular septation defects (He et al. 2012a). This is perhaps as a result of impaired proliferation, as a result of the derepression of negative regulators of the cell cycle. Interestingly, cardiac progenitor genes that would normally no longer be expressed in the heart are still on, indicating that PRC2 function is essential to shut off progenitor genes permanently once they are no longer needed in differentiating cardiomyocytes. A slightly different ablation of *Ezh2*, in a more restricted domain of cardiac progenitors, leads not to defects in cardiac morphogenesis, but instead to cardiac enlargement after birth (Delgado-Olguin et al. 2012). This results mainly from lack of repression of one transcription factor that functions in cardiac precursors, the homeodomain transcription factor encoded by *Six1*. When its expression persists, Six1 can still activate noncardiac genes, including genes normally restricted to skeletal muscle. Therefore, epigenetic repression of developmental regulators by PRC2 is an essential component of early cardiac development. Ezh2 may also function independently of histone methylation, as a direct methylator of GATA4; GATA4 methylation represses its transcription activation potential (He et al. 2012b).

2.5 Lessons Learned from Early Heart Development

Studying early heart development yielded many important lessons that are relevant to lineage specification and differentiation. Transcriptional regulation of cardiac differentiation underscored the importance of combinatorial factor

interactions in robust regulation of tissue-specific gene expression. Furthermore, from modeling human CHD in the mouse, these interactions are dosage sensitive, indicating a very fine integration of transcriptional inputs (Bruneau et al. 2001b; Jerome and Papaioannou 2001; Lindsay et al. 2001; Merscher et al. 2001). Understanding how transcription factors and chromatin remodelers interface contributes to a model by which transcription factors promote or repress target gene expression in a specific cell type. The study of early gene expression and its regulators has also led to important insights into mechanisms of lineage determination and lineage allocation that are relevant to most mammalian cell types. Integrating these investigations will yield a comprehensive view of cell lineage determination and regulation of differentiation.

3 CARDIAC ORGANOGENESIS

Heart formation includes several important steps that precisely organize the various cellular subtypes into chambers and associated structures. Along the way, precise patterning of gene expression underlies many of the events that drive organogenesis (see Fig. 3 for examples).

3.1 Establishing the Heart Field

On differentiation, the bilateral regions of mesoderm that will form the heart migrate toward the midline to form a linear heart tube. Defective convergence of the two sides of the heart-forming region results in cardia bifida, in which cardiac differentiation proceeds independently in two separate areas, and a normal heart does not form. In striking cases, cardiac development proceeds some distance. Sometimes, the paired hearts form primitive chambers (Li et al. 2004). Although the cellular mechanisms required for bilateral migration and fusion are not well understood, several transcriptional regulators are known to be important for this process. *Mesp1* is required early on, presumably to allow migration of cardiac precursors to their appropriate location (Saga et al. 1999). *Gata4* is also essential for cardiac fusion (Kuo et al. 1997; Molkentin et al. 1997), although it is not clear whether this is a cell-autonomous requirement in early cardiomyocytes or a function in adjacent endoderm. The proprotein convertase furin also has essential functions in regulating embryonic ventral closure, and in its absence cardia bifida is a common phenotype (Roebroek et al. 1998; Constam and Robertson 2000). Furin may activate several TGF-β family members, including Nodal, Lefty, and BMPs. Perhaps related to Furin's function, BMP2 secreted from visceral endoderm is required for embryonic patterning events, including normal migration of cardiac precursors (Madabhushi and Lacy 2011).

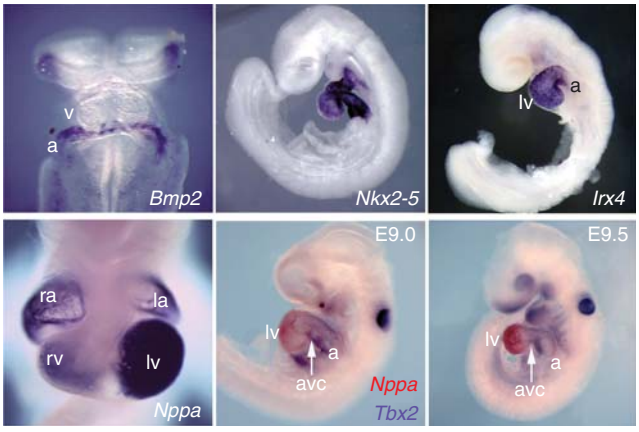

Figure 3. Patterning the developing heart. In situ hybridization in mouse embryos illustrates the complexity and precision of patterning. Shown are in situ hybridizations for *Bmp2* (with expression in atrial precursors at E8.5), *Nkx2-5* (expressed in all cardiac cells, at E9.25), *Irx4* (expressed in ventricular [lv] but not atrial [a] cells at E9.25), *Nppa* (with a complex chamber expression pattern at E10.5), and the mutually exclusive pattern of *Nppa* and *Tbx2* (*Nppa* in chamber myocardium and *Tbx2* in AV canal [Venter et al. 2001]) at E9.25 and E9.5. a, atrium; avc, atrioventricular canal; lv, left ventricle; rv, right ventricle; v, ventricle. (Adapted from Bruneau 2003, 2011.)

3.2 Chamber Formation

At the onset of heart looping, the chambers begin to form. The first evident chamber is the single ventricle that bulges out from the looping heart tube. As this chamber grows, a morphologically recognizable right ventricle begins to form, and the atria begin to grow into the laterally paired appendages visible behind the more evident ventricular chambers. One interesting hypothesis suggests how the ventricular and atrial chambers grow, whereas the rest of the heart tube remains more or less in its original tubular form. Based on patterns of gene expression, and later on examining patterns of cell proliferation, it was proposed that the chambers of the heart "balloon" out from the primitive heart tube (Christoffels et al. 2000; Moorman and Christoffels 2003). This ballooning model is attractive as it neatly explains what appeared to be an overly complex means of allocating cells to a particular structure. The model of directional growth leading to morphogenesis of structures of specific shape and cellular orientation is directly supported by the same retrospective lineage analyses that defined the early cardiac lineages: By examining smaller clones of labeled cells, the patterns of cell division that cells had followed could be understood during recent organogenesis (Meilhac et al. 2003; Meilhac et al. 2004b; Bajolle et al. 2006). For example, ventricular growth was observed as radial, explaining the large rounded shape of the primitive ventricles. Conversely, growth in the outflow tract is

initially linear, corresponding to its elongation, and then is radial, which corresponds to the broadening and rotation of the outflow tract.

Lineage analyses confirmed that the chambers form as thought (Meilhac et al. 2003, 2004b), but allocating cells from the primitive heart tube to the forming chambers might be more complicated. For example, *Tbx2*-expressing cells, which are predominantly confined to the AV canal (Fig. 3), contribute many cells to the adjacent left ventricle (Aanhaanen et al. 2009).

Importantly, "primitive" segments of the heart are retained and result in regions of slowed conduction and minimal contraction. This is apparent during chamber morphogenesis as the AV canal forms. Patterning cues establish boundaries along the primitive heart tube, within which the AV canal forms. This region of the heart expresses a distinct complement of genes, especially those involved in conduction of impulses, such as connexins. The AV canal myocardium is important in coordinated impulse propagation: It forms the insulating conduction system tissue that directs the impulses that initiate in the atrium along a specific insulated path (Hoogaars et al. 2004; Bakker et al. 2010; Aanhaanen et al. 2011). Signals that initiate and maintain formation of the AV valves arise from the AV canal. The specialization of the conduction system and valve formation will be reviewed later in this section.

Little is understood about what regulates these growth processes, except that differential rates of proliferation may drive early chamber morphogenesis (Soufan et al. 2006; van den Berg et al. 2009; Aanhaanen et al. 2011). The Tbx2 transcription factor, which is restricted to the AV canal, is important for maintaining a lower level of proliferation in this segment of the heart (Aanhaanen et al. 2011).

3.3 Trabeculation

Formation of trabeculae, the fingerlike projections that protrude inside the developing chambers, is important in cardiac chamber morphogenesis. The trabeculae ensure the growth of the heart from a thin-walled organ to one that can pump against high pressures. A trabeculated heart can also exert force, while being easily oxygenated before development of coronary circulation. Trabeculae are highly conserved in evolution and, in more primitive hearts, such as amphibians and reptiles, are retained into adulthood. Several signaling pathways are deployed to ensure trabeculation, including neuregulin/ErbB, Notch, Ephrin/Eph, and Bmp10. *Bmp10* is expressed within developing trabeculae and is essential for proliferation of trabecular myocardium (Chen et al. 2004). Nrg1, produced from the endocardium, signals to ErbB receptors in the myocardium to allow trabeculation (Gassmann et al. 1995; Meyer and Birchmeier 1995), in a pathway that appears independent of Bmp10 (Grego-Bessa et al. 2007). EphrinB2 and its receptor EphB4 are also important for trabeculation (Wang et al. 1998). Notch signaling in the endocardium adjacent to the trabeculae is also essential for trabeulation, and is implicated in many signaling pathways: Notch signaling sustains *Bmp10* expression and promotes expression of *Nrg1* and *EphrinB2* in the endocardium (Grego-Bessa et al. 2007).

3.4 Chamber Septation

Chamber septation involves separation of the left and right sides of the atria and ventricles by growth of specific regions of myocardium (Fig. 4). Septation of the ventricles is simpler and occurs at the junction of the two heart fields. Ventricular septation defects are the second most common CHDs. However, the patterning events that dictate ventricular septation and the subsequent cellular changes that govern septal morphogenesis are not understood.

Early embryological studies determined that the interventricular septum (IVS) is formed by outgrowth of two adjacent populations of cells in a small segment of the left and right ventricles. By genetic lineage analysis, the left and right components of the IVS do not mix during IVS outgrowth (Meilhac et al. 2004a; Franco et al. 2006). These observations suggest patterning of the developing ventricles that dictates the location of the IVS.

Several genes have been implicated in forming of the IVS, including *Tbx5*, *Gata4*, *Nkx2-5*, *Sall4*, and the *Hand* family of transcription factors. Although these genes are all important for forming the IVS, their loss results in pleiotropic effects on the heart as a whole or in defects in, but not a complete absence of, IVS formation. Therefore, the molecular determinants that pattern the location of the IVS and promote its formation are not well understood.

At least one gene might be directly related to patterning the myocardium guiding IVS formation. *Tbx5* is expressed in a dynamic pattern that extends, in the ventricles, just up to the region where the IVS forms (Bruneau et al. 1999; Koshiba-Takeuchi et al. 2009). Genetic manipulation of *Tbx5* in the chick and mouse suggested that the position of the IVS relative to the ventricles is determined by the boundary of cells that do and do not express *Tbx5* (Fig. 5) (Takeuchi et al. 2003; Koshiba-Takeuchi et al. 2009). Misexpression of *Tbx5* across the boundary between the left and right ventricles eliminates IVS formation (Koshiba-Takeuchi et al. 2009). In mice with *Tbx5* deleted from ventricular myocardium or a segment of the ventricular myocardium that includes the cells that will contribute to the IVS, grossly normal hearts form, but fail to initiate IVS formation (Koshiba-Takeuchi et al. 2009). This suggests *Tbx5* in the specific region of ventricular myocardium

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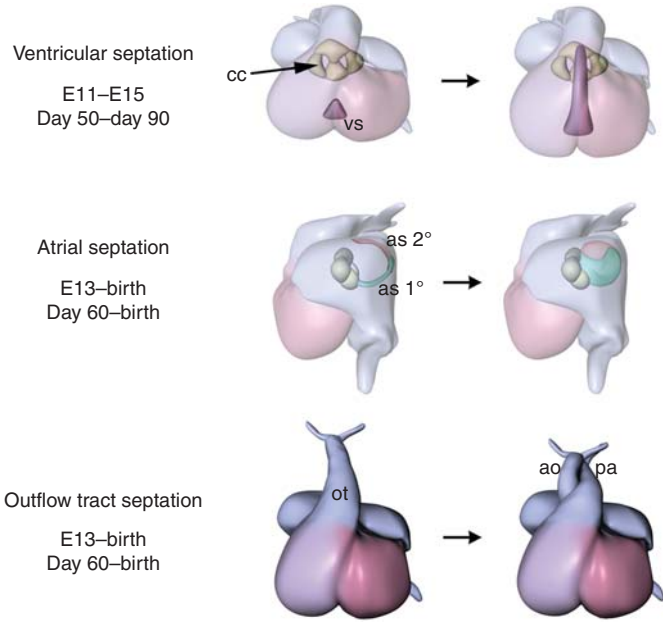

Figure 4. Later steps in cardiac morphogenesis. Shown are ventricular septation, atrial septation, and septation of the outflow tracts. ao, aorta; as 1°, primum atrial septum; as 2°, secundum atrial septum, cc, cardiac cushions; ot, outflow tract; pa, pulmonary artery; vs, ventricular septum. Days of development for mouse and human are shown below. (From Bruneau 2008, with permission.)

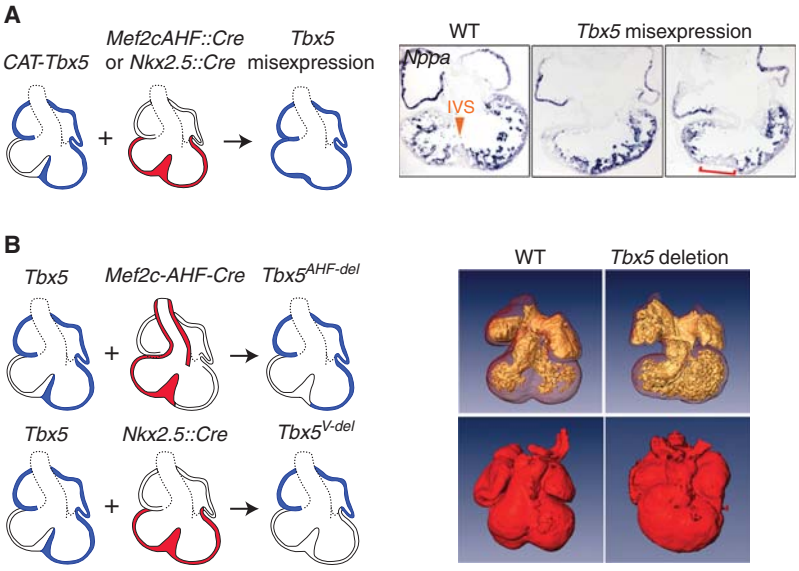

Figure 5. Function of a Tbx5 boundary in ventricular septation. (*A*) Effect of misexpression of *Tbx5* on septum formation. *Left*: diagrammatic representation of the experimental design and resulting phenotype. *Right*: in situ hybridization for *Nppa* on sections from wild-type (WT) and *Tbx5* misexpression hearts, showing absence of septum formation and expansion of expression of *Nppa*. (*B*) Phenotype resulting from deletion of Tbx5 from ventricular myocardium (V-del). Two distinct strategies are shown. *Left*: Optical projection tomography scans of heart from WT and Tbx5 deletion hearts. Note the lack of septation in the absence of Tbx5. (From Koshiba-Takeuchi et al. 2009, with permission.)

that contributes to the IVS is critical for IVS formation. Therefore, the patterning of *Tbx5* expression is essential for IVS formation, but how this boundary functions to form an IVS is unknown.

Tbx5 is also critical for atrial septation, via the integration of diverse cell signaling pathways. Its function in the endocardium is required for establishing signaling cascades that ensure septal formation (Nadeau et al. 2010). An earlier role in the origin of the atrial septum primum from the SHF implicates *Tbx5* in regulating response of the posterior SHF to Hedgehog signaling, and provides a mechanism for the dosage-sensitive defects in atrial septation that result from *Tbx5* haploinsufficiency (Xie et al. 2012). Deletion of one copy of *Tbx5* in endocardium or myocardium does not reproduce the septation defects seen in $Tbx5^{+/-}$ mice, but deletion in the Hh-responsive cells of the SHF does, showing that *Tbx5* is required very early on to establish atrial septum precursors. Tbx5 directly regulates Hh-pathway genes, and also may act in parallel to regulate Osr1 (Xie et al. 2012).

3.5 Cushions and Valves

As the heart septates, connections between the atria and ventricles are maintained, and valves ensure unidirectional blood flow. Valve formation arises from complex coordinated signaling between the myocardium and the overlying endocardium. Signals from the myocardium initiate local endothelial-to-mesenchymal transformation (EMT). EMT location depends on the initial patterning of the heart segments and thus the localized expression of EMT-inducing factors, such as Bmp2 (Ma et al. 2005). *Bmp2* is also required for patterning the AV canal and outflow tract, in a feedback loop involving *Tbx2*, and for the expression of genes required for the establishment of the cardiac jelly, in which transformed endocardial cells migrate (Harrelson et al. 2004; Ma et al. 2005). Patterning of the AV canal also involves Notch signaling in the endocardium, which represses *Bmp2* outside of its domain of expression (Luna-Zurita et al. 2010). TGFβ signaling is critical for initiating expression of the Snail-family transcription factors Snai1 and Snai2, which are essential for conferring invasive properties to cells undergoing EMT (Tao et al. 2011). Finally, a fourth pathway involving myocardial calcineurin/NFAT signaling represses VEGF expression in the valve-forming region of the AV canal (Chang et al. 2004). In the endocardium, *Gata4* activity is required to activate EMT-inducing factors, including ErbB3 (Rivera-Feliciano et al. 2006). Notch and BMP are essential for endocardial EMT, with BMP signaling functioning upstream of Notch (Timmerman et al. 2004; Luna-Zurita et al. 2010).

Septation of the outflow tract (OFT) and establishment of the pulmonary and aortic valves follows a similar yet distinct set of signaling pathways as AV valve formation. For example, although VEGF signaling is required for complete EMT in the AV canal and OFT, this role is more important in the OFT than for AVC endocardial cushion formation (Stankunas et al. 2010). The OFT elongates from the SHF, where intricate signaling events are coordinated. Within the forming OFT, cells of the cardiac neural crest migrate in, establishing themselves as an important source of cells for the cushions of the distal outflow tract. The proximal cushions of the OFT are derived from the underlying endothelium. A fine interplay between signals secreted from the mesoderm of the SHF to the adjacent neural crest and endocardial cells is essential for their survival and differentiation (Park et al. 2006, 2008). Fgf8 signals, under control of the T-box transcription factor *Tbx1* (Hu et al. 2004), are essential for an autocrine signaling loop that activates production of other growth factors, including BMPs, which subsequently signal to the adjacent neural crest and endothelial cells (Stottmann et al. 2004; Park et al. 2008). The role of *Tbx1* in initiating the signaling is particularly interesting, as heterozygous loss of human *TBX1* underlies 22q11 microdeletion syndrome or DiGeorge syndrome (Jerome and Papaioannou 2001; Lindsay et al. 2001; Merscher et al. 2001); the cardiovascular manifestations of this syndrome were thought to be owing to a defect in neural crest formation, but *Tbx1* is also expressed in branchial arch and SHF mesoderm (Hu et al. 2004; Xu et al. 2004). Instead of a cell-autonomous function of Tbx1 within neural crest cells, it activates autocrine and paracrine signals that influence OFT development and cardiac morphogenesis.

After cushion formation is initiated in the OFT and AV canal, subsequent steps in valve formation involve elongation and maturation of the valve tissue (Hinton et al. 2006). Similar signaling is deployed. For example, calcineurin/NFAT signaling in the endocardium is deployed again, following its initial function in the myocardium (Chang et al. 2004). Although less well understood than the early stages of valvulogenesis, maturation of valves involves profound changes in gene expression and cellular identity, with the loss of early markers such as *Twist1* and *Tbx20*, and the acquisition of a tendonlike phenotype, with expression of *Sox9* and *Scleraxis* (Lincoln et al. 2007; Levay et al. 2008).

3.6 Cell-Type Specialization and Formation of the Conduction System

During heart formation, various cell types specialize. Myocardial cells, in particular, specialize into atrial, ventricular, pacemaker, AV node, and His-Purkinje cells, the latter three cell types being the major constituents of the cardiac conduction system. Patterning and intrinsic cellular

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programs are responsible for this diversity of cell-type specialization.

The factors leading to establishment of atrial and ventricular cells, corresponding to the left and right chambers, are unclear. The determinants of atrial versus ventricular identity are also unclear, although candidates have been proposed (Pereira et al. 1999; Xavier-Neto et al. 1999; Bruneau et al. 2000, 2001a). Diversification into left and right ventricular myocytes is likely owing to antero-posterior patterning of the heart tube and specific expression of transcription factors, such as *Tbx5* and *Hand1* (Bruneau et al. 1999; McFadden et al. 2004). The left and right atria are patterned based on left-right signaling cascades that initiate early on in establishing the embryonic body plan (Shiratori and Hamada 2006; Galli et al. 2008). In particular, left-sided expression of the *Pitx2* transcription factor establishes left and right atrial identity, in part by suppressing in the left atrium the initiation of pacemaker tissue differentiation, which is critical for normal initiation and conduction of the heartbeat (Mommersteeg et al. 2007; Wang et al. 2010).

Within the atrial and ventricular tissues, cell-type specialization forms the fibers that conduct impulses coordinately for each heartbeat. The patterning of the sinoatrial node, where impulses initiate, is dictated by the localized expression of *Tbx3*, which partly represses a working myocardium phenotype, while promoting the expression of ion channels that are essential for the spontaneous depolarization that is a key feature of sinoatrial node pacemaker cells (Hoogaars et al. 2007; Frank et al. 2012). *Tbx3* is also important for forming the atrioventricular conduction system, which includes the atrioventricular node, where impulses slow to ensure coordination between atrial and ventricular contraction. *Tbx3* expression outlines the atrioventricular conduction system (Hoogaars et al. 2004), partly overlapping the expression of *Tbx2* (Aanhaanen et al. 2009). *Tbx3* is important for forming the atrioventricular node (Frank et al. 2012), but *Tbx2* specifies the insulating tissues that ensure that impulses pass from the atria to the ventricles only via the atrioventricular node (Aanhaanen et al. 2011). Without this important electrical insulation, accessory pathways develop that lead to lethal arrhythmias (Aanhaanen et al. 2011). The distal conduction system, the fast conducting fibers of the His-Purkinje system, is patterned by the concerted dosage-sensitive function of *Tbx5* and *Nkx2-5*, which act upstream of a third transcription factor, *Id2* (Moskowitz et al. 2004, 2007). It is partly established by the function of the *Irx3* transcription factor, which acts predominantly to maintain a balance between gap junction proteins to ensure electrical isolation of the fibers from the rest of the myocardium to ensure fast impulse propagation (Zhang et al. 2011). Finally, the patterning of cardiac repolarization coordinates the resetting of currents so that the next heartbeat can spread; this is accomplished by a transmural gradient of the *Irx5* transcription factor, which establishes an inverse gradient of ion channels (Costantini et al. 2005).

4 HEART REPAIR AND MAINTENANCE

The mammalian heart cannot regenerate after injury. Because the hearts of other vertebrates, such as fish, regenerate (Poss et al. 2002; Poss 2010), mammalian hearts might have a hidden regenerative potential. Indeed, mammalian hearts have a short-lived capacity for regeneration, which disappears a few days after birth (Porrello et al. 2011). This temporary regenerative capacity seems related to proliferative potential of existing cardiomyocytes (Porrello et al. 2011), which exit the cell cycle almost permanently shortly thereafter, although other experimental approaches have led to suggestions of a role for c-kit$^+$ progenitors in neonatal cardiac regeneration (Jesty et al. 2012). The knowledge acquired from understanding specification and allocation of cardiac lineages may provide strategies for cardiac regeneration (Fig. 6). Regeneration in zebrafish is based on proliferation of cardiomyocytes (Jopling et al. 2010; Kikuchi et al. 2010), and parallel mechanisms might exist between the adult zebrafish and the neonatal mammalian heart. The turnover of cardiac myocytes in the uninjured human heart has been calculated to be ~50% over a lifetime, indicating very little if any ongoing proliferation (Bergmann et al. 2009) (although this estimate has been questioned and is unresolved [Anversa et al. 2012]). After injury, such as after a myocardial infarction, there also does not seem to be significant myocardial proliferation. Several approaches have been suggested to increase cardiac proliferation, and although some success has been reported (Kuhn et al. 2007; Bersell et al. 2009), the numbers of proliferating adult cardiomyocytes are far from those needed to restore an injured heart.

4.1 Endogenous Stem Cells: Evidence for and against

Endogenous cardiac stem cells in the adult heart might be an excellent starting point for devising strategies to promote cardiac regeneration. Several studies claimed to isolate cardiac progenitors of various types from the mouse heart, using a variety of surface markers (Laflamme and Murry 2005, 2011; Leri et al. 2005). For the most part, cells with cardiogenic properties are difficult to identify in the endogenous heart, and thus their identity and lineage origin are not clear. When reintroduced into a damaged heart, these cells seldom become new cardiac myocytes. Thus, under these conditions, they do not possess

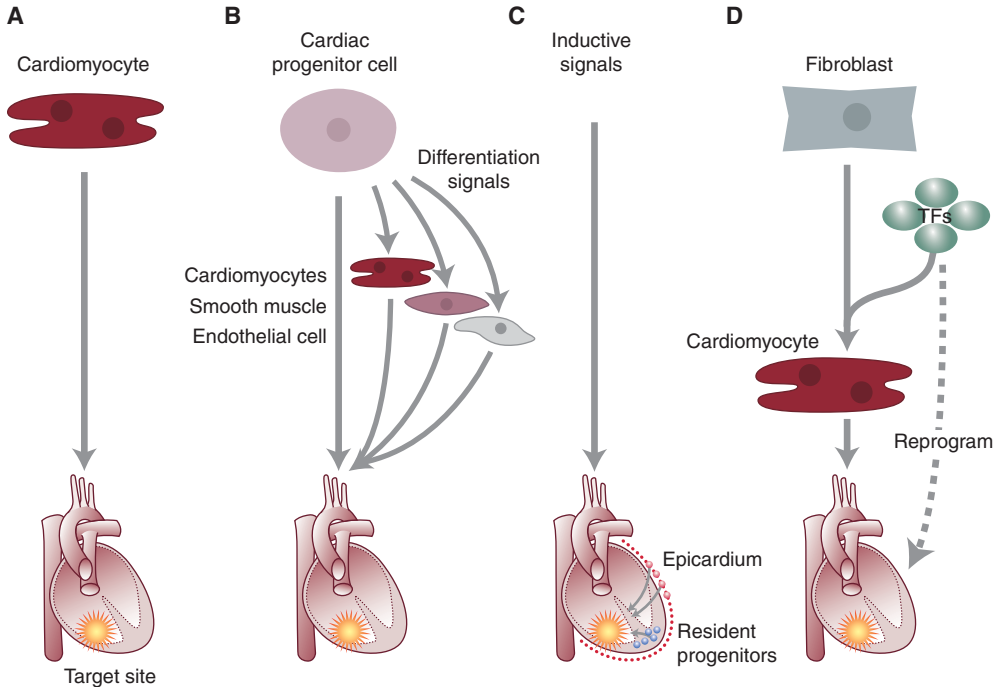

Figure 6. Strategies for cardiac regeneration. Various strategies that have been suggested are shown, including (*A*) implantation of in vitro-generated cardiomyocytes, (*B*) differentiation and implantation of cardiac progenitors, (*C*) mobilization of endogenous precursors by inductive signals, or (*D*) direct reprogramming. (From Alexander and Bruneau 2010.)

cardiogenic properties. For example, c-kit-expressing cells were thought to be resident cardiac stem cells (Beltrami et al. 2003). In the neonatal heart, these cells can function as bona fide cardiac precursors (Jesty et al. 2012). However, their ability to contribute to regenerating adult heart was not confirmed (Tallini et al. 2009; Zaruba et al. 2010; Jesty et al. 2012). In vitro, when differentiated from ES cells, the c-kit$^+$ population varies in its potential for cardiogenesis (Wu et al. 2006; Yang et al. 2008; Zaruba et al. 2010). Isl1-expressing progenitors have been detected postnatally, but their numbers are very small and all but gone in adulthood (Laugwitz et al. 2005, 2008). Finally, a cardiac mesenchymal stem cell-like population was identified in the perivascular niche of the adult mouse heart (Chong et al. 2011). These cells are distinct from c-kit$^+$ cells and express PDGFRα; careful genetic tracing determined that they are not bone marrow derived, and are from a mesodermal lineage, likely originating from the epicardium. Whether these cells can participate in endogenous heart repair is not clear.

The epicardium has been suggested broadly as a source of cells that can regenerate myocardium after injury. Wilm's tumor-1 (WT1)-expressing cells, carefully pulse-chase labeled using the Cre-loxP system, have been shown to contribute to some extent to producing new cardiomyocytes after injury and priming with Thymosin β4 (TB4) (Smart et al. 2011). In these experiments, following myocardial infarction and TB4 treatment, subsets of WT1$^+$ cells expressed Isl1 and Nkx2-5, and lineage-marked cells contributed new cTnT$^+$, functionally integrated cardiomyocytes. The number of new cardiomyocytes that appear to be epicardially derived is very small, but it is intriguing that a population of cells that would normally not contribute new myocardium could be coaxed into doing so under the right conditions.

The best evidence for endogenous renewal of cardiac myocytes and, by extrapolation, the existence of cardiac stem cells comes from a genetic experiment in the mouse. Cardiac myocytes were genetically labeled with cardiac-specific inducible Cre recombinase transgenes (*Myh6::MerCreMer* [Sohal et al. 2001]) during a short time, and the persistence of the label was followed over the lifetime of the mouse (Hsieh et al. 2007). If the number of labeled cells does not change, the originally labeled cells would still be present, and few or no new cardiomyocytes would have been generated. If the number of labeled cells decreases, new cells that did not receive the original genetic tag would have been generated. Although the origin of these cells cannot be ascertained, they would necessarily have arisen later than the early pulse of genetic recombination. During normal aging, there was little change in the number of labeled cells. However, after injuring the heart by imposing a myocardial infarction, the heart had fewer labeled cells. Thus, in

response to injury, over time, cardiac myocytes were renewed from sources other than the genetically labeled cells.

4.2 Strategies for Enhancing Tissue Repair/Regeneration

The knowledge accumulated in studies of the developing heart has become useful in developing strategies to induce the formation of new cardiomyocytes (Fig. 6). Two distinct approaches based on pathways that regulate cardiac differentiation have been deployed to attempt to regenerate injured myocardium.

One approach has been to differentiate ES cells, or induced pluripotent stem (iPS) cells, into cardiomyocytes that could then be implanted into an injured heart. Based on the endogenous signaling cues that are important for inducing cardiac cells in vivo, protocols have been derived that achieve varying degrees of cardiac differentiation from ES and iPS cells (Laflamme et al. 2007; Yang et al. 2008; Kattman et al. 2011). When implanted in an injured mouse or rat heart, however, human ES cell-derived cardiomyocytes cannot achieve long-term contribution to the myocardium, or do not couple effectively (Laflamme et al. 2007; Laflamme and Murry 2011). The lack of long-term engraftment has been considered to be an important hindrance to any potential for use of pluripotent cell-derived cardiomyocytes. It may be that the use of small, rapidly beating rodent hearts is the main source of failure in these sets of experiments. Use of a guinea-pig infarct model has shown that, in fact, human ES cell-derived cardiomyocytes can very effectively incorporate into injured myocardium, couple to their endogenous neighbors, and survive long term (Shiba et al. 2012). Importantly, in this experimental setting, the implantation of in vitro-generated human cardiomyocytes does not lead to arrhythmogenic events, and in fact, is antiarryhthmic.

An attractive scenario in regenerative medicine is to induce one cell type to become another to replace lost or damaged cells. This is known as direct reprogramming (Graf and Enver 2009). In the heart, loss of cardiomyocytes after infarct is accompanied by an overproliferation of fibroblasts, which contribute to formation of scar tissue that impairs the proper contraction of the heart. A desirable strategy would be to convert the excess of fibroblasts into functional cardiomyocytes, which would both replenish the heart with functional units and reduce the amount of scar tissue. Recent studies have shown great promise (Ieda et al. 2010; Qian et al. 2012; Song et al. 2012). In cell culture, introducing three transcription factors (Gata4, Mef2c, and Tbx5 [GMT]), or these three factors plus Hand2 (GHMT), induced cardiac fibroblasts to radically change phenotypes to resemble cardiomyocytes (Ieda et al. 2010; Song et al. 2012). These factors induced fibroblasts to express a full battery of cardiac genes, form sarcomeres, develop cardiomyocyte-like electrical activity, and in a few cases even elicit beating activity. This approach also worked in dermal fibroblasts, indicating that the potential of the introduced factors was not limited to only one cell type. Thus, a limited set of transcription factors could impose an entire cardiac program on a noncardiac cell. This approach has also been extended to human ES cells, to determine if initial success with driving mesoderm to become cardiomyocytes (Takeuchi and Bruneau 2009) could be applied to pluripotent cell in culture. The combination of GATA4, TBX5, NKX2-5, and BAF60c was indeed effective in inducing cardiomyocytes from human ES cells, but at low efficiency (Dixon et al. 2011).

Would a directed differentiation approach be effective in the endogenous heart? This has turned out to be promising (Qian et al. 2012; Song et al. 2012). Injection of viruses overexpressing GMT or GHMT into a mouse heart immediately after a myocardial infarction led to expression of the added factors only in the highly proliferating fibroblasts as ascertained by Cre-loxP-based cell marking. Overexpressing GMT or GHMT in vivo led to generation of new functional cardiomyocytes. A large percentage (~25%) of infected fibroblasts changed their phenotype to become remarkably similar to endogenous cardiomyocytes: They had defined sarcomeres, were rod shaped and binucleated, expressed gap junction proteins and connected to their neighbors, and contracted. Therefore, these factors converted endogenous fibroblasts into functional cardiomyocytes. The increased efficiency in vivo contrasts with the initial foray in cell culture, and predicts that the environment in vivo is more favorable for directed differentiation than culture conditions. The de novo production of cardiomyocytes significantly affected the heart. Cardiac function was partially restored and infarct scar size was greatly reduced. The reduction in scar size cannot be accounted for solely by the production of new myocytes, so there must be some paracrine effects on fibroblast proliferation that contributes as well. This study suggests functional myocardium can be reliably regenerated in vivo by direct reprogramming of endogenous cardiac fibroblasts (Qian et al. 2012; Song et al. 2012). Combined with additional factors, specific types of cardiomyocytes might be generated, such as biological pacemakers, by the inclusion of Tbx3 (Bakker et al. 2012).

4.3 Outlook to the Future: Epigenetic Reprogramming?

The outlook for cardiac regeneration is encouraging. Nevertheless, significant challenges remain, and it is not clear if

strategies that work in a small heart, such as mouse, will work in the much larger hearts of humans. Furthermore, safe delivery of factors that repair the heart will be needed. Direct reprogramming is an exciting and promising approach; lessons learned from reprogramming of somatic cells to induced pluripotent cells may be instructive to develop small-molecule-based strategies of cardiac reprogramming (Xu et al. 2008; Lin et al. 2009; Yamanaka and Blau 2010; Zhu et al. 2010; Robinton and Daley 2012). Reprogramming strategies are likely to benefit from epigenetic manipulation of the genome, to broadly affect or enhance direct conversion of one cell type to another. Significant knowledge of the epigenetic blueprint of the heart will be necessary for this approach to become practical.

5 CONCLUSIONS

The developing heart is a paradigm of organogenesis, and its study has yielded important insights into progenitor and stem cell biology, lineage commitment, and morphogenesis. It illustrates how little we still understand about the formation of this complex and important organ. The study of heart development has also provided key insights into the basis of CHD, which is still prevalent and devastating for the children suffering from them, and their families. Finally, the knowledge obtained from deciphering the cues that form the heart will be critical to developing strategies for cardiac regeneration.

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