HHS Public Access

Author manuscript

Circ Res. Author manuscript; available in PMC 2016 January 16.

Published in final edited form as:

Circ Res. 2015 January 16; 116(2): 341–353. doi:10.1161/CIRCRESAHA.116.302752.

Molecular Regulation of Cardiomyocyte Differentiation

Sharon L. Paige[1], **Karolina Plonowska**[2], **Adele Xu**[2], and **Sean M. Wu**[1,2,3,4,5]

[1]Department of Pediatrics and Division of Pediatric Cardiology, Stanford University School of Medicine, Stanford, CA, 94305, USA

[2]Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA, 94305, USA

[3]Division of Cardiovascular Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, 94305, USA

[4]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, 94305, USA

[5]Child Health Research Institute, Stanford University School of Medicine, Stanford, CA, 94305, USA

Abstract

The heart is the first organ to form during embryonic development. Given the complex nature of cardiac differentiation and morphogenesis, it is not surprising that some form of congenital heart disease is present in approximately one percent of newborns. The molecular determinants of heart development have received much attention over the past several decades. This has been driven in large part by an interest in understanding the etiology of congenital heart disease coupled with the potential of utilizing knowledge from developmental biology to generate functional cells and tissues that could be used for regenerative medicine purposes. In this review, we highlight the critical signaling pathways and transcription factor networks that regulate cardiomyocyte lineage specification in both *in vivo* and *in vitro* models. Special focus will be given to epigenetic regulators that drive the commitment of cardiomyogenic cells from nascent mesoderm and their differentiation into chamber-specific myocytes as well as regulation of myocardial trabeculation.

Keywords

cardiac development; cardiomyocyte; transcription factor; molecular regulation

Introduction

Molecular regulation of heart formation has fascinated biologists for more than a century. Driven by both the need for greater understanding of the etiology of congenital heart disease and its potential in regenerative medicine, investigators in cardiac developmental biology

Correspondence: Sean M. Wu, MD, PhD, Lokey Stem Cell Research Building, Rm G1120A, 265 Campus Dr. Stanford, CA 94305, P: 650-724-4498, F: 650-724-4689, smwu@stanford.edu.

Financial Disclosure and Conflict of Interest

None

have devoted significant efforts in recent years to address fundamental mechanisms that underlie the complex patterning of the developing heart. In this review, we aim to summarize succinctly the critical regulators of this process. In particular, we focus on interactions between signaling molecules and transcription factor networks during cardiomyocyte lineage commitment and myocardial chamber specification. Furthermore, we highlight recent articles in this area and draw particular attention to epigenetic factors that play a role in these processes. For a detailed discussion of earlier works on this topic, we recommend several excellent reviews that address myocardial development in different model organisms including mouse, fish, frogs, and chick.[1, 2] We hope this article will inform and inspire greater interest in cardiac development among investigators in cardiovascular biology and spur new collaborations between basic and clinical scientists interested in congenital heart disease.

Overview of Morphological Development

The following overview is intended to orient the reader with respect to major morphological processes that will be discussed in further detail in subsequent sections. The heart is formed from bilateral heart fields that are established during the gastrulation process (see Figure 1). The heart field precursors migrate anteriorly and laterally from the primitive streak to form the antero-lateral plate mesoderm which undergoes rapid migration towards the midline. Subsequent fusion at the midline, led by closure of the foregut,[3] gives rise to the linear heart tube. Notably, elongation of the heart tube occurs primarily via addition of second heart field (SHF)-derived cardiomyocytes to both poles, producing an embryonic organ that consists of the truncus arteriosus most cranially followed by the bulbus cordis, ventricle, atrium and sinus venosus. It is at this stage that the heart becomes functional as blood is pumped from the venous pole to the arterial pole.[4] The heart's ability to generate blood flow at this stage is critical to the continuation of cardiogenesis and to other processes in embryogenesis that respond to hemodynamic signals.[5–9] The process of rightward looping occurs in conjunction with the addition of the SHF cells to both poles of the linear heart tube. Concurrently, the developing atria migrate and become cranially positioned relative to the ventricles. Complex remodeling, selective proliferation, myocardial subpopulation specification, septation, and valve development then result in the formation of the four-chambered heart.

Specification of cardiac mesoderm and formation of the cardiac crescent

During gastrulation, the three primary germ layers are formed with mesoderm sandwiched between ventral endoderm and dorsal ectoderm. Myocardial precursor cells expressing the T-box transcription factor *T* (*Brachyury*) migrate from the posterior epiblast through the anterior portion of the primitive streak. These cells subsequently move laterally and, as the embryonic coelom forms, will occupy bilateral regions of lateral splanchnic mesoderm that is adjacent to underlying endoderm.[10] The population of cardiac progenitor cells then converges at the midline and organizes into a crescent, with the peak of the crescent located cranially and the tails of the crescent extending caudally. Pro-cardiogenic signals from underlying foregut endoderm and anti-cardiogenic signals from midline structures and the neural plate determine the precise size and shape of the heart fields (Figure 2).[10]

A cascade of transcription factor activation ultimately results in cardiac specification and differentiation. During the primitive streak stage of mouse embryonic development, the T-box transcription factor *Eomesodermin* (*Eomes*) activates the basic helix-loop-helix transcription factor *Mesoderm Posterior 1* (*Mesp1*).[11, 12] *Mesp1* expression at the start of gastrulation represents the first known molecular step towards cardiogenesis. *Mesp1* and family member *Mesp2* are the earliest markers of cardiovascular specification in the developing embryo.[13] The mechanism by which Mesp1 promotes cardiac specification is multi-faceted in that Mesp1 drives expression of cardiac transcription factors while also repressing genes that maintain pluripotency.[14] As cardiac precursors migrate away from the primitive streak to form the cardiac crescent, they down-regulate *Mesp1* and *Mesp2* while activating other transcription factor networks that drive cardiac specification.

Among the pro-cardiogenic factors expressed in endoderm and mesoderm are Hedgehog, bone morphogenetic proteins (BMPs)[15, 16], fibroblast growth factors (FGFs), and non-canonical Wnt/JNK.[17] Canonical Wnt ligands, including Wnt1 and Wnt3a, secreted from the neural tube inhibit cardiac mesoderm specification[18], as do BMP antagonists Noggin and Chordin secreted from the notochord. Canonical Wnt/β-catenin inhibitors, including Dkk-1 and crescent, are also secreted by the endoderm underlying cardiac mesoderm and serve to counteract the inhibitory Wnt signals emanating from the neural plate.[19, 20] Interestingly, selective elimination of canonical Wnt/β-catenin signaling in endoderm via deletion of *β-catenin* results in formation of ectopic cardiac tissue in overlying mesoderm.[21] The mechanism by which inhibition of Wnt/β-catenin signaling in endoderm induces cardiac specification is thought to be due to activation of the homeodomain transcription factor *Hex* in endoderm.[22] Notably, *Hex* is associated with induction of cardiac transcription factors such as *Nkx2-5* and *Tbx5* but does not directly activate expression of cardiac contractile genes, such as those encoding myofilament proteins.[22]

First and Second Heart Fields

The earliest cells to express *Mesp1* form the first heart field (FHF), which makes up the cardiac crescent. A later wave of *Mesp1* produces cardiac progenitors of the second heart field (SHF), which are derived from pharyngeal mesoderm and lie anterior and medial to the cells of the FHF.[23–26] Clonal analysis in mice suggests that these progenitor cells are committed to the FHF or SHF early, likely prior to the onset of *Mesp1* expression.[27] As the cells of the FHF differentiate and proliferate during the formation of the linear heart tube, the cells of the SHF retain their undifferentiated myocardial progenitor identity and become positioned dorsally relative to the heart tube[28]. Lineage tracing studies in both chick and mouse embryos have demonstrated that the cells of the FHF contribute primarily to the left ventricle with small contributions to the atria while the SHF will form the right ventricle, outflow tract, atria and inflow myocardium (see Figure 3).[24, 25, 29–32] Within the SHF, progenitor cells that will give rise to the right ventricle and outflow tract are considered anterior SHF, while precursors of the atria and inflow tract are termed posterior SHF.[33] SHF progenitors demonstrate increased proliferation and delayed differentiation compared to their FHF counterparts[34] and differentiate as they contribute to both the venous and arterial poles of the linear heart tube, promoting its elongation.

The FHF cells are identified by the expression of *Tbx5* and the first wave of *Nkx2-5*.[35, 36] Recently, early expression of ion channel gene *HCN4* was also reported to be FHF-specific, leading to identification of the atrioventricular (AV) node and parts of the early conduction system as FHF-derived structures.[37, 38] Of note, early FHF-specific *HCN4* expression is distinct from later expression in the cardiac conduction system and sinoatrial node.[37] The second heart field is marked by expression of the LIM homeodomain transcription factor *Islet1* (*Isl1*).[31, 35, 39] Recently, expression of *Tbx1* in the second heart field was shown to coordinate SHF contribution to the two poles of the linear heart tube.[40] Other genes expressed in SHF progenitor cells include *Prdm1*, *Pitx2*, *Six1*, *Fgf8*, and *Fgf10*.[28, 31–33, 41, 42] As cardiac differentiation progresses during addition of SHF cells to the linear heart tube, these genes will be down-regulated as the next wave of cardiac transcription factors becomes activated, including *Nkx2-5*, *Gata4* and *Mef2c*.[24, 32] A summary of the key transcription factor interactions that regulate FHF and SHF differentiation is shown in Figure 4.

In both human and mouse embryonic stem (ES) cell models, *Isl1*-expressing cells have been shown to give rise to cardiomyocytes as well as smooth muscle cells and endothelium (Figure 3).[39, 43] The existence of a multipotent *Isl1*+ progenitor cell *in vivo* is postulated from the contribution of *Isl1*-expressing SHF to the smooth muscle cells of the great vessels (aorta and pulmonary trunk) and endothelial cells of the proximal coronary artery. However, limited data exist to support the diversification of cardiovascular lineage cells from a single *Isl1*+ cell. Nevertheless, a population of *Isl1*+ progenitors has been identified in both mouse and human hearts in the fetal and early postnatal stages.[43, 44] Reportedly, these cells represent residual SHF progenitor cells given the significant decline in the number of these cells after early fetal development and the absence of these cells in the adult heart. The potential that these cells may participate in myocardial injury repair has motivated a number of investigators to examine the presence of *Isl1*+ cells in the heart after infarction.[45] Thus far, no reactivation of *Isl1*+ cells or their contribution to new cardiomyocyte formation has been found.

The characteristic delayed differentiation and continued proliferation of SHF progenitors are regulated by FGF, Hedgehog and canonical Wnt/β- catenin signaling pathways. Fgf8 has been identified as a necessary signaling molecule for SHF deployment as evidenced by the observation that conditional ablation of *Fgf8* in mesoderm results in formation of hearts that are without a right ventricle or outflow tract.[46] Other FGF family members involved in SHF development include *Fgf10*[47] and *Fgf3*.[48] *Shh* is expressed in adjacent pharyngeal endoderm and is required for SHF proliferation.[49] Interestingly, Shh has been shown to activate a set of forkhead-containing transcription factors including *Foxa2*, *Foxc1* and *Foxc2*, which then activate *Tbx1*.[50, 51] *Tbx1* in turn is critical for activation of *Fgf3*, *Fgf8* and *Fgf10*, and for suppression of *Mef2c* and SRF.[46, 47, 52–54] Canonical Wnt/β- catenin pathways activate proliferation in SHF cells in mouse embryos and also promote maintenance of multipotency of *Isl1*+ progenitors derived from ES cells.[43, 55]

Neural Crest and Proepicardial Contributions

Other cell lineages also contribute significantly to heart development alongside the first and second heart fields. Classically, the postotic neural crest has been described to give rise to a population of cells, known as the cardiac neural crest, that migrate from the neural tube in a dorsolateral fashion to reach the caudal pharyngeal arches, from where they continue on to migrate into the cardiac outflow tract.[56] These cells are responsible for establishing the aorticopulmonary septum, which divides the outflow tract into the aorta and pulmonary artery, and give rise to the smooth muscle cells in the tunica media of both vessels in the distal region of the arterial pole.[29, 57] Recently, Arima *et al.* have demonstrated that the preotic neural crest also contributes to the conotruncal region and interventricular septum.[58] In addition, Escot et al. have identified SDF1 as a chemotactic agent driving the cardiac neural crest cell migration towards the pharyngeal arches.[59] Further, Holler et al. observed a marked reduction in the neural crest-derived cells found in the outflow tract following loss of *Hand2* expression in murine embryos.[60] Ablation of these cells results in persistent truncus arteriosus, arterial pole alignment defects, and aberrant myocardium calcium transients stemming from elevated *Fgf8* expression, which normally appears to be modulated by the cardiac neural crest cells.[61] In addition, cardiac neural crest will contribute the autonomic and sensory innervation of the heart. Anomalies involving the cardiac neural crest cells are of clinical interest as they are responsible for a multitude of human cardiocraniofacial defects, such as DiGeorge Syndrome (DGS) and velocardiofacial syndrome (VCFS) due to the loss of Tbx1 in the context of 22q11 deletion.[62] The deployment of cardiac neural crest cells in a coordinated fashion and their dependence on exchanges of signal with SHF cells is highlighted by the requirement of Jagged-1/Notch signaling in the SHF cells to regulate Fgf8 expression that is essential for cardiac neural crest cell migration and endothelial-mesenchymal transition. The exogenous replacement of Fgf8 appears to rescue the developmental defect in explant assay of endocardial cushion.[63, 64]

Cells of the proepicardial organ migrate to cover the surface of the developing heart. Though little is known about the molecular drivers of this migration, studies in mouse embryos suggest that the interplay between the α4 integrins and vascular adhesion molecule 1 (VCAM-1) is essential in maintaining the epicardial lining after embryonic day 11.[65, 66] Some of these cells undergo an epithelial-to-mesenchymal transition and invade the underlying myocardium. Del Monte et al. have shown that Notch1 activity may play a critical role in this process by promoting blood vessel progenitor migration through the compact myocardium.[67] These cells then differentiate into cardiac fibroblasts and vascular smooth muscle cells of the coronary vessels.[68, 69] Specific subpopulations from within the proepicardium may also contribute to coronary endothelium.[70] Interestingly, two studies report the differentiation of epicardial cells into cardiomyocytes,[71, 72] however, significant controversies exist in this area. For additional discussion, the readers are encouraged to review articles that cover these lineages in greater detail.[73–76]

Specification of Chamber Myocardium

The cardiomyocytes that make up the linear heart tube are termed “primary myocardium” based on their slow proliferation rate, limited automaticity, slow conduction velocity and poor contractility.[77, 78] The cardiomyocytes that make up the outflow tract, inner curvature, atrioventricular canal and sinus horns retain this primary phenotype. As atrial and ventricular cells selectively proliferate and differentiate, “ballooning” of the outer curvature of the heart tube occurs. The linear heart tube’s peristaltic pumping may drive this ballooning process, as endocardial and myocardial cells alter their shape, size, and proliferation in response to mechanical stress from blood flow and contraction.[79–81] The atrial and ventricular cells obtain a chamber myocardium identity, with associated fast conduction velocity, increased automaticity, increased contractility and enhanced sarcomere organization.[82]

Multiple transcription factors have been shown to regulate cardiac chamber morphogenesis including the T-box transcription factors *Tbx2*, *Tbx3*, *Tbx5* and *Tbx20* as well as *Nkx2-5*, *Gata4*, *Cited1*, *Irx4*, *Irx1*/*Irx3*/*Irx5* and *Hand1* (Figure 5). *Tbx5* and *Tbx20* act in combination with *Nkx2-5* and *Gata4* to promote specification of chamber myocardium, which includes induction of atrial natriuretic factor (encoded by *Nppa*), gap junction proteins *connexin 40* (*Cx40*) and *connexin 43 (Cx43*) as well as the cytoskeletal protein Chisel (encoded by *Smpx*).[4, 82, 83] Additionally, Tbx5 cooperates with Nkx2-5 to regulate expression of the transcriptional repressor Id2, which contributes to patterning of the right and left ventricular bundle branches.[84, 85] The multiple roles of *Tbx5* are consistent with the varied cardiac phenotypes observed in patients with Holt-Oram syndrome.[86] In contrast, *Tbx2* and *Tbx3* can also interact with *Nkx2-5* and *Gata4* to repress chamber specification.[87, 88] Suppression of chamber specification by *Tbx3* allows for the expression of the pacemaker channel *HCN4* in cells at the sinoatrial junction that will give rise to the sinoatrial node.[85, 89]

Regions of chamber versus primary myocardium are exquisitely regulated by overlapping regions of these various T-box transcription factors, leading to the proposed T-box code hypothesis.[1] Whereas *Nkx2-5* and *Gata4* are highly expressed throughout the linear heart tube, the T-box transcription factors have more narrow expression patterns.[78, 83] As a result of retinoic acid signaling, *Tbx5* is expressed in a graded fashion with peak expression caudally in the area of the inflow tract and primitive atrium with diminished expression in the primitive ventricle and absence in the outflow tract.[86, 90] *Tbx2* and *Tbx3* are expressed in the primary myocardium of the outflow tract, inner curvature, AV canal and inflow tract, with *Tbx2* expression extending more rostrally compared to *Tbx3*.[78] *Tbx2* has been shown to inhibit proliferation and suppress expression of chamber myocardium-specific genes including *Cx40*, *Cx43*, *Smpx* and *Nppa*.[87, 91] Notably, *Tbx20* is broadly expressed throughout the linear heart tube and deletion of *Tbx20* in mice results in defects in chamber development, at least in part due to expansion of *Tbx2* expression (Figure 5).[92–95]

Within the chamber myocardium, each of the four chambers is marked by unique gene expression patterns. The mouse atria and ventricles express myosin light chain isoforms *MLC2a* and *MLC2v*, respectively.[96] Both ventricles additionally express the homeobox gene

Irx4, which activates ventricular myosin heavy chain 1 (*MHC1*) and suppresses the atrial *MHC1* isoform in chick.[97] In both mouse and chick, *Tbx5* expression is absent in the right ventricle.[86] In chick, the right ventricle is also distinguished by *Tbx20* expression, which the left ventricle lacks.[35] The basic helix-loop-helix transcription factors *eHAND* and *dHAND* are expressed in the systemic and pulmonary ventricles, respectively, even in situs inversus mouse embryos.[98] In atrial development, high expression of the homeobox gene *Pitx2* in the left atrium suppresses *Shox2*, thereby inhibiting genes associated with sinoatrial node development and preventing ectopic pacemaker specification.[99]

Trabeculation of the Ventricular Myocardium

Trabeculation and subsequent compaction of the ventricular myocardium facilitate septation, confer greater contractility and conductivity, and help establish the coronary circulation system in the developing heart.[100–104] Paracrine signaling between the endocardium and the maturing myocardium is an essential driver for the formation of the organized muscular ridges lining the ventricular wall (Figure 6), a process initiated towards the end of cardiac looping.[105]

Central to endocardium-to-myocardium regulation of trabeculation is the transmembrane receptor Notch1.[101, 106] When the extracellular domain of Notch1 binds to ligands such as Delta4 and Jagged, proteolytic cleavage releases the intracellular domain (N1ICD) into the cytoplasm.[107] N1ICD then associates with its transcriptional cofactor RBPJK and translocates to the nucleus[108, 109] N1ICD/RBPJk upregulates myocardial expression of *BMP10*, which stimulates proliferation by decreasing the activity of cell cycle inhibitor $P57^{kip2}$ and by up-regulating Tbx20 and Hey2 activity.[110, 111] Since *Notch1* is predominantly expressed in endocardial cells lining the base of trabeculae at E9.0, Notch activity therefore promotes growth of the trabecular myocardium.[101] Endothelial-specific ablation of a N1ICD inhibitor, *Fkbp1a*, accordingly results in hypertrabeculation.[112] Curiously, inactivation of Mib1, an ubiquitinase that promotes ligand binding and subsequent activation of Notch1, in the myocardium also leads to hypertrabeculation. Additionally, *Mib1*-deficient mice have an abnormally thin compact myocardial zone.[113] The apparently contradictory effects of Notch1 regulator manipulation suggest that Notch1 plays a complex role in spatiotemporal regulation of trabecular growth.

Notch1 signaling also promotes neuregulin-1 (Nrg1) activity through EphrinB2.[101] Nrg1, one of the endothelial growth factors produced by the endocardium, binds to myocardial tyrosine kinase receptor ErbB4 and leads to its dimerization with ErbB2, thus activating signaling cascades modulating cell growth and migration[114]. *Nrg1*, *ErbB4*, and *ErbB2* null mice die *in utero* around E9.5–10.5 due to embryonic heart failure, with histological analyses revealing ventricular chambers lacking trabeculae.[115–118] Liu et al. have identified ErbB2 as a dual factor in cardiomyocyte proliferation and directional delamination during ventricular trabeculation.[117] Their assessment of the cardiac function in *ErbB2* mutants revealed decreased ventricular cardiac contractility and bradycardia, supporting previous reports that Nrg1/ErbB2/4 signaling modulating trabeculae formation may play an important physiological role in cardiac contractility.[119] Additionally, high-resolution imaging of cardiomyocytes in zebrafish embryos suggests that ErbB2 contributes to robust formation of

cardiomyocyte surface protrusions and contacts between non-neighboring cardiomyocytes, which may be required for initiating trabeculation.[120] Intriguingly, *EphrinB2* expression is altered by shear stress in embryonic stem cells *in vitro*, suggesting that trabeculation may simultaneously contribute to cardiac contractility while responding to the consequential increase in blood flow.[121] This interdependence between cardiogenesis, blood flow, and other embryonic processes presents challenges for experimental design.

Growth factor signaling from the myocardium to the endocardium also plays an essential role in sustaining trabeculation: mice that lack expression of *Angiopoietin-1*, the primary ligand produced in the myocardium for the endocardial Tie2 receptor, display ventricular morphology similar to that of the *Neuregulin/ErbB4* mutants with further aberrant phenotypes such as defective angiogenesis, a less intricately-folded immature endocardium, and embryonic lethality at E12.5.[122] Vascular endothelial growth factor (VEGF) is also produced in the myocardium and binds to the endocardial receptor Flk1. *Flk1*-deficient mice lack some endothelial lineages and therefore do not form trabeculae, causing embryonic lethality by E9.5.[106, 123–125] Conversely, overexpression of *VEGF* results in an attenuated compact zone and hypertrabeculation.[126]

The modes of communication between the endocardium and myocardium may be moderated or complemented by the cardiac jelly, the extracellular matrix between the two layers. Beginning at E7.5 in the normal embryo, expression of hyaluronic acid synthase-2 (*Has2*) contributes to formation of the extracellular matrix, while global *Has2* deficiency manifests as compact ventricular walls lacking trabeculation.[127] A potential mechanism through which *Has2* regulates trabeculation is offered by the example of Has2 activation of ErbB2/3 in cushion mesenchyme formation.[128] In later stages of normal cardiac development, the metalloproteinase ADAMTS1 is highly expressed during E12.5–14.5.[129] During this time, ADAMTS1 eliminates cardiac jelly by cleaving versican, an extracellular matrix proteoglycan.[129, 130] Since versican appears to promote trabecular proliferation, ADAMTS1 expression thereby signals the conclusion of trabecular growth.[130] Based on investigations of similar versican cleavage activity in other embryonic processes, versican proteolysis not only disrupts functions served by intact versican but may also unmask distinct morphogenetic functions of the resulting fragments.[131, 132] Cleavage of versican by ADAMTS1 is facilitated by fibulin-1, another extracellular matrix protein that binds both ADAMTS1 and versican, and global deletion of ADAMTS1 or fibulin-1 results in hypertrabeculation.[129, 130] Endocardial-specific loss around E9.0 of Brg1, an ADAMTS1 inhibitor, allows ADAMTS1 to prematurely thin the cardiac jelly and terminates trabeculation.[129]

The development of the cardiac conduction system provides an additional example of cardiac jelly-mediated endocardium-to-myocardium signaling in relation to trabeculation. Recent work suggests that endocardial signals promote *Cx40* expression in the myocardium and that the cardiac jelly reduces the intensity of these molecular signals. This model is supported by the observation that the atrioventricular junction, where the endocardium and myocardium are separated by relatively thick cardiac jelly during valve formation, has low *Cx40* expression and delayed conduction through the atrioventricular node.[133] Endocardial promotion of connexin expression may also explain why the peripheral ventricular

conduction system, marked by *Cx40* in the mature heart, forms from trabecular cardiomyocytes closest to the endocardium.[134]

To further support the interdependence of trabeculation and cardiac conduction system development, three recent reports have identified mutations in either ion channel *HCN4* or ryanodine receptor *RYR2* in comorbid cases of left ventricular non-compaction and certain familial arrhythmias.[135–137] Whereas earlier surveys of LVNC patients suggested that the non-compact phenotype was arrhythmogenic, these reports motivate further investigation into the mechanisms by which *HCN4* and *RYR2*, genes previously associated with arrhythmic disorders, may also perturb myocardial structure.[138]

Epigenetic Regulation of Heart Development

Acting in concert with signaling pathways and transcription factors, several epigenetic factors play a critical role in modulating cardiac lineage specification and cardiac morphogenesis during development. Included in the category of epigenetic regulators are histone modifications, ATP-dependent chromatin remodeling complexes and DNA methylation. Each of these regulatory modes influences gene expression by modulating the accessibility of regulatory DNA sequences to DNA binding proteins, such as transcription factors. As a result, epigenetic regulation adds an additional layer of complexity to the tight temporal and spatial control of cardiac gene expression during development.

Covalent modifications to histone proteins alter DNA-histone interactions, resulting in the establishment of looser or more accessible versus tighter or more restricted chromatin. Histone acetylation primarily promotes chromatin accessibility, thereby leading to transcriptional activation. The specific role of histone acetyltransferases (HAT) in heart development is still unclear. However, mice who carry a global loss-of-function mutation in the HAT gene *p300* die between E12.5 and E15.5, possibly due to the development of severe heart defects.[139] Interestingly, this effect may be due to acetylation and activation of Gata4 by p300.[140] In addition, mice globally lacking the HAT Moz show aortic arch abnormalities and ventricular septal defects similar to those found in 22q11 deletion syndrome due to decreased acetylation and therefore transcription of *Tbx1*.[141, 142] In contrast to HATs, histone deacetylases (HDACs) remove acetyl groups from histones, resulting in formation of condensed chromatin and transcriptional repression. Cardiac-specific loss of either *Hdac1* or *Hdac2* has no significant effect while double mutants lacking both *Hdac1* and *Hdac2* in myocardium die in the neonatal period due to cardiac arrhythmias and dilated cardiomyopathy.[143] The deacetylase activity of these epigenetic factors may not be limited to histone modification. For example, Hdac2 is thought to limit myocardial proliferation via a partnership with Hopx (homeodomain-only protein) that results in deacetylation of Gata4, thereby decreasing its transcriptional activity.[144, 145] Similarly, Hdac3 may suppress Tbx5 activity by altering acetylation of Tbx5 residues.[146]

In contrast to histone acetylation, the effect of histone methylation is highly residue-specific, with methylation of histone H3 at lysine 4, 36 and 79 leading to gene activation while methylation at lysine 9 and 27 generally results in gene silencing. Both histone methyltransferases and demethylases have been shown to play critical roles in cardiac

development, and *de novo* mutations in genes associated with regulation of histone 3 lysine 4 and lysine 27 methylation have been detected in excess in children with non-familial congenital heart disease.[147] Loss of the histone methyltransferase *Smyd1* results in right ventricular hypoplasia, possibly due to diminished expression of key cardiac transcription factors *Hand2* and *Irx4*.[148] *Jarid2*, a member of the Jumonji family of histone demethylases, is a key regulator of cardiac trabeculation (discussed in the preceding section) via repression of endocardial Notch and Nrg1.[149]

ATP-dependent chromatin remodeling complexes harness energy from ATP hydrolysis to alter nucleosome packaging, and thus DNA accessibility.[140] Of the four families of ATP-dependent chromatin remodelers, the family best studied in cardiac development is the switching defective/sucrose non-fermenting (SWI/SNF) family. The Brg1/BRM-associated factor (BAF) complex contains twelve subunits, including the ATPase subunit encoded by either brahma (*Brm*) or brahma-related gene 1 (*Brg1*).[150] *Brg1* has been shown to genetically interact with several cardiac transcription factors, including *Nkx2-5*, *Gata4*, *Tbx5* and *Tbx20*.[151, 152] Mice that are globally haploinsufficient for *Brg1* show a wide range of mild cardiac defects, including mild septal defects and aberrant conduction. Interestingly, mice with both haploinsufficiency for *Brg1* as well as haploinsufficiency for *Nkx2-5*, *Tbx5* or *Tbx20* show more severe and often lethal cardiac defects than single mutants alone.[153–155] *Brg1* also promotes myocardial proliferation through stimulation of *Bmp10*, and the thin myocardium phenotype observed in the absence of *Brg1* can be rescued with *Bmp10*.[156] Also of note, murine *Brg1* activates fetal beta-myosin heavy chain while repressing adult alpha-myosin heavy chain, thereby maintaining a fetal phenotype during development.[156] Finally, perhaps the most striking evidence demonstrating a central role for chromatin remodeling in cardiomyocyte lineage specification is the observation that overexpression of *Tbx5*, *Gata4*, and the BAF subunit *Baf60c* can promote transdifferentiation of non-cardiac mesoderm into cardiac tissue.[157]

DNA methylation involves the addition of a methyl group to cytosine in so-called CpG islands. Notably, DNA methylation patterns are wiped clean at the time of conception and are gradually re-introduced during embryogenesis. Recent genome-wide analysis of DNA methylation patterns in developing mouse hearts demonstrated overall stability in the amount of DNA methylation between E11.5 and E14.5.[158] However, less than 1% of methylation sites did show differential methylation patterns between the two time-points, of which about two-thirds were more highly methylated at E14.5.[158] Cardiac regulatory genes were highly enriched in this subset, including signaling molecules such as *Wnt2* and *Fgf2* as well as transcription factors such as *Gata6* and *Mef2c*.[158] Among those sites with the greatest amount of differential methylation, half also showed corresponding changes in expression of the associated gene.[158]

Summary

As our understanding of the molecular regulation of cardiac development continues to improve, so will our ability to clarify the determinants of congenital heart disease as well as our capacity to generate cells for regenerative medicine therapies. In recent years, the identification of key transcription factors and their requirement in distinct stages of

cardiomyogenesis has helped lay the groundwork for determining the precise molecular mechanism involved in commitment of cardiac progenitor cells from their mesodermal precursors, chamber-specific cardiomyocytes from heart field progenitors, and trabecular versus compact myocardium from chamber myocytes. Furthermore, the elucidation of critical epigenetic modifiers that regulate expression of cardiac genes in a spatiotemporal manner has helped reveal essential mechanisms that modify chromatin at specific locations for transcription factors to execute their designated roles during development.

Future Perspectives

In order to realize the tremendous potential made by our cumulative investment in cardiac development biology in recent decades to identify the pathogenic mechanisms of congenital heart diseases such as hypoplastic left heart syndrome, transposition of great vessels, double outlet right ventricle, and ventricular non-compaction, further clarification is needed on a number of key unresolved issues. For example, with regards to the understanding of heart field precursor commitment, we need greater insight into the differences in epigenetic regulation between FHF and SHF cells as they may explain the differences in the response to failure between the right and left ventricle. Specifically, the lack of requirement for Isl1 expression in FHF cells and the differential effects of FGF and BMP signaling in SHF cells suggest that the biology of right ventricular cardiomyocytes at the single cell level may be very different from those in the left ventricle.

In the formation of chamber-specific cardiomyocytes, the distinct expression of T-box and Hand transcription factors along the linear and looping heart tube suggest differences in epigenetic regulation between atrial versus ventricular, as well as right versus left ventricular myocyte development. It would be of great value to elucidate the gene expression and epigenetic profile of all chamber myocyte precursors during the linear heart tube stage of development at the single cell level. This level of resolution could help us determine the key regulators at the earliest steps of chamber myocyte formation and their link to chamber-specific malformations.

Finally, in the context of trabecular versus compact myocardium formation for chamber-specific myocytes, we need a greater understanding of the role of specific signaling pathway and biomechanical influences on myocardial maturation. Identifying the answers to these questions may hold the key to our ability to accurately predict and diagnose congenital heart diseases and treat them in utero using novel interventional or molecular therapies. Indeed, one of the most significant barriers to translating pluripotent stem cell (PSC)-derived cardiomyocytes into regenerative therapies is the inability of these cells to reach full maturity both *in vitro* and *in vivo* after transplantation. Despite significant efforts to enhance the maturation of these cells by pacing, stretching, and co-culturing with non-myocytes, they remain largely immature. A better understanding of the key factors that regulate normal cardiomyocyte maturation during embryonic development should help guide, conceptually, the designing of new approaches to enhance the maturation of PSC-derived cardiomyocytes in vitro. If we are successful in this endeavor, the potential for screening and discovering new drugs using PSC-base disease models to treat defective signaling pathways in congenital heart disease could be significantly increased.

In summary, we believe that while major strides have been made in recent years to unravel the molecular underpinnings of cardiomyogenic lineage commitment, expansion, and maturation, there remain significant gaps in our knowledge that await further investigation. With the continuous advancements in stem cell and genome-editing technology, as well as detailed mechanistic analysis using model organisms, we shall be able to identify and pinpoint key regulators of cardiomyogenesis and devise approaches to correct their functional deficiency prior to the development of disease.

Acknowledgments

We apologize to colleagues whose work we could not cite in this brief review article. We thank Dr. Anthony Sturzu for manuscript critique. This article is funded in part by the NIH/NHLBI Progenitor Cell Biology Consortium (U01HL009976-05), NIH Director's Pioneer Award (DP1LM012179-01), the California Institute for Regenerative Medicine (RB3-05129), the American Heart Association (14GRNT18630016), and an Endowed Faculty Scholar Award from the Child Health Research Institute at Stanford (to S.M.W.)

Non-standard abbreviations and acronyms

Mesp1	Mesoderm Posterior 1
BMP	bone morphogenetic protein
FGF	fibroblast growth factor
FHF	first heart field
SHF	second heart field
AV	atrioventricular
LIM	Lin11, Isl1, Mec3
Isl1	Islet1
ES	embryonic stem
DGS	DiGeorge syndrome
VCFS	velocardiofacial syndrome
VCAM-1	vascular adhesion molecule 1
Cx	connexin
MHC	myosin heavy chain
N1ICD	Notch1 intracellular domain
Nrg1	neuregulin-1
VEGF	vascular endothelial growth factor
Has2	hyaluronic acid synthase-2
HAT	histone acetyltransferase
HDAC	histone deacetylase
Hopx	homeodomain-only protein

SWI/SNF	switching defective/sucrose non-fermenting
BAF	Brg1/BRM-associated factor
Brm	brahma
Brg1	brahma-related gene 1
Shh	sonic hedgehog
AVC	atrioventricular canal
p-OFT	proximal outflow tract
d-OFT	distal outflow tract

References

1. Evans SM, Yelon D, Conlon FL, Kirby ML. Myocardial lineage development. Circ Res. 2010; 107:1428–1444. [PubMed: 21148449]
2. Vincent SD, Buckingham ME. How to make a heart: The origin and regulation of cardiac progenitor cells. Curr Top Dev Biol. 2010; 90:1–41. [PubMed: 20691846]
3. Kuo CT, Morrisey EE, Anandappa R, Sigrist K, Lu MM, Parmacek MS, Soudais C, Leiden JM. Gata4 transcription factor is required for ventral morphogenesis and heart tube formation. Genes Dev. 1997; 11:1048–1060. [PubMed: 9136932]
4. Moorman AF, Christoffels VM. Cardiac chamber formation: Development, genes, and evolution. Physiol Rev. 2003; 83:1223–1267. [PubMed: 14506305]
5. Cho CH, Kim SS, Jeong MJ, Lee CO, Shin HS. The na+ -ca2+ exchanger is essential for embryonic heart development in mice. Mol Cells. 2000; 10:712–722. [PubMed: 11211878]
6. Wakimoto K, Kobayashi K, Kuro-O M, Yao A, Iwamoto T, Yanaka N, Kita S, Nishida A, Azuma S, Toyoda Y, Omori K, Imahie H, Oka T, Kudoh S, Kohmoto O, Yazaki Y, Shigekawa M, Imai Y, Nabeshima Y, Komuro I. Targeted disruption of na+/ca2+ exchanger gene leads to cardiomyocyte apoptosis and defects in heartbeat. J Biol Chem. 2000; 275:36991–36998. [PubMed: 10967099]
7. North TE, Goessling W, Peeters M, Li P, Ceol C, Lord AM, Weber GJ, Harris J, Cutting CC, Huang P, Dzierzak E, Zon LI. Hematopoietic stem cell development is dependent on blood flow. Cell. 2009; 137:736–748. [PubMed: 19450519]
8. Reckova M, Rosengarten C, deAlmeida A, Stanley CP, Wessels A, Gourdie RG, Thompson RP, Sedmera D. Hemodynamics is a key epigenetic factor in development of the cardiac conduction system. Circ Res. 2003; 93:77–85. [PubMed: 12775585]
9. Hove JR, Köster RW, Forouhar AS, Acevedo-Bolton G, Fraser SE, Gharib M. Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis. Nature. 2003; 421:172–177. [PubMed: 12520305]
10. Harvey RP. Patterning the vertebrate heart. Nat Rev Genet. 2002; 3:544–556. [PubMed: 12094232]
11. Costello I, Pimeisl IM, Dräger S, Bikoff EK, Robertson EJ, Arnold SJ. The t-box transcription factor eomesodermin acts upstream of mesp1 to specify cardiac mesoderm during mouse gastrulation. Nat Cell Biol. 2011; 13:1084–1091. [PubMed: 21822279]
12. van den Ameele J, Tiberi L, Bondue A, Paulissen C, Herpoel A, Iacovino M, Kyba M, Blanpain C, Vanderhaeghen P. Eomesodermin induces mesp1 expression and cardiac differentiation from embryonic stem cells in the absence of activin. EMBO Rep. 2012; 13:355–362. [PubMed: 22402664]
13. Saga Y, Kitajima S, Miyagawa-Tomita S. Mesp1 expression is the earliest sign of cardiovascular development. Trends Cardiovasc Med. 2000; 10:345–352. [PubMed: 11369261]
14. Bondue A, Lapouge G, Paulissen C, Semeraro C, Iacovino M, Kyba M, Blanpain C. Mesp1 acts as a master regulator of multipotent cardiovascular progenitor specification. Cell Stem Cell. 2008; 3:69–84. [PubMed: 18593560]

15. Schultheiss TM, Burch JB, Lassar AB. A role for bone morphogenetic proteins in the induction of cardiac myogenesis. Genes Dev. 1997; 11:451–462. [PubMed: 9042859]
16. Andrée B, Duprez D, Vorbusch B, Arnold HH, Brand T. Bmp-2 induces ectopic expression of cardiac lineage markers and interferes with somite formation in chicken embryos. Mech Dev. 1998; 70:119–131. [PubMed: 9510029]
17. Pandur P, Lasche M, Eisenberg LM, Kuhl M. Wnt-11 activation of a non-canonical wnt signalling pathway is required for cardiogenesis. Nature. 2002; 418:636–641. [PubMed: 12167861]
18. Tzahor E. Wnt/beta-catenin signaling and cardiogenesis: Timing does matter. Dev Cell. 2007; 13:10–13. [PubMed: 17609106]
19. Marvin MJ, Di Rocco G, Gardiner A, Bush SM, Lassar AB. Inhibition of wnt activity induces heart formation from posterior mesoderm. Genes Dev. 2001; 15:316–327. [PubMed: 11159912]
20. Schneider VA, Mercola M. Wnt antagonism initiates cardiogenesis in xenopus laevis. Genes Dev. 2001; 15:304–315. [PubMed: 11159911]
21. Lickert H, Kutsch S, Kanzler B, Tamai Y, Taketo MM, Kemler R. Formation of multiple hearts in mice following deletion of beta-catenin in the embryonic endoderm. Dev Cell. 2002; 3:171–181. [PubMed: 12194849]
22. Foley AC, Mercola M. Heart induction by wnt antagonists depends on the homeodomain transcription factor hex. Genes Dev. 2005; 19:387–396. [PubMed: 15687261]
23. Kelly RG, Brown NA, Buckingham ME. The arterial pole of the mouse heart forms from fgf10-expressing cells in pharyngeal mesoderm. Dev Cell. 2001; 1:435–440. [PubMed: 11702954]
24. Waldo KL, Kumiski DH, Wallis KT, Stadt HA, Hutson MR, Platt DH, Kirby ML. Conotruncal myocardium arises from a secondary heart field. Development. 2001; 128:3179–3188. [PubMed: 11688566]
25. Mjaatvedt CH, Nakaoka T, Moreno-Rodriguez R, Norris RA, Kern MJ, Eisenberg CA, Turner D, Markwald RR. The outflow tract of the heart is recruited from a novel heart-forming field. Dev Biol. 2001; 238:97–109. [PubMed: 11783996]
26. Lescroart F, Chabab S, Lin X, Rulands S, Paulissen C, Rodolosse A, Auer H, Achouri Y, Dubois C, Bondue A, Simons BD, Blanpain C. Early lineage restriction in temporally distinct populations of mesp1 progenitors during mammalian heart development. Nat Cell Biol. 2014; 16:829–840. [PubMed: 25150979]
27. Devine WP, Wythe JD, George M, Koshiba-Takeuchi K, Bruneau BG. Early patterning and specification of cardiac progenitors in gastrulating mesoderm. Elife. 2014; 3
28. Buckingham M, Meilhac S, Zaffran S. Building the mammalian heart from two sources of myocardial cells. Nat Rev Genet. 2005; 6:826–835. [PubMed: 16304598]
29. Waldo KL, Hutson MR, Ward CC, Zdanowicz M, Stadt HA, Kumiski D, Abu-Issa R, Kirby ML. Secondary heart field contributes myocardium and smooth muscle to the arterial pole of the developing heart. Dev Biol. 2005; 281:78–90. [PubMed: 15848390]
30. Zaffran S, Kelly RG, Meilhac SM, Buckingham ME, Brown NA. Right ventricular myocardium derives from the anterior heart field. Circ Res. 2004; 95:261–268. [PubMed: 15217909]
31. Cai CL, Liang X, Shi Y, Chu PH, Pfaff SL, Chen J, Evans S. Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. Dev Cell. 2003; 5:877–889. [PubMed: 14667410]
32. Verzi MP, McCulley DJ, De Val S, Dodou E, Black BL. The right ventricle, outflow tract, and ventricular septum comprise a restricted expression domain within the secondary/anterior heart field. Dev Biol. 2005; 287:134–145. [PubMed: 16188249]
33. Kelly RG. The second heart field. Curr Top Dev Biol. 2012; 100:33–65. [PubMed: 22449840]
34. Rochais F, Mesbah K, Kelly RG. Signaling pathways controlling second heart field development. Circ Res. 2009; 104:933–942. [PubMed: 19390062]
35. Takeuchi JK, Ohgi M, Koshiba-Takeuchi K, Shiratori H, Sakaki I, Ogura K, Saijoh Y, Ogura T. Tbx5 specifies the left/right ventricles and ventricular septum position during cardiogenesis. Development. 2003; 130:5953–5964. [PubMed: 14573514]
36. Stanley EG, Biben C, Elefanty A, Barnett L, Koentgen F, Robb L, Harvey RP. Efficient cre-mediated deletion in cardiac progenitor cells conferred by a 3'utr-ires-cre allele of the homeobox gene nkx2–5. Int J Dev Biol. 2002; 46:431–439. [PubMed: 12141429]

37. Liang X, Wang G, Lin L, Lowe J, Zhang Q, Bu L, Chen Y, Chen J, Sun Y, Evans SM. Hcn4 dynamically marks the first heart field and conduction system precursors. Circ Res. 2013; 113:399–407. [PubMed: 23743334]
38. Später D, Abramczuk MK, Buac K, Zangi L, Stachel MW, Clarke J, Sahara M, Ludwig A, Chien KR. A hcn4+ cardiomyogenic progenitor derived from the first heart field and human pluripotent stem cells. Nat Cell Biol. 2013; 15:1098–1106. [PubMed: 23974038]
39. Moretti A, Caron L, Nakano A, Lam JT, Bernshausen A, Chen Y, Qyang Y, Bu L, Sasaki M, Martin-Puig S, Sun Y, Evans SM, Laugwitz KL, Chien KR. Multipotent embryonic isl1+ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. Cell. 2006; 127:1151–1165. [PubMed: 17123592]
40. Rana MS, Theveniau-Ruissy M, De Bono C, Mesbah K, Francou A, Rammah M, Dominguez JN, Roux M, Laforest B, Anderson RH, Mohun TJ, Zaffran S, Christoffels VM, Kelly RG. Tbx1 coordinates addition of posterior second heart field progenitor cells to the arterial and venous poles of the heart. Circ Res. 2014
41. Vincent SD, Mayeuf-Louchart A, Watanabe Y, Brzezinski JA, Miyagawa-Tomita S, Kelly RG, Buckingham M. Prdm1 functions in the mesoderm of the second heart field, where it interacts genetically with tbx1, during outflow tract morphogenesis in the mouse embryo. Hum Mol Genet. 2014
42. Ma HY, Xu J, Eng D, Gross MK, Kioussi C. Pitx2-mediated cardiac outflow tract remodeling. Dev Dyn. 2013; 242:456–468. [PubMed: 23361844]
43. Bu L, Jiang X, Martin-Puig S, Caron L, Zhu S, Shao Y, Roberts DJ, Huang PL, Domian IJ, Chien KR. Human isl1 heart progenitors generate diverse multipotent cardiovascular cell lineages. Nature. 2009; 460:113–117. [PubMed: 19571884]
44. Laugwitz KL, Moretti A, Lam J, Gruber P, Chen Y, Woodard S, Lin LZ, Cai CL, Lu MM, Reth M, Platoshyn O, Yuan JX, Evans S, Chien KR. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature. 2005; 433:647–653. [PubMed: 15703750]
45. Weinberger F, Mehrkens D, Friedrich FW, Stubbendorff M, Hua X, Müller JC, Schrepfer S, Evans SM, Carrier L, Eschenhagen T. Localization of islet-1-positive cells in the healthy and infarcted adult murine heart. Circ Res. 2012; 110:1303–1310. [PubMed: 22427341]
46. Park EJ, Ogden LA, Talbot A, Evans S, Cai CL, Black BL, Frank DU, Moon AM. Required, tissue-specific roles for fgf8 in outflow tract formation and remodeling. Development. 2006; 133:2419–2433. [PubMed: 16720879]
47. Watanabe Y, Zaffran S, Kuroiwa A, Higuchi H, Ogura T, Harvey RP, Kelly RG, Buckingham M. Fibroblast growth factor 10 gene regulation in the second heart field by tbx1, nkx2–5, and islet1 reveals a genetic switch for down-regulation in the myocardium. Proc Natl Acad Sci U S A. 2012; 109:18273–18280. [PubMed: 23093675]
48. Zaffran S, Kelly RG. New developments in the second heart field. Differentiation. 2012; 84:17–24. [PubMed: 22521611]
49. Dyer LA, Kirby ML. Sonic hedgehog maintains proliferation in secondary heart field progenitors and is required for normal arterial pole formation. Dev Biol. 2009; 330:305–317. [PubMed: 19361493]
50. Yamagishi H, Maeda J, Hu T, McAnally J, Conway SJ, Kume T, Meyers EN, Yamagishi C, Srivastava D. Tbx1 is regulated by tissue-specific forkhead proteins through a common sonic hedgehog-responsive enhancer. Genes Dev. 2003; 17:269–281. [PubMed: 12533514]
51. Seo S, Kume T. Forkhead transcription factors, foxc1 and foxc2, are required for the morphogenesis of the cardiac outflow tract. Dev Biol. 2006; 296:421–436. [PubMed: 16839542]
52. Aggarwal VS, Liao J, Bondarev A, Schimmang T, Lewandoski M, Locker J, Shanske A, Campione M, Morrow BE. Dissection of tbx1 and fgf interactions in mouse models of 22q11ds suggests functional redundancy. Hum Mol Genet. 2006; 15:3219–3228. [PubMed: 17000704]
53. Pane LS, Zhang Z, Ferrentino R, Huynh T, Cutillo L, Baldini A. Tbx1 is a negative modulator of mef2c. Hum Mol Genet. 2012; 21:2485–2496. [PubMed: 22367967]
54. Chen L, Fulcoli FG, Tang S, Baldini A. Tbx1 regulates proliferation and differentiation of multipotent heart progenitors. Circ Res. 2009; 105:842–851. [PubMed: 19745164]

55. Lin L, Cui L, Zhou W, Dufort D, Zhang X, Cai CL, Bu L, Yang L, Martin J, Kemler R, Rosenfeld MG, Chen J, Evans SM. Beta-catenin directly regulates islet1 expression in cardiovascular progenitors and is required for multiple aspects of cardiogenesis. Proc Natl Acad Sci U S A. 2007; 104:9313–9318. [PubMed: 17519333]
56. Kirby ML, Hutson MR. Factors controlling cardiac neural crest cell migration. Cell Adh Migr. 2010; 4
57. Hutson MR, Kirby ML. Model systems for the study of heart development and disease. Cardiac neural crest and conotruncal malformations. Semin Cell Dev Biol. 2007; 18:101–110. [PubMed: 17224285]
58. Arima Y, Miyagawa-Tomita S, Maeda K, Asai R, Seya D, Minoux M, Rijli FM, Nishiyama K, Kim KS, Uchijima Y, Ogawa H, Kurihara Y, Kurihara H. Preotic neural crest cells contribute to coronary artery smooth muscle involving endothelin signalling. Nat Commun. 2012; 3:1267. [PubMed: 23232397]
59. Escot S, Blavet C, Härtle S, Duband JL, Fournier-Thibault C. Misregulation of sdf1-cxcr4 signaling impairs early cardiac neural crest cell migration leading to conotruncal defects. Circ Res. 2013; 113:505–516. [PubMed: 23838132]
60. Holler KL, Hendershot TJ, Troy SE, Vincentz JW, Firulli AB, Howard MJ. Targeted deletion of hand2 in cardiac neural crest-derived cells influences cardiac gene expression and outflow tract development. Dev Biol. 2010; 341:291–304. [PubMed: 20144608]
61. Farrell MJ, Burch JL, Wallis K, Rowley L, Kumiski D, Stadt H, Godt RE, Creazzo TL, Kirby ML. Fgf-8 in the ventral pharynx alters development of myocardial calcium transients after neural crest ablation. J Clin Invest. 2001; 107:1509–1517. [PubMed: 11413158]
62. Keyte A, Hutson MR. The neural crest in cardiac congenital anomalies. Differentiation. 2012; 84:25–40. [PubMed: 22595346]
63. High FA, Jain R, Stoller JZ, Antonucci NB, Lu MM, Loomes KM, Kaestner KH, Pear WS, Epstein JA. Murine jagged1/notch signaling in the second heart field orchestrates fgf8 expression and tissue-tissue interactions during outflow tract development. J Clin Invest. 2009; 119:1986–1996. [PubMed: 19509466]
64. de la Pompa JL, Epstein JA. Coordinating tissue interactions: Notch signaling in cardiac development and disease. Dev Cell. 2012; 22:244–254. [PubMed: 22340493]
65. Yang JT, Rayburn H, Hynes RO. Cell adhesion events mediated by alpha 4 integrins are essential in placental and cardiac development. Development. 1995; 121:549–560. [PubMed: 7539359]
66. Kwee L, Baldwin HS, Shen HM, Stewart CL, Buck C, Buck CA, Labow MA. Defective development of the embryonic and extraembryonic circulatory systems in vascular cell adhesion molecule (vcam-1) deficient mice. Development. 1995; 121:489–503. [PubMed: 7539357]
67. del Monte G, Casanova JC, Guadix JA, MacGrogan D, Burch JB, Pérez-Pomares JM, de la Pompa JL. Differential notch signaling in the epicardium is required for cardiac inflow development and coronary vessel morphogenesis. Circ Res. 2011; 108:824–836. [PubMed: 21311046]
68. Olivey HE, Compton LA, Barnett JV. Coronary vessel development: The epicardium delivers. Trends Cardiovasc Med. 2004; 14:247–251. [PubMed: 15451517]
69. Red-Horse K, Ueno H, Weissman IL, Krasnow MA. Coronary arteries form by developmental reprogramming of venous cells. Nature. 2010; 464:549–553. [PubMed: 20336138]
70. Katz TC, Singh MK, Degenhardt K, Rivera-Feliciano J, Johnson RL, Epstein JA, Tabin CJ. Distinct compartments of the proepicardial organ give rise to coronary vascular endothelial cells. Dev Cell. 2012; 22:639–650. [PubMed: 22421048]
71. Zhou B, Ma Q, Rajagopal S, Wu SM, Domian I, Rivera-Feliciano J, Jiang D, von Gise A, Ikeda S, Chien KR, Pu WT. Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. Nature. 2008; 454:109–113. [PubMed: 18568026]
72. Cai CL, Martin JC, Sun Y, Cui L, Wang L, Ouyang K, Yang L, Bu L, Liang X, Zhang X, Stallcup WB, Denton CP, McCulloch A, Chen J, Evans SM. A myocardial lineage derives from tbx18 epicardial cells. Nature. 2008; 454:104–108. [PubMed: 18480752]
73. Masters M, Riley PR. The epicardium signals the way towards heart regeneration. Stem Cell Res. 2014

74. Nakajima Y, Imanaka-Yoshida K. New insights into the developmental mechanisms of coronary vessels and epicardium. Int Rev Cell Mol Biol. 2013; 303:263–317. [PubMed: 23445813]
75. Ruiz-Villalba A, Pérez-Pomares JM. The expanding role of the epicardium and epicardial-derived cells in cardiac development and disease. Curr Opin Pediatr. 2012; 24:569–576. [PubMed: 22890066]
76. Pérez-Pomares JM, de la Pompa JL. Signaling during epicardium and coronary vessel development. Circ Res. 2011; 109:1429–1442. [PubMed: 22158650]
77. Soufan AT, van den Berg G, Ruijter JM, de Boer PA, van den Hoff MJ, Moorman AF. Regionalized sequence of myocardial cell growth and proliferation characterizes early chamber formation. Circ Res. 2006; 99:545–552. [PubMed: 16888243]
78. Rana MS, Christoffels VM, Moorman AF. A molecular and genetic outline of cardiac morphogenesis. Acta Physiol (Oxf). 2013; 207:588–615. [PubMed: 23297764]
79. Lin YF, Swinburne I, Yelon D. Multiple influences of blood flow on cardiomyocyte hypertrophy in the embryonic zebrafish heart. Dev Biol. 2012; 362:242–253. [PubMed: 22192888]
80. Dietrich AC, Lombardo VA, Abdelilah-Seyfried S. Blood flow and bmp signaling control endocardial chamber morphogenesis. Dev Cell. 2014; 30:367–377. [PubMed: 25158852]
81. Auman HJ, Coleman H, Riley HE, Olale F, Tsai HJ, Yelon D. Functional modulation of cardiac form through regionally confined cell shape changes. PLoS Biol. 2007; 5:e53. [PubMed: 17311471]
82. Christoffels VM, Smits GJ, Kispert A, Moorman AF. Development of the pacemaker tissues of the heart. Circ Res. 2010; 106:240–254. [PubMed: 20133910]
83. Hoogaars WM, Barnett P, Moorman AF, Christoffels VM. T-box factors determine cardiac design. Cell Mol Life Sci. 2007; 64:646–660. [PubMed: 17380306]
84. Moskowitz IP, Pizard A, Patel VV, Bruneau BG, Kim JB, Kupershmidt S, Roden D, Berul CI, Seidman CE, Seidman JG. The t-box transcription factor tbx5 is required for the patterning and maturation of the murine cardiac conduction system. Development. 2004; 131:4107–4116. [PubMed: 15289437]
85. Hatcher CJ, Basson CT. Specification of the cardiac conduction system by transcription factors. Circ Res. 2009; 105:620–630. [PubMed: 19797194]
86. Bruneau BG, Logan M, Davis N, Levi T, Tabin CJ, Seidman JG, Seidman CE. Chamber-specific cardiac expression of tbx5 and heart defects in holt-oram syndrome. Dev Biol. 1999; 211:100–108. [PubMed: 10373308]
87. Christoffels VM, Hoogaars WM, Tessari A, Clout DE, Moorman AF, Campione M. T-box transcription factor tbx2 represses differentiation and formation of the cardiac chambers. Dev Dyn. 2004; 229:763–770. [PubMed: 15042700]
88. Singh R, Hoogaars WM, Barnett P, Grieskamp T, Rana MS, Buermans H, Farin HF, Petry M, Heallen T, Martin JF, Moorman AF, ‘t Hoen PA, Kispert A, Christoffels VM. Tbx2 and tbx3 induce atrioventricular myocardial development and endocardial cushion formation. Cell Mol Life Sci. 2012; 69:1377–1389. [PubMed: 22130515]
89. Mommersteeg MT, Hoogaars WM, Prall OW, de Gier-de Vries C, Wiese C, Clout DE, Papaioannou VE, Brown NA, Harvey RP, Moorman AF, Christoffels VM. Molecular pathway for the localized formation of the sinoatrial node. Circ Res. 2007; 100:354–362. [PubMed: 17234970]
90. Liberatore CM, Searcy-Schrick RD, Yutzey KE. Ventricular expression of tbx5 inhibits normal heart chamber development. Dev Biol. 2000; 223:169–180. [PubMed: 10864469]
91. Habets PE, Moorman AF, Clout DE, van Roon MA, Lingbeek M, van Lohuizen M, Campione M, Christoffels VM. Cooperative action of tbx2 and nkx2.5 inhibits anf expression in the atrioventricular canal: Implications for cardiac chamber formation. Genes Dev. 2002; 16:1234–1246. [PubMed: 12023302]
92. Cai CL, Zhou W, Yang L, Bu L, Qyang Y, Zhang X, Li X, Rosenfeld MG, Chen J, Evans S. T-box genes coordinate regional rates of proliferation and regional specification during cardiogenesis. Development. 2005; 132:2475–2487. [PubMed: 15843407]
93. Singh MK, Christoffels VM, Dias JM, Trowe MO, Petry M, Schuster-Gossler K, Bürger A, Ericson J, Kispert A. Tbx20 is essential for cardiac chamber differentiation and repression of tbx2. Development. 2005; 132:2697–2707. [PubMed: 15901664]

94. Stennard FA, Harvey RP. T-box transcription factors and their roles in regulatory hierarchies in the developing heart. Development. 2005; 132:4897–4910. [PubMed: 16258075]

95. Takeuchi JK, Mileikovskaia M, Koshiba-Takeuchi K, Heidt AB, Mori AD, Arruda EP, Gertsenstein M, Georges R, Davidson L, Mo R, Hui CC, Henkelman RM, Nemer M, Black BL, Nagy A, Bruneau BG. Tbx20 dose-dependently regulates transcription factor networks required for mouse heart and motoneuron development. Development. 2005; 132:2463–2474. [PubMed: 15843409]

96. Small EM, Krieg PA. Molecular regulation of cardiac chamber-specific gene expression. Trends Cardiovasc Med. 2004; 14:13–18. [PubMed: 14720469]

97. Bao ZZ, Bruneau BG, Seidman JG, Seidman CE, Cepko CL. Regulation of chamber-specific gene expression in the developing heart by irx4. Science. 1999; 283:1161–1164. [PubMed: 10024241]

98. Thomas T, Yamagishi H, Overbeek PA, Olson EN, Srivastava D. The bhlh factors, dhand and ehand, specify pulmonary and systemic cardiac ventricles independent of left-right sidedness. Dev Biol. 1998; 196:228–236. [PubMed: 9576835]

99. Wang J, Klysik E, Sood S, Johnson RL, Wehrens XH, Martin JF. Pitx2 prevents susceptibility to atrial arrhythmias by inhibiting left-sided pacemaker specification. Proc Natl Acad Sci U S A. 2010; 107:9753–9758. [PubMed: 20457925]

100. Ben-Shachar G, Arcilla RA, Lucas RV, Manasek FJ. Ventricular trabeculations in the chick embryo heart and their contribution to ventricular and muscular septal development. Circ Res. 1985; 57:759–766. [PubMed: 4053307]

101. Grego-Bessa J, Luna-Zurita L, del Monte G, Bolos V, Melgar P, Arandilla A, Garratt AN, Zang H, Mukouyama YS, Chen H, Shou W, Ballestar E, Esteller M, Rojas A, Perez-Pomares JM, de la Pompa JL. Notch signaling is essential for ventricular chamber development. Developmental cell. 2007; 12:415–429. [PubMed: 17336907]

102. Rentschler S, Zander J, Meyers K, France D, Levine R, Porter G, Rivkees SA, Morley GE, Fishman GI. Neuregulin-1 promotes formation of the murine cardiac conduction system. Proc Natl Acad Sci U S A. 2002; 99:10464–10469. [PubMed: 12149465]

103. Tian X, Hu T, Zhang H, He L, Huang X, Liu Q, Yu W, He L, Yang Z, Yan Y, Yang X, Zhong TP, Pu WT, Zhou B. Vessel formation. De novo formation of a distinct coronary vascular population in neonatal heart. Science (New York, NY). 2014; 345:90–94.

104. Moorman AF, Christoffels VM. Development of the cardiac conduction system: A matter of chamber development. Novartis Found Symp. 2003; 250:25–34. discussion 34–43, 276–279. [PubMed: 12956322]

105. Moorman AFM, Christoffels VM. Cardiac chamber formation: Development, genes, and evolution. 2003

106. Zhang W, Chen H, Qu X, Chang CP, Shou W. Molecular mechanism of ventricular trabeculation/compaction and the pathogenesis of the left ventricular noncompaction cardiomyopathy (lvnc). Am J Med Genet C Semin Med Genet. 2013; 163C:144–156. [PubMed: 23843320]

107. Kopan R. Notch: A membrane-bound transcription factor. J Cell Sci. 2002; 115:1095–1097. [PubMed: 11884509]

108. Tamura K, Taniguchi Y, Minoguchi S, Sakai T, Tun T, Furukawa T, Honjo T. Physical interaction between a novel domain of the receptor notch and the transcription factor rbp-j kappa/su(h). Curr Biol. 1995; 5:1416–1423. [PubMed: 8749394]

109. Jarriault S, Brou C, Logeat F, Schroeter EH, Kopan R, Israel A. Signalling downstream of activated mammalian notch. Nature. 1995; 377:355–358. [PubMed: 7566092]

110. Chen H, Shi S, Acosta L, Li W, Lu J, Bao S, Chen Z, Yang Z, Schneider MD, Chien KR, Conway SJ, Yoder MC, Haneline LS, Franco D, Shou W. Bmp10 is essential for maintaining cardiac growth during murine cardiogenesis. Development. 2004; 131:2219–2231. [PubMed: 15073151]

111. Zhang W, Chen H, Wang Y, Yong W, Zhu W, Liu Y, Wagner GR, Payne RM, Field LJ, Xin H, Cai CL, Shou W. Tbx20 transcription factor is a downstream mediator for bone morphogenetic protein-10 in regulating cardiac ventricular wall development and function. J Biol Chem. 2011; 286:36820–36829. [PubMed: 21890625]

112. Chen H, Zhang W, Sun X, Yoshimoto M, Chen Z, Zhu W, Liu J, Shen Y, Yong W, Li D, Zhang J, Lin Y, Li B, VanDusen NJ, Snider P, Schwartz RJ, Conway SJ, Field LJ, Yoder MC, Firulli

AB, Carlesso N, Towbin JA, Shou W. Fkbp1a controls ventricular myocardium trabeculation and compaction by regulating endocardial notch1 activity. Development. 2013; 140:1946–1957. [PubMed: 23571217]

113. Luxán G, Casanova JC, Martínez-Poveda B, Prados B, D'Amato G, MacGrogan D, Gonzalez-Rajal A, Dobarro D, Torroja C, Martinez F, Izquierdo-García JL, Fernández-Friera L, Sabater-Molina M, Kong YY, Pizarro G, Ibañez B, Medrano C, García-Pavía P, Gimeno JR, Monserrat L, Jiménez-Borreguero LJ, de la Pompa JL. Mutations in the notch pathway regulator mib1 cause left ventricular noncompaction cardiomyopathy. Nat Med. 2013; 19:193–201. [PubMed: 23314057]
114. Yarden Y, Sliwkowski MX. Untangling the erbb signalling network. Nat Rev Mol Cell Biol. 2001; 2:127–137. [PubMed: 11252954]
115. Meyer D, Birchmeier C. Multiple essential functions of neuregulin in development. Nature. 1995; 378:386–390. [PubMed: 7477375]
116. Gassmann M, Casagranda F, Orioli D, Simon H, Lai C, Klein R, Lemke G. Aberrant neural and cardiac development in mice lacking the erbb4 neuregulin receptor. Nature. 1995; 378:390–394. [PubMed: 7477376]
117. Liu J, Bressan M, Hassel D, Huisken J, Staudt D, Kikuchi K, Poss KD, Mikawa T, Stainier DY. A dual role for erbb2 signaling in cardiac trabeculation. Development. 2010; 137:3867–3875. [PubMed: 20978078]
118. Chan R, Hardy WR, Laing MA, Hardy SE, Muller WJ. The catalytic activity of the erbb-2 receptor tyrosine kinase is essential for embryonic development. Molecular and cellular biology. 2002; 22:1073–1078. [PubMed: 11809799]
119. Liu X, Gu X, Li Z, Li X, Li H, Chang J, Chen P, Jin J, Xi B, Chen D, Lai D, Graham RM, Zhou M. Neuregulin-1/erbb-activation improves cardiac function and survival in models of ischemic, dilated, and viral cardiomyopathy. Journal of the American College of Cardiology. 2006; 48:1438–1447. [PubMed: 17010808]
120. Staudt DW, Liu J, Thorn KS, Stuurman N, Liebling M, Stainier DY. High-resolution imaging of cardiomyocyte behavior reveals two distinct steps in ventricular trabeculation. Development. 2014; 141:585–593. [PubMed: 24401373]
121. Masumura T, Yamamoto K, Shimizu N, Obi S, Ando J. Shear stress increases expression of the arterial endothelial marker ephrinb2 in murine es cells via the vegf-notch signaling pathways. Arterioscler Thromb Vasc Biol. 2009; 29:2125–2131. [PubMed: 19797707]
122. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, Yancopoulos GD. Requisite role of angiopoietin-1, a ligand for the tie2 receptor, during embryonic angiogenesis. Cell. 1996; 87:1171–1180. [PubMed: 8980224]
123. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC. Failure of blood-island formation and vasculogenesis in flk-1-deficient mice. Nature. 1995; 376:62–66. [PubMed: 7596435]
124. Milgrom-Hoffman M, Harrelson Z, Ferrara N, Zelzer E, Evans SM, Tzahor E. The heart endocardium is derived from vascular endothelial progenitors. Development. 2011; 138:4777–4787. [PubMed: 21989917]
125. Tian Y, Morrisey EE. Importance of myocyte-nonmyocyte interactions in cardiac development and disease. Circ Res. 2012; 110:1023–1034. [PubMed: 22461366]
126. Miquerol L, Langille BL, Nagy A. Embryonic development is disrupted by modest increases in vascular endothelial growth factor gene expression. Development. 2000; 127:3941–3946. [PubMed: 10952892]
127. Camenisch TD, Spicer AP, Brehm-Gibson T, Biesterfeldt J, Augustine ML, Calabro A, Kubalak S, Klewer SE, McDonald JA. Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. J Clin Invest. 2000; 106:349–360. [PubMed: 10930438]
128. Camenisch TD, Schroeder JA, Bradley J, Klewer SE, McDonald JA. Heart-valve mesenchyme formation is dependent on hyaluronan-augmented activation of erbb2-erbb3 receptors. Nat Med. 2002; 8:850–855. [PubMed: 12134143]

129. Stankunas K, Hang CT, Tsun ZY, Chen H, Lee NV, Wu JI, Shang C, Bayle JH, Shou W, Iruela-Arispe ML, Chang CP. Endocardial brg1 represses adamts1 to maintain the microenvironment for myocardial morphogenesis. Dev Cell. 2008; 14:298–311. [PubMed: 18267097]

130. Cooley MA, Fresco VM, Dorlon ME, Twal WO, Lee NV, Barth JL, Kern CB, Iruela-Arispe ML, Argraves WS. Fibulin-1 is required during cardiac ventricular morphogenesis for versican cleavage, suppression of erbb2 and erk1/2 activation, and to attenuate trabecular cardiomyocyte proliferation. Dev Dyn. 2012; 241:303–314. [PubMed: 22183742]

131. Kern CB, Wessels A, McGarity J, Dixon LJ, Alston E, Argraves WS, Geeting D, Nelson CM, Menick DR, Apte SS. Reduced versican cleavage due to adamts9 haploinsufficiency is associated with cardiac and aortic anomalies. Matrix Biol. 2010; 29:304–316. [PubMed: 20096780]

132. Kern CB, Norris RA, Thompson RP, Argraves WS, Fairey SE, Reyes L, Hoffman S, Markwald RR, Mjaatvedt CH. Versican proteolysis mediates myocardial regression during outflow tract development. Dev Dyn. 2007; 236:671–683. [PubMed: 17226818]

133. Bressan M, Yang PB, Louie JD, Navetta AM, Garriock RJ, Mikawa T. Reciprocal myocardial-endocardial interactions pattern the delay in atrioventricular junction conduction. Development. 2014; 141:4149–4157. [PubMed: 25273084]

134. Christoffels VM, Moorman AF. Development of the cardiac conduction system: Why are some regions of the heart more arrhythmogenic than others? Circ Arrhythm Electrophysiol. 2009; 2:195–207. [PubMed: 19808465]

135. Milano A, Vermeer AM, Lodder EM, Barc J, Verkerk AO, Postma AV, van der Bilt IA, Baars MJ, van Haelst PL, Caliskan K, Hoedemaekers YM, Le Scouarnec S, Redon R, Pinto YM, Christiaans I, Wilde AA, Bezzina CR. Hcn4 mutations in multiple families with bradycardia and left ventricular noncompaction cardiomyopathy. J Am Coll Cardiol. 2014; 64:745–756. [PubMed: 25145517]

136. Schweizer PA, Schröter J, Greiner S, Haas J, Yampolsky P, Mereles D, Buss SJ, Seyler C, Bruehl C, Draguhn A, Koenen M, Meder B, Katus HA, Thomas D. The symptom complex of familial sinus node dysfunction and myocardial noncompaction is associated with mutations in the hcn4 channel. J Am Coll Cardiol. 2014; 64:757–767. [PubMed: 25145518]

137. Ohno S, Omura M, Kawamura M, Kimura H, Itoh H, Makiyama T, Ushinohama H, Makita N, Horie M. Exon 3 deletion of ryr2 encoding cardiac ryanodine receptor is associated with left ventricular non-compaction. Europace. 2014

138. Brescia ST, Rossano JW, Pignatelli R, Jefferies JL, Price JF, Decker JA, Denfield SW, Dreyer WJ, Smith O, Towbin JA, Kim JJ. Mortality and sudden death in pediatric left ventricular noncompaction in a tertiary referral center. Circulation. 2013; 127:2202–2208. [PubMed: 23633270]

139. Shikama N, Lutz W, Kretzschmar R, Sauter N, Roth JF, Marino S, Wittwer J, Scheidweiler A, Eckner R. Essential function of p300 acetyltransferase activity in heart, lung and small intestine formation. EMBO J. 2003; 22:5175–5185. [PubMed: 14517255]

140. Chang CP, Bruneau BG. Epigenetics and cardiovascular development. Annu Rev Physiol. 2012; 74:41–68. [PubMed: 22035349]

141. Voss AK, Vanyai HK, Collin C, Dixon MP, McLennan TJ, Sheikh BN, Scambler P, Thomas T. Moz regulates the tbx1 locus, and moz mutation partially phenocopies digeorge syndrome. Dev Cell. 2012; 23:652–663. [PubMed: 22921202]

142. McElhinney DB, Driscoll DA, Levin ER, Jawad AF, Emanuel BS, Goldmuntz E. Chromosome 22q11 deletion in patients with ventricular septal defect: Frequency and associated cardiovascular anomalies. Pediatrics. 2003; 112:e472. [PubMed: 14654648]

143. Montgomery RL, Davis CA, Potthoff MJ, Haberland M, Fielitz J, Qi X, Hill JA, Richardson JA, Olson EN. Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and contractility. Genes Dev. 2007; 21:1790–1802. [PubMed: 17639084]

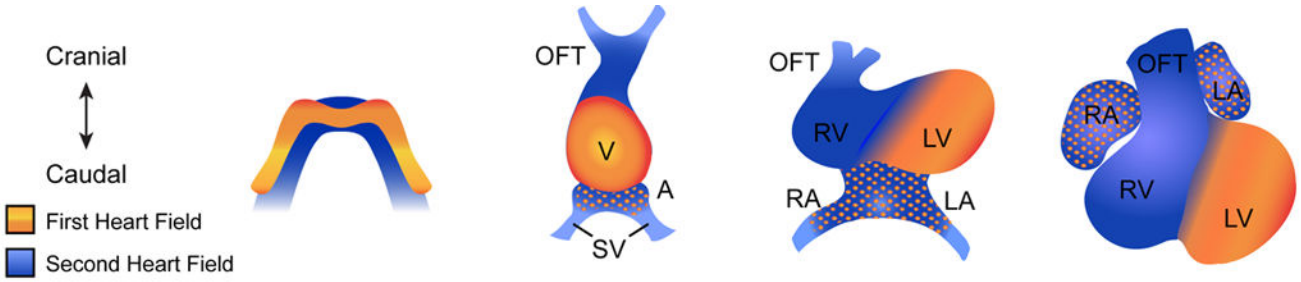
144. Trivedi CM, Zhu W, Wang Q, Jia C, Kee HJ, Li L, Hannenhalli S, Epstein JA. Hopx and hdac2 interact to modulate gata4 acetylation and embryonic cardiac myocyte proliferation. Dev Cell. 2010; 19:450–459. [PubMed: 20833366]

145. Chen F, Kook H, Milewski R, Gitler AD, Lu MM, Li J, Nazarian R, Schnepp R, Jen K, Biben C, Runke G, Mackay JP, Novotny J, Schwartz RJ, Harvey RP, Mullins MC, Epstein JA. Hop is an

unusual homeobox gene that modulates cardiac development. Cell. 2002; 110:713–723. [PubMed: 12297045]

146. Lewandowski SL, Janardhan HP, Smee KM, Bachman M, Sun Z, Lazar MA, Trivedi CM. Histone deacetylase 3 modulates tbx5 activity to regulate early cardiogenesis. Hum Mol Genet. 2014; 23:3801–3809. [PubMed: 24565863]
147. Zaidi S, Choi M, Wakimoto H, Ma L, Jiang J, Overton JD, Romano-Adesman A, Bjornson RD, Breitbart RE, Brown KK, Carriero NJ, Cheung YH, Deanfield J, DePalma S, Fakhro KA, Glessner J, Hakonarson H, Italia MJ, Kaltman JR, Kaski J, Kim R, Kline JK, Lee T, Leipzig J, Lopez A, Mane SM, Mitchell LE, Newburger JW, Parfenov M, Pe'er I, Porter G, Roberts AE, Sachidanandam R, Sanders SJ, Seiden HS, State MW, Subramanian S, Tikhonova IR, Wang W, Warburton D, White PS, Williams IA, Zhao H, Seidman JG, Brueckner M, Chung WK, Gelb BD, Goldmuntz E, Seidman CE, Lifton RP. De novo mutations in histone-modifying genes in congenital heart disease. Nature. 2013; 498:220–223. [PubMed: 23665959]
148. Gottlieb PD, Pierce SA, Sims RJ, Yamagishi H, Weihe EK, Harriss JV, Maika SD, Kuziel WA, King HL, Olson EN, Nakagawa O, Srivastava D. Bop encodes a muscle-restricted protein containing mynd and set domains and is essential for cardiac differentiation and morphogenesis. Nat Genet. 2002; 31:25–32. [PubMed: 11923873]
149. Mysliwiec MR, Bresnick EH, Lee Y. Endothelial jarid2/jumonji is required for normal cardiac development and proper notch1 expression. J Biol Chem. 2011; 286:17193–17204. [PubMed: 21402699]
150. Bevilacqua A, Willis MS, Bultman SJ. Swi/snf chromatin-remodeling complexes in cardiovascular development and disease. Cardiovasc Pathol. 2014; 23:85–91. [PubMed: 24183004]
151. Lickert H, Takeuchi JK, Von Both I, Walls JR, McAuliffe F, Adamson SL, Henkelman RM, Wrana JL, Rossant J, Bruneau BG. Baf60c is essential for function of baf chromatin remodelling complexes in heart development. Nature. 2004; 432:107–112. [PubMed: 15525990]
152. Takeuchi JK, Lou X, Alexander JM, Sugizaki H, Delgado-Olguín P, Holloway AK, Mori AD, Wylie JN, Munson C, Zhu Y, Zhou YQ, Yeh RF, Henkelman RM, Harvey RP, Metzger D, Chambon P, Stainier DY, Pollard KS, Scott IC, Bruneau BG. Chromatin remodelling complex dosage modulates transcription factor function in heart development. Nat Commun. 2011; 2:187. [PubMed: 21304516]
153. Biben C, Weber R, Kesteven S, Stanley E, McDonald L, Elliott DA, Barnett L, Köentgen F, Robb L, Feneley M, Harvey RP. Cardiac septal and valvular dysmorphogenesis in mice heterozygous for mutations in the homeobox gene nkx2-5. Circ Res. 2000; 87:888–895. [PubMed: 11073884]
154. Stennard FA, Costa MW, Lai D, Biben C, Furtado MB, Solloway MJ, McCulley DJ, Leimena C, Preis JI, Dunwoodie SL, Elliott DE, Prall OW, Black BL, Fatkin D, Harvey RP. Murine t-box transcription factor tbx20 acts as a repressor during heart development, and is essential for adult heart integrity, function and adaptation. Development. 2005; 132:2451–2462. [PubMed: 15843414]
155. Nührenberg T, Gilsbach R, Preissl S, Schnick T, Hein L. Epigenetics in cardiac development, function, and disease. Cell Tissue Res. 2014; 356:585–600. [PubMed: 24817102]
156. Hang CT, Yang J, Han P, Cheng HL, Shang C, Ashley E, Zhou B, Chang CP. Chromatin regulation by brg1 underlies heart muscle development and disease. Nature. 2010; 466:62–67. [PubMed: 20596014]
157. Takeuchi JK, Bruneau BG. Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors. Nature. 2009; 459:708–711. [PubMed: 19396158]
158. Chamberlain AA, Lin M, Lister RL, Maslov AA, Wang Y, Suzuki M, Wu B, Greally JM, Zheng D, Zhou B. DNA methylation is developmentally regulated for genes essential for cardiogenesis. J Am Heart Assoc. 2014; 3
159. Bruneau BG. The developmental genetics of congenital heart disease. Nature. 2008; 451:943–948. [PubMed: 18288184]
160. Pérez-Pomares JM, Carmona R, González-Iriarte M, Atencia G, Wessels A, Muñoz-Chápuli R. Origin of coronary endothelial cells from epicardial mesothelium in avian embryos. Int J Dev Biol. 2002; 46:1005–1013. [PubMed: 12533024]

161. Wu B, Zhang Z, Lui W, Chen X, Wang Y, Chamberlain AA, Moreno-Rodriguez RA, Markwald RR, O'Rourke BP, Sharp DJ, Zheng D, Lenz J, Baldwin HS, Chang CP, Zhou B. Endocardial cells form the coronary arteries by angiogenesis through myocardial-endocardial vegf signaling. Cell. 2012; 151:1083–1096. [PubMed: 23178125]



Stage	Cardiac Crescent	Linear Heart Tube	Looping Heart	Chamber Specialization/ Septation
Mouse Embryo Stage	E7.5	E8.0	E9-12	E10+
Human Embryo Stage	Day 15	Day 20	Day 28-30	Day 32+
Key Regulators	GATA4 NKX2.5 MESP1/2 ISL1 BAF60C MEF2C	GATA4 NKX2.5 TBX5 TBX20 MEF2C	GATA4 NKX2.5 TBX5 MEF2C HAND1/2	GATA4 NKX2.5 TBX5 PITX2 TBX1

Figure 1. Overview of heart development

The cardiac crescent consisting of the first and second heart field cardiac progenitors is established during late gastrulation. The subsequent proliferation and differentiation of cells in the first heart field (FHF) leads to the formation of a linear heart tube, giving rise primarily to the left ventricle and a portion of the atria. The cardiac progenitors in the anterior second heart field (SHF) contribute to the right ventricle and the outflow tract while the posterior SHF cells give rise to the atria and the inflow tract.[159] Extension and rightward looping of the linear heart tube allow cranial positioning of the atria with respect to the ventricles. Remodeling events modulate chamber formation, septation, and valve development, resulting in formation of the four-chambered heart. Transcription factors that regulate each stage of heart development are listed. FHF and its derivatives are shown in orange. SHF and its derivatives are shown in blue.

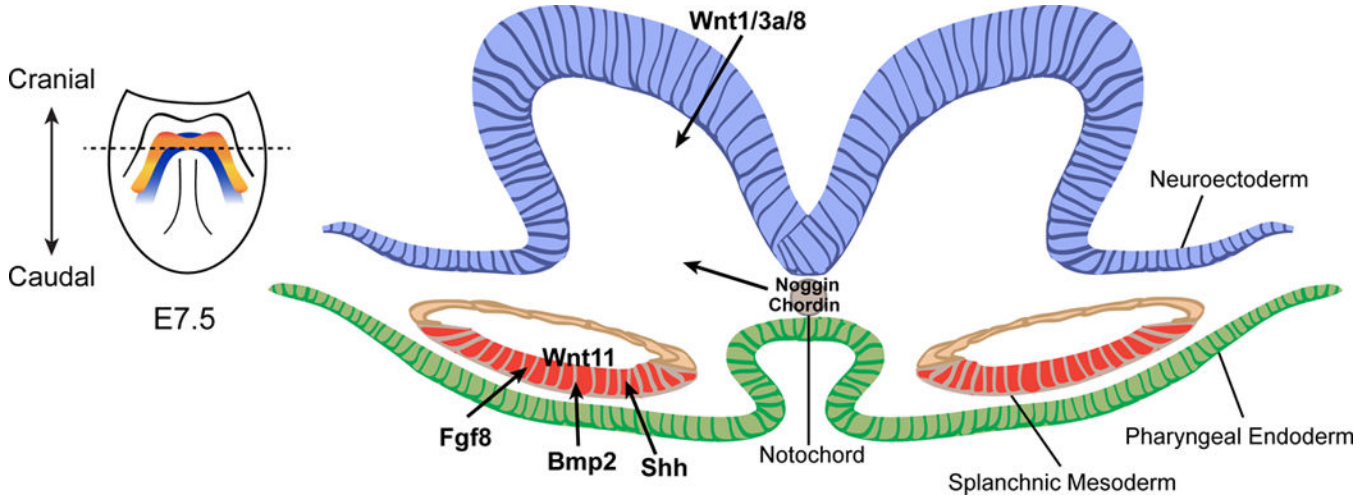


Figure 2. Regulation of cardiac mesoderm specification

Shown is a cross-section of an E7.5 mouse embryo detailing the signaling pathways that regulate cardiac specification within splanchnic mesoderm. Factors secreted by the adjacent endoderm that support cardiac mesoderm specification include FGF, BMP and Shh. Additionally, non-canonical Wnt ligands, such as Wnt11, expressed in splanchnic mesoderm also promote cardiac differentiation. Conversely, canonical Wnt ligands, including Wnt1, Wnt3a and Wnt8, secreted from the overlying neuroectoderm as well as BMP antagonists Noggin and Chordin secreted from the notochord inhibit cardiac mesoderm specification, thereby limiting the size of the cardiogenic fields. Abbreviations: FGF, fibroblast growth factor; BMP, bone morphogenetic protein; Shh, sonic hedgehog.

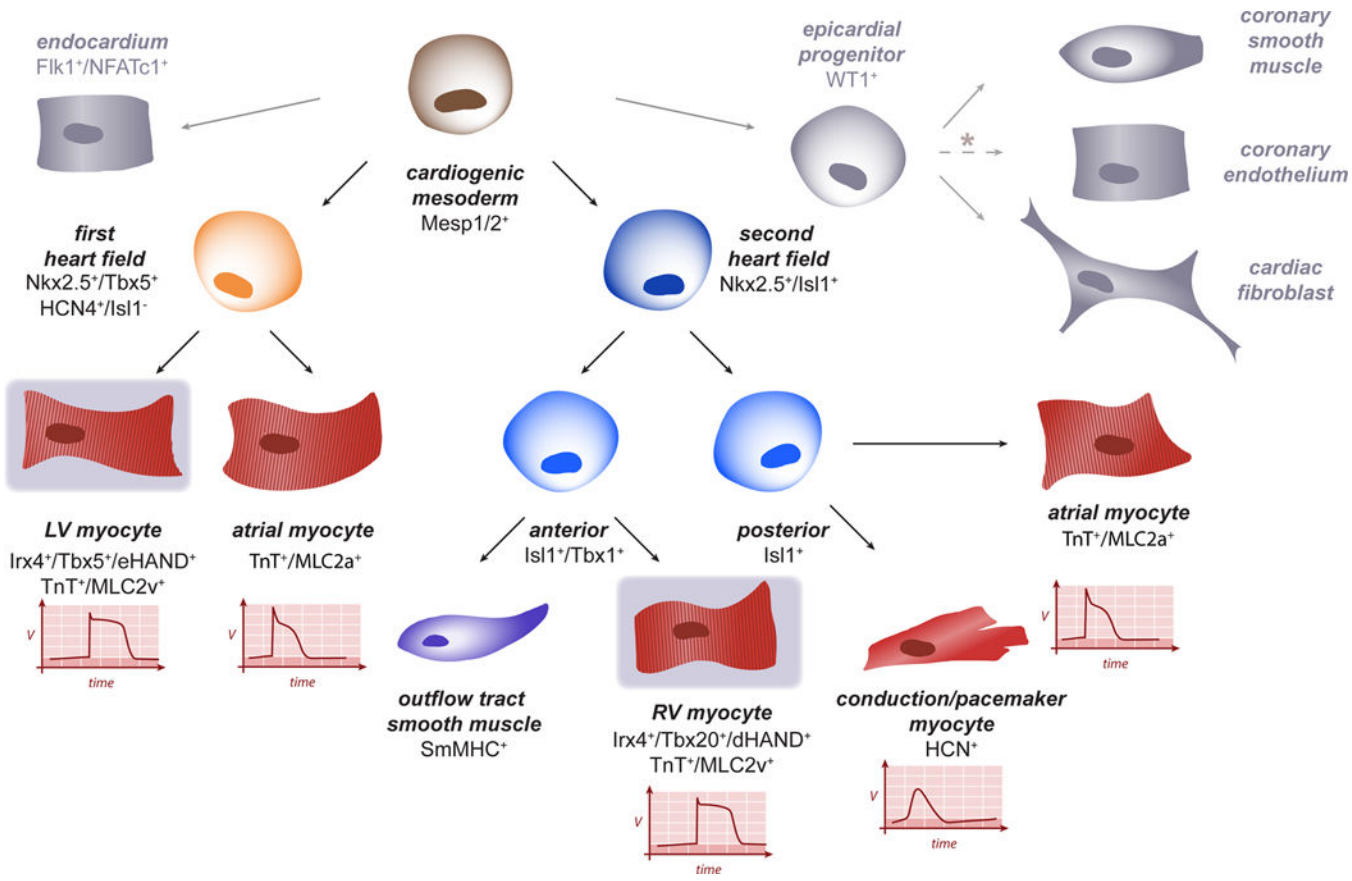


Figure 3. Schematic of cardiovascular lineage diversification

The specification of cardiomyocytes in the first and second heart fields is shown in the context of other cardiac cells that also derive from a common cardiogenic mesoderm progenitor. In particular, Isl1 expression distinguishes the first and second heart fields. Comparison of action potentials for mature myocytes reveals the range of function generated from each heart field. *Developmental origin of the coronary endothelium is an active topic of investigation. While some evidence points to partial contributions to the coronary endothelium from the epicardium[70, 160], other sources such as the endocardium[161] and the sinus venouses[69] have also been reported.

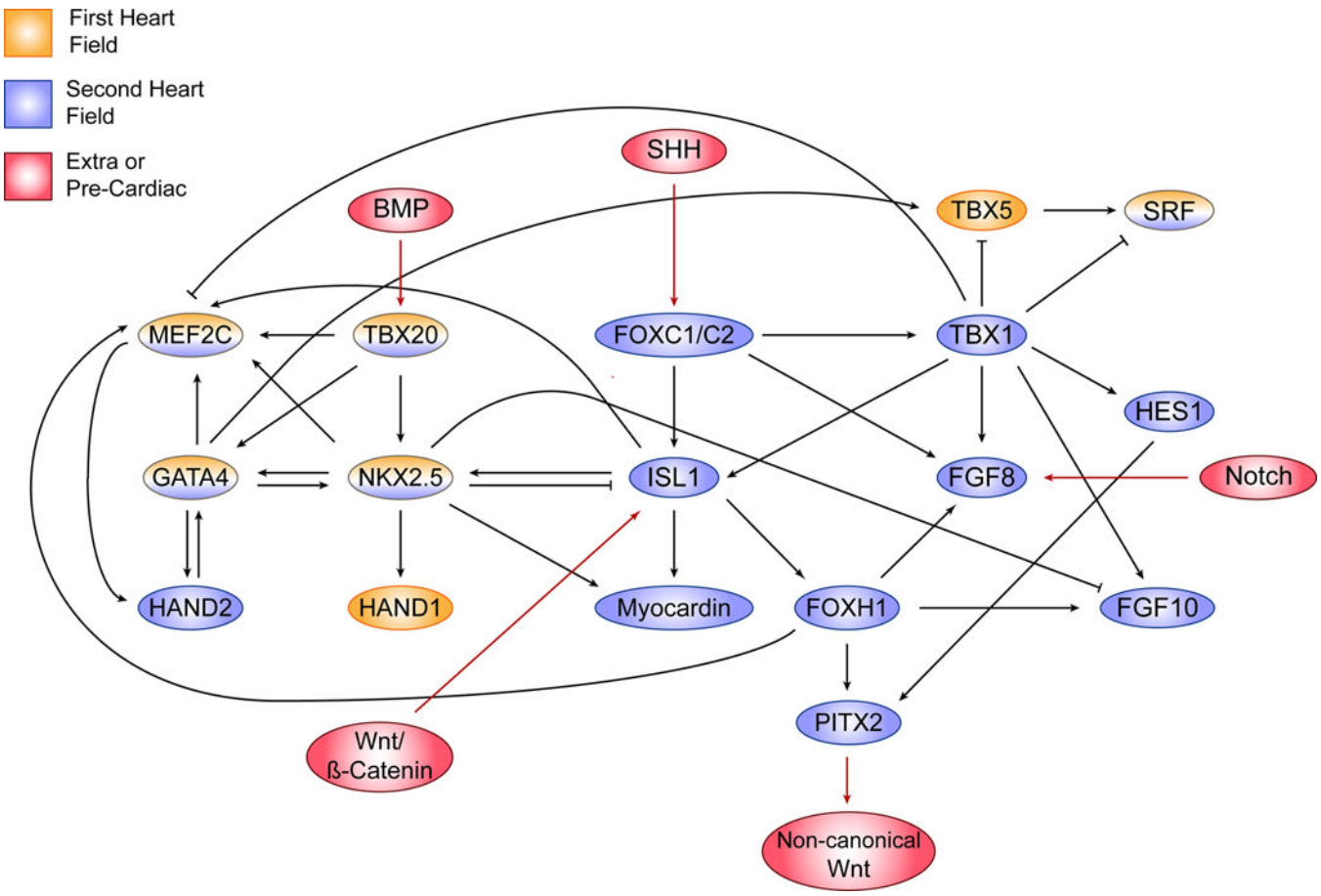


Figure 4. Cardiac gene regulatory network

The diagram shown is a brief overview of a subset of all known transcription factor interactions and signaling pathways that drive the differentiation of FHF (orange) and SHF (blue) cardiac progenitor cells during development. Factors colored by both orange and blue represent regulators of both FHF and SHF. Arrows indicate increased expression of one transcription factor or signaling molecule due to activity of another transcription factor. Signaling pathways that activate expression of certain transcription factors are shown in red.

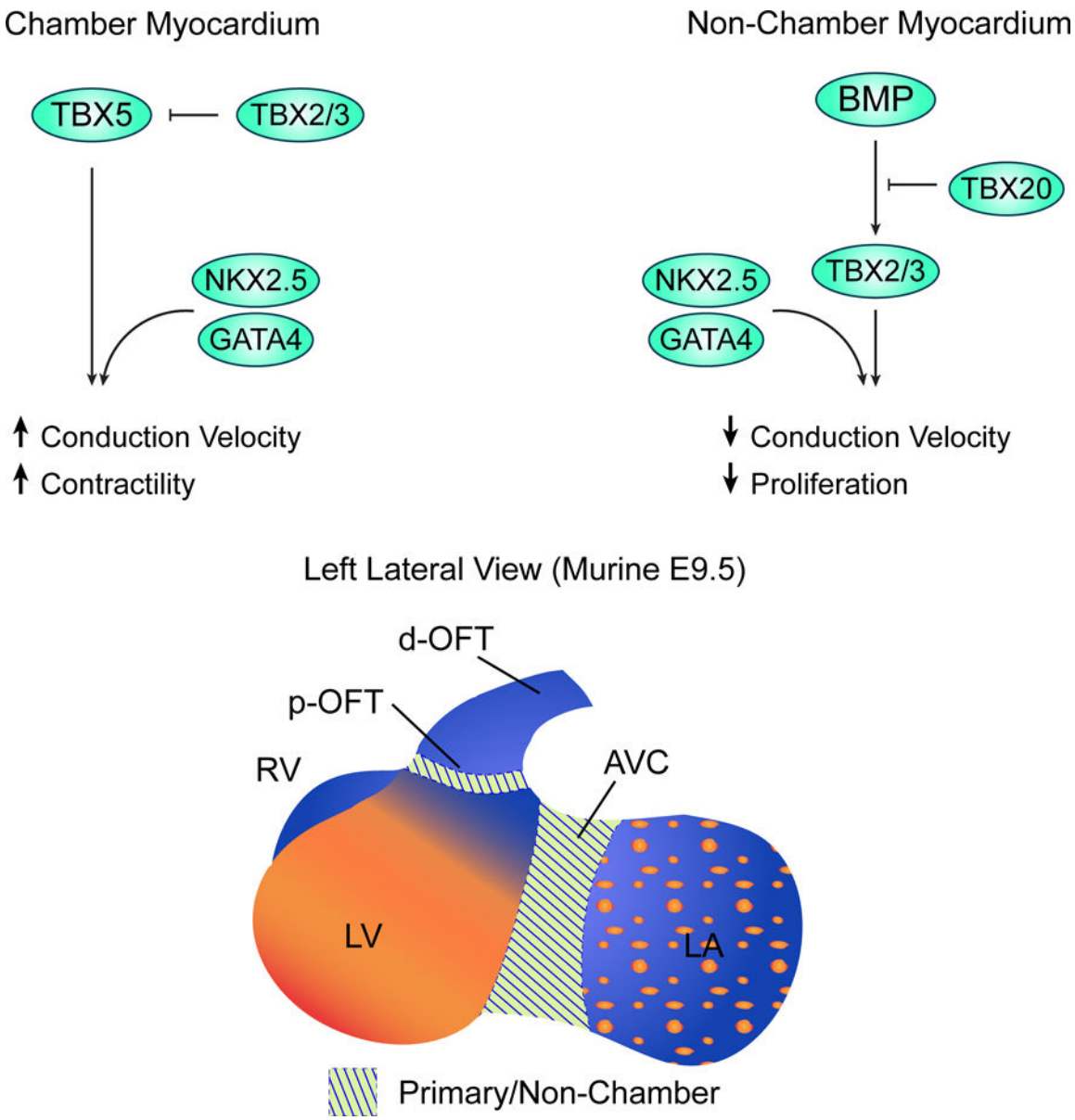


Figure 5. Specification of chamber myocardium

The specification of cells to the chamber myocardium is modulated by Tbx5 and Tbx20 in tandem with more broadly expressed factors Nkx2-5 and Gata4. Tbx2 and Tbx3 suppress the expression of chamber myocardium-specific genes, resulting in low proliferation rate, slow conduction velocity, and poor contractibility characteristic to the primary myocardium. The primary myocardium phenotype becomes restricted to the AVC and p-OFT. Abbreviations: AVC, atrioventricular canal; p-OFT, proximal outflow tract; d-OFT, distal outflow tract.

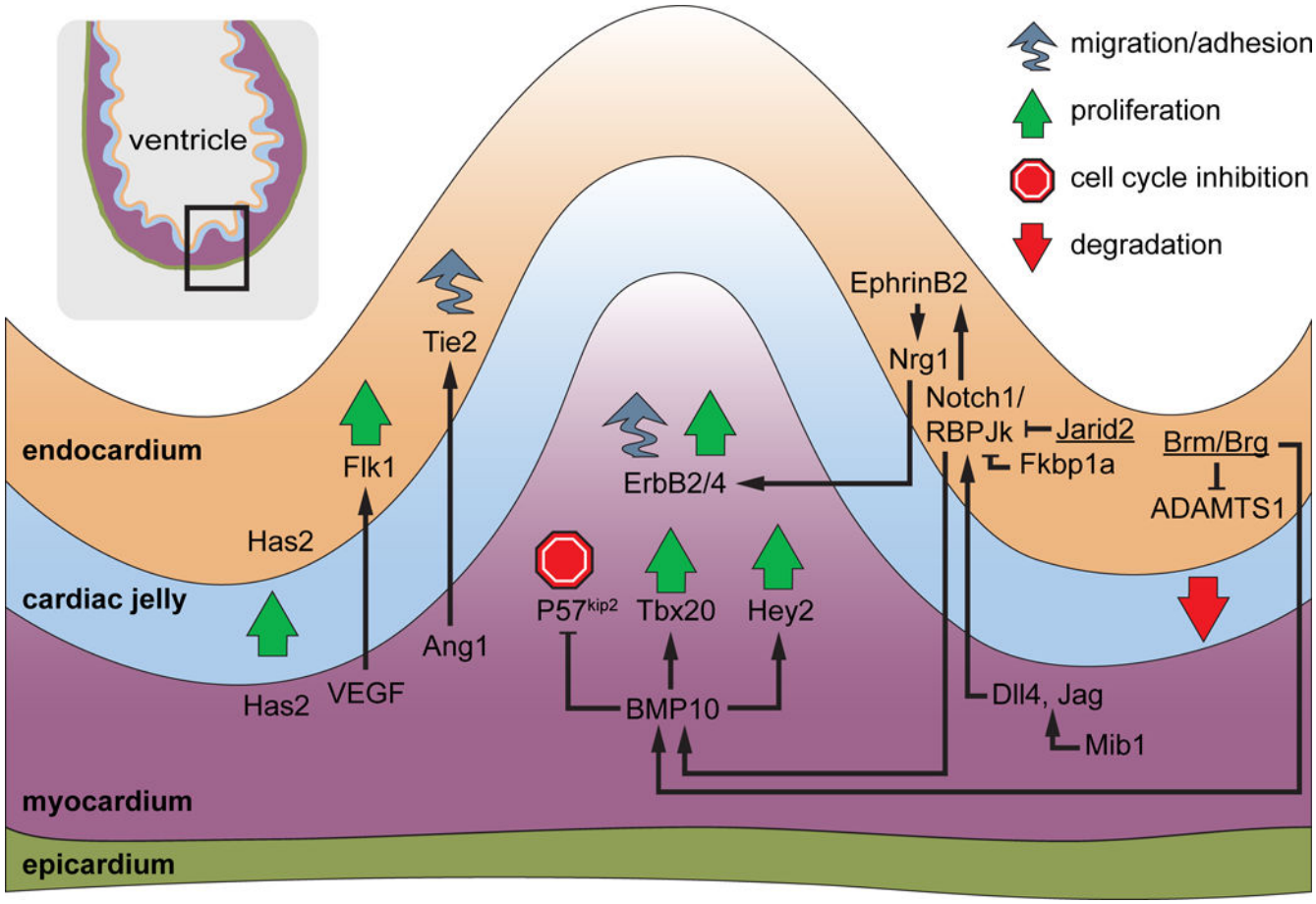


Figure 6. Spatiotemporal regulation of trabeculation

The development of ventricular trabeculae is governed by signal transduction involving the endocardium, myocardium, and cardiac jelly. Pathways implicated in trabeculation are depicted in approximate chronological order of expression from left to right. Beginning with establishment of the cardiac jelly and endothelium, trabeculation progresses via cellular proliferation and migration, and ends with degradation of the cardiac jelly. Factors involved in epigenetic mechanisms are underlined.