

Chapter 2

Molecular Mechanisms of Cardiac Development

Patricia Roche, Michael P. Czubryt and Jeffrey T. Wigle

Abstract The heart is the first organ to develop in order to supply the ever-increasing metabolic demands of the growing embryo. The heart is a unique structure in the body as it is derived from four distinct pools of progenitors: the first heart field (cardiac crescent), the second heart field (SHF), the proepicardial organ and the cardiac neural crest. These progenitors differentiate into the different cell types that comprise the adult heart: cardiomyocytes, endothelial cells, vascular smooth muscle cells, fibroblasts, and the conduction system. This complex program of differentiation is controlled by different molecular signaling pathways. A key component of the cardiac development program is the exquisitely coordinated expression of various genes in a spatially and temporally controlled fashion. Genes must be activated or repressed within restricted regions at specific times in order for normal cardiac development to proceed. In large part, this regulation of gene expression is controlled by an evolutionarily conserved set of transcription factors and microRNAs (miRNAs). Historically, the study of cardiac transcription factors has been very informative in understanding the early events in cardiogenesis. The rapidly evolving field of cardiac miRNAs promises to further extend our understanding of cardiac development. In this chapter, we will describe essential cardiac transcription factors and miRNAs and their role in controlling cardiac development.

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2.1 Introduction to Cardiogenesis

Cardiogenesis begins during embryonic development. In humans, the heart is the first functional organ to develop, at around the third week of gestation. Subsequent embryonic development is dependent on the ability of the primordial heart to meet the embryo's ever-increasing demands for oxygen and nutrients. Heart development requires the precise migration, proliferation, and differentiation of a myriad of different cell types deriving from distinct embryonic origins. For proper cardiac development to occur, these processes need to be tightly controlled by a well-conserved set of molecular pathways. Even minor deviations from the cardiogenesis program can have severe consequences. Abnormalities in cardiac development are the most common form of birth defect, with congenital heart disease (CHD) affecting nearly 1 % of newborns. It is estimated that the number of affected pregnancies is tenfold greater if spontaneous abortions due to heart defects are accounted for [1].

The cardiogenic program involves the expression of evolutionarily-conserved transcription factors, many of which appear to have chamber-specific expression patterns. Cardiac development differs from organogenesis of other major organs, such as skeletal muscle, in that it involves intricate temporal and spatial interplay between a variety of factors. Another unique feature of heart development is evident in adult cardiac disease (e.g. hypertrophy), in which the fetal cardiac gene program is reactivated. In this chapter, we will review the transcriptional control of cardiac development. We will focus on the early pioneering studies on key transcription factors and recent developments in the field including the delineation of the roles of the second heart field (SHF), the proepicardial organ and microRNAs (miRNA).

2.2 Transcriptional Regulators of Cardiogenesis

Early during gastrulation, cardiac progenitor cells arise from the anterior lateral mesoderm and migrate through the primitive node and primitive streak to the cranial and cranio-lateral regions of the embryo. These early progenitors comprise a distinct epithelial cell population called the cardiac crescent that expresses cardiac-specific transcription factors [2] (Fig. 2.1). Positive and negative influences from the underlying endoderm act to induce cardiac specification in these progenitor cells. *Wnt*, the mammalian ortholog of the *Drosophila* gene *Wingless* (required for dorsal vessel formation) [3], is one of the earliest markers of cardiac specification in mesodermal progenitors [4]. Generally, anti-cardiogenic

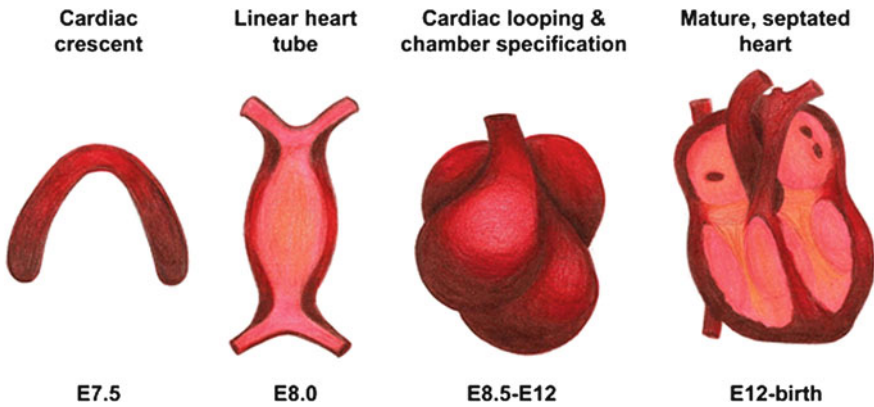


Fig. 2.1 Four major stages in mouse heart development: cardiac crescent formation (E7.5), formation of the linear heart tube (E8.0), cardiac looping (E8.5–E9.5) and specification of chamber identities (E10–E12), and maturation and septation of the heart (E12–birth). Transcription factors critical for modulating these processes are discussed in the text

Wnt3a and *Wnt8* signaling appears to exert its effects via the canonical signaling pathway that inhibits cardiac specification, which is relieved by endogenous *Wnt* antagonists (*Crescent*, *Dickkopf-1*) [5–9]. The progenitors comprising the heart fields coalesce to form a parallel pair of vessels which fuse to form the cardiac tube. This tube undergoes rightward looping, followed by a series of septation and fusion events to give rise to the four-chambered heart, which further matures prior to birth (Fig. 2.1). Shortly after birth, cardiomyocytes undergo terminal differentiation, losing their proliferative capacity.

Each of these events relies on specific orchestrated roles of conserved cardiogenic transcription factors. Our focus will be on the most critical transcriptional modulators of heart development, most of which are evolutionarily conserved and implicated in the development of CHD. Here, we discuss some of the major players in the early stages of cardiogenesis (*Nkx2-5*, *MEF2C*, *Hand1*, *Hand2*, *Tbx5* and *GATA4*).

2.2.1 *Nkx2-5*

Our current understanding of the mechanisms of vertebrate cardiogenesis originates from the study of model organisms, the most notable being the fruit fly *Drosophila*. Vertebrate orthologs of *Drosophila* genes instrumental to the formation of the primitive fly heart are expressed in the earliest stages of cardiac development—the establishment of cardiac lineage in the primary heart field and formation of the cardiac crescent. In some cases, maintained expression of these genes is crucial for the later stages of cardiac morphogenesis. Bodmer demonstrated that fruit flies, which were mutant for the homeobox gene *tinman*, lacked a

heart [10]. The mammalian ortholog, *Nkx2-5* or *Csx*, unlike *tinman*, is not required for the initial stages of cardiogenesis in higher animals, suggesting functional redundancy with other genes which have not yet been elucidated. Although *Nkx2-5* null mice have a heart, the heart does not loop properly and there is decreased expression of ventricular markers [11, 12]. In humans, an autosomal dominant form of CHD (atrial septal defects with atrioventricular conduction defects) was shown to be caused by mutations that altered the DNA binding ability of *Nkx2-5* [13]. Further studies have identified over 40 different mutations in *Nkx2-5* that give rise to dominant forms of human CHD [14].

2.2.2 *MEF2C*

Myocyte enhancer binding factor 2C (*MEF2C*) is one of the four mammalian orthologs of the *Drosophila* *MEF2* gene required for muscle formation in the fruit fly [15]. *MEF2C* is expressed at E7.5 in the procardiogenic mesodermal cells of the mouse embryo prior to formation of the linear heart tube. *MEF2C*-null mouse embryos fail to properly develop a right ventricle (RV), do not exhibit cardiac looping, and fail to express a subset of cardiomyocyte-specific genes [16, 17]. At the presumed onset of cardiac looping, *MEF2C*-null mice also displayed downregulation of *Hand2* expression and distorted *Hand1* expression, which supports the role of *MEF2C* in ventricular development [17]. A dominant-negative form of *MEF2C* causes decreased post-natal myocardial growth in transgenic mice [18]. Targeted deletion of *MEF2C* also affects development of the embryonic vasculature, attenuating the expression of endothelial cytokines by the heart, which results in severe vascular anomalies and embryonic lethality by E9.5 [19, 20]. In addition to its role in cardiac myogenesis and right ventricular development, *MEF2C* expression is upregulated in response to both physiological and pathological cardiomyocyte hypertrophy [18]. *MEF2C* mutations in humans may contribute to non-syndromic congenital heart defects [21]. Recent reports from the Srivastava lab indicate that ectopic expression of *MEF2C*, along with the developmentally-important transcription factors *GATA4* and *Tbx5*, can induce reprogramming of fibroblasts into cardiomyocyte-like cells and improve heart function after myocardial infarction, demonstrating the potency of these factors for governing cell fate [22, 23].

2.2.3 *Hand1/Hand2*

Proper development of the left and right ventricular myocardium requires expression of two chamber-specific basic helix-loop-helix transcription factors, *eHAND/Hand1* and *dHAND/Hand2*. During the linear heart tube stage of murine cardiogenesis, *Hand2* expression is higher in the RV while *Hand1* is higher in the left ventricle (LV) [24, 25]. In *Hand1*-null mice, embryonic lethality occurred

between E7.5 and E9.5 due to extraembryonic mesodermal and yolk sac defects, accompanied by an arrest of cardiac development. Rescue of extra-embryonic *Hand1* expression by aggregation of mutant and tetraploid wild-type embryonic stem (ES) cells extended the survival of null embryos to E10.5, however, the linear heart tube failed to undergo rightward looping [26, 27]. In hypomorphic *Hand1* mouse lines, embryos are viable to between E10.5 and E12.5 and cardiac looping is rescued, but these mice display a thin LV myocardium and reduced expression of LV-specific markers [28]. Whereas ES cell-derived cardiomyocytes in a *Hand1*-null background display increased differentiation, its overexpression in vivo causes increased cardioblast proliferation resulting in an extended heart tube and extra-neous looping [29]. It is also important to note that *Hand1* expression and cardiac looping are abolished in *Nkx2.5*-null mice [24].

Targeted deletion of *Hand2* in mice causes embryonic lethality by E10.5 and hypoplasia of the RV [25]. Double-knockout mice lacking *Hand2* and *Nkx2.5* develop a single atrial cardiac chamber [30]. The ability of *Hand1* and *Hand2* to form both homo- and heterodimers in vivo suggests some degree of functional redundancy during cardiac development [31]. This is confirmed by the recent report that genetic deletion of *Hand2* specifically from *Hand1*-expressing cells resulted in mutants with impaired formation of epicardial tissues and a failure to develop coronary arteries [32].

Despite the critical roles of *Hand1* and *Hand2* in the developing mouse myocardium, the influence of these transcription factors on human congenital disease is far less clear. *Hand2* deletion from neural crest cells results in impaired outflow tract formation in mice, and *Hand2* mutations have been associated with right outflow tract stenosis in humans [33, 34]. Mutations of *Hand1* in Chinese patients were associated with ventricular septal defects, however, *Hand1* mutations were not associated with Tetralogy of Fallot, one of the most common congenital heart defects [35–37]. The impact of *Hand1* and *Hand2* mutations on other cardiac congenital conditions remains to be determined.

2.2.4 *Tbx5*

Studies in humans and mouse models have shown that the various atrial and ventricular septal defects associated with Holt-Oram syndrome result from loss of function of one allele of the transcription factor *Tbx5* [38]. The T-box domain via which *Tbx5* binds its target genes is highly conserved amongst mammals. Early in development, *Tbx5* is expressed uniformly throughout the cardiac crescent. Temporal and spatial changes in *Tbx5* expression throughout cardiac development indicate a critical role for this transcription factor in formation of the linear heart tube, cardiac looping, and chamber septation. Upon formation of the linear heart tube, *Tbx5* expression becomes graded (with higher levels posteriorly). With cardiac looping, its expression is further restricted to the atria and left ventricle, where it marks the boundary at which the ventricular septum is formed [39].

Unlike other transcription factors discussed earlier, *Tbx5* is not required for the earliest steps of cardiogenesis (i.e. cardiac crescent and heart tube development) though it is instrumental in later processes including chamber specification, septation and cardiomyocyte differentiation [40–42]. For example, *Tbx5*-null mice at E9.5 display arrested cardiac development as demonstrated by the failure of cardiac looping, and hypoplasia of the left ventricle, resulting in embryonic lethality at E10.5 [40]. Studies with embryonic stem cells overexpressing *Tbx5* suggest that this transcription factor is involved in driving the expression of atrial and conduction system-specific markers [40, 43–46].

2.2.5 *Gata4*

The GATA family of double zinc finger transcription factors have been identified as crucial mediators of vertebrate cardiogenesis, with potential functional redundancy with one another [47–50]. *GATA4* widely expressed in the developing heart and in adult cardiomyocytes, and plays a critical role in cardiac differentiation and morphogenesis [51–54]. In *GATA4*-null mice, gross cardiac defects (e.g., failure of the linear heart tube to form) result in embryonic lethality around E9.0 [53, 54]. However, for normal heart development, *GATA4* expression is required only in the underlying endoderm and not within the cardiac mesoderm itself [54–57]. *GATA4* has been shown to directly regulate the transcriptional activity of numerous cardiac-restricted genes that control cardiac progenitor cell differentiation, including *Mef2C*, *Hand2* and *GATA6*. *GATA4* associates with *Tbx5* and *Nkx2-5* to form multiprotein transcriptional complexes that regulate the expression of a set of downstream genes involved in cardiac development [40, 58, 59]. *Nkx2-5* and *GATA4* are in turn themselves regulated by *Nkx2-5* and *GATA* factors in a positive feedback loop, which amplifies and maintains their expression in cells of the cardiac lineage [60, 61]. Thus, it appears as though the role of GATA factors in cardiogenesis is widespread and involves many other (and more cardiac specific) transcription factors. In humans, heterozygous *GATA4* mutations result in familial septal defects and are involved in a range of cardiac defects including right ventricular hypoplasia and cardiomyopathy [62].

2.2.6 *Second Heart Field*

Historically, all cardiomyocytes were believed to originate from the cardiac crescent. This view of cardiac development was radically changed upon the discovery of a second source of cardiac cells in 2001, the second heart field (SHF), (Reviewed in [63]). Two studies in chick demonstrated by in vivo labeling the existence of a SHF that contributed to the outflow tract region of the heart [64, 65]. In addition, tissue isolated from this SHF could be differentiated into beating

myocytes. Whereas the first heart field contributes primarily to the left ventricle, the SHF contributes primarily to the RV and the outflow tract. The SHF in mammals was identified by studying a transgenic mouse where *LacZ* had been knocked in upstream of the *Fgf10* gene [66]. The expression of this integrated transgene recapitulated that of endogenous *Fgf10* and labeled the RV, outflow tract, and pharyngeal mesoderm. In vivo labeling studies in mice demonstrated that progenitor cells migrated from the pharyngeal mesoderm into the outflow tract during heart development. The SHF was shown to contribute to the RV and the outflow tract of the developing mammalian heart but not to the left ventricle [67, 68]. We will discuss below three transcription factors (*Isl1*, *Tbx1* and *Hand2*) that have important roles in determining the fate of the SHF derivatives.

2.2.7 *Isl1*

Islet-1 (*Isl1*) is a LIM homeodomain-containing protein that was first isolated through binding to the enhancer region of the insulin gene [69]. More recently, *Isl1* expression was shown to label the SHF in mice [70]. In vivo *Isl1* expression is dependent on β -catenin and Forkhead transcription factors [71, 72]. Interestingly, *Isl1* was shown to directly regulate expression of *Fgf10*, the gene that was first used to label the SHF in mice [73]. However, *Isl1* is not exclusive to the SHF as it was also recently shown to label cardiac neural crest derivatives [74]. Knockout of *Isl1* resulted in a heart that lacked expression of right ventricular markers [70]. The migration, proliferation, and survival of the SHF progenitors were negatively affected by loss of *Isl1* function, indicating the key role of this transcription factor in the fate of the SHF.

Isl1 expression labels a pool of cardiac progenitors found in postnatal mouse and human hearts. These progenitor cells could be differentiated into cardiomyocytes with high efficiency [75]. A recent study by Bu et al. [76] established that *Isl1* positive progenitors could give rise to three different cardiac lineages: cardiomyocytes, vascular smooth muscle cells, and endothelial cells. *Isl1* expression was required for the differentiation of cardiac progenitors into cardiomyocytes and vascular smooth muscle in vivo but not required for endothelial differentiation [77].

2.2.8 *Tbx1*

T-box transcription factor 1 (*Tbx1*) has been implicated as being a cause of DiGeorge Syndrome and mice heterozygous for *Tbx1* have cardiac outflow tract abnormalities similar to human patients [78–80]. Tissue-specific deletion of *Tbx1* demonstrated that loss of *Tbx1* function in endothelial cells or cardiomyocytes did not affect development of the outflow tract. In contrast, loss of *Tbx1* function in the SHF (using *Nkx2.5* Cre) resulted in outflow tract abnormalities that phenocopied

those seen in *Tbx1*-null mice [81]. These defects likely arise from reduced proliferation of progenitors resulting from decreased expression of *Fgf8* and *Fgf10* [81, 82]. In addition, the non-canonical *Wnt* family member, *Wnt5a*, has also been shown to be a direct target of *Tbx1* in the SHF [83]. Gain of *Tbx1* function in cardiomyocytes resulted in an expansion of the *Fgf10* domain, a marker of the outflow tract [82]. Loss of *Tbx1* expression in the mesoderm, but not in the pharyngeal endoderm, was sufficient to result in cardiac outflow tract abnormalities [84].

Fate mapping studies demonstrated that *Tbx1*-expressing cells differentiated into cardiac endothelial cells, vascular smooth muscle cells, and cardiomyocytes [85]. In vitro studies established that a single *Tbx1*-expressing progenitor could be differentiated into all three cell lineages [86]. *Tbx1* was shown to increase the proliferative capacity of the cardiac progenitor cells but at the same time decrease their differentiation [86].

2.2.9 *Hand2*

The phenotype of the *Hand2* knockout mice (above) was suggestive of a potential role for *Hand2* in the SHF, since the RVs of these mice are hypoplastic [25, 30]. To determine the role of *Hand2* in different progenitor populations, a conditional allele of *Hand2* was crossed with a variety of Cre transgenic mice, which were expressed in different cardiac subpopulations [87]. Deletion of *Hand2* via *Nkx2-5* Cre, which is expressed in both ventricles but not in early cardiac progenitors, did not dramatically affect the initial formation of the ventricles. However, this deletion did affect the proliferation of cardiomyocytes, resulting in lethality by E12.5. In contrast, deletion of *Hand2* with *Isl1*-driven Cre expression (deletion in the SHF) resulted in defective RV and outflow tract growth that phenocopied the original *Hand2* null mice [25, 30]. These elegant experiments clearly demonstrated that *Hand2* expression is required in the SHF progenitors in order for proper cardiogenesis to occur.

2.3 Proepicardial Organ and Epicardium

In addition to the first and second heart fields, the proepicardial organ (in the embryo) and the epicardium (in the adult) also contribute multipotent cardiac progenitors to the heart. The epicardium is the outer epithelial layer of the heart and is the last region of the heart to form during embryonic development. The epicardium is derived from a transient structure, the proepicardial organ, which forms at the sinus venosus of the developing heart [88, 89]. Cells from the proepicardial organ protrude via an extracellular matrix bridge to cover the developing heart, forming the epicardium [90]. Bone morphogenetic protein (BMP) signalling was

shown to be essential for the proepicardium to protrude and fuse with the heart tube [91]. Early retroviral lineage and chick/quail chimera studies demonstrated that cells of the proepicardium could differentiate into coronary vascular smooth muscle cells and endothelial cells [92, 93]. The transcription factors *Tbx18*, *Scleraxis* (*Scx*) and Wilms Tumor 1 (*WT1*) are important for the regulation of the differentiation of cells derived from the epicardium, as described below.

2.3.1 *Tbx18*

Analysis of the expression of *Tbx18* in the mouse demonstrated that it was expressed predominantly in the proepicardial organ and the epicardium [94]. Knockout studies suggested a critical role for *Tbx18*-expressing precursors in generating the venous pole of the developing heart and for the formation of the sinoatrial node [95, 96]. In adult zebrafish, the epicardially-derived cells, marked by *Tbx18* expression, differentiate into cardiomyocytes [97]. In mice, fate mapping studies showed that *Tbx18*-positive progenitors could differentiate into cardiomyocytes and vascular smooth muscle cells, but not into endothelial cells [98]. This study identified the proepicardial organ/pericardium as being a novel source of cardiac cells. However, a subsequent paper demonstrated that *Tbx18* is itself expressed in cardiomyocytes and this expression may impact the lineage tracing findings of Cai et al. [99].

2.3.2 *Wilms Tumor 1*

Wilms Tumor 1 (*WT1*) is expressed in the proepicardial organ and the pericardium in the mouse. Loss of *WT1* function resulted in epicardial defects and a lack of proper intramyocardial blood vessels [100, 101]. Lineage mapping studies demonstrated that *WT1* was expressed in the mouse epicardium but that these cells could differentiate into vascular smooth muscle cells, cardiomyocytes, and a very small minority of endothelial cells [102]. *WT1* has been proposed to repress the epithelial nature of the epicardial cells to promote their transition into a more mesenchymal phenotype, which is required for the formation of cardiac progenitors [103]. *WT1* was also shown to mark a population of resident cardiac progenitor cells in the adult mouse heart that could differentiate into functionally integrated cardiomyocytes [104].

2.3.3 *Epicardially-Derived Cardiac Endothelial Cells*

Unlike the chick model, mouse epicardium progenitors were not observed to efficiently differentiate into endothelial cells [98, 102]. The reasons for this discrepancy were not clear until recently. A study by Katz et al. [105] demonstrated

that a second subpopulation existed in the proepicardial organ that was neither *Tbx18*- nor *WT1*-positive but instead expressed the transcription factor *Scleraxis* and Semaphorin3D receptor. These progenitors differentiate into coronary endothelial cells, sinus venosum, and cardiac endocardium. Thus the proepicardium is an important new source of cardiac endothelial progenitors in both chick and mouse.

2.4 miRNAs and Cardiac Development

2.4.1 Introduction to miRNAs

The field of miRNA biology has recently exploded, with over 15,000 papers published on the subject since the first definitive identification of this class of small regulatory RNAs in *C. elegans* in 2001 [106–108]. Virtually all branches of physiology and pathophysiology have been revolutionized with the increasing recognition of the key role played by miRNAs in health and disease, and cardiovascular science is no exception. This new-found understanding of how miRNAs regulate protein translation has helped to define novel mechanisms governing cardiac development, growth, and dysfunction.

miRNAs are a novel post-transcriptional mechanism of gene regulation, and serve to target specific mRNAs for silencing or degradation, providing an additional layer of control governing protein production beyond mRNA transcription and translation. At the same time, miRNA expression itself is tightly regulated, thus providing for exquisite and fine-grained control over gene transcriptional outputs.

A comprehensive explanation of the mechanism by which miRNAs regulate gene expression is beyond the scope of this chapter, and the reader is directed to recent excellent reviews on the subject [109, 110]. In brief, genes encoding miRNAs are transcribed by RNA polymerase II to generate long primary miRNAs (pri-miRNAs); these initially single-stranded molecules self-hybridize to form complex stem-loop structures, and may encode more than one miRNA in a polycistronic structure. The pri-miRNA is processed in the nucleus by a large complex that includes the RNase Drosha and numerous cofactors, which cleave off individual stem-loop structures as nascent precursor miRNAs (pre-miRNAs). Another class of miRNAs is encoded by special introns within genes (mirtrons) which produce a pri-miRNA that directly forms a pre-miRNA via splicing, and thus does not require processing by the Drosha complex. Pre-miRNAs are exported from the nucleus via Exportin-5, and once in the cytosol are further cleaved by the Dicer complex to produce mature miRNAs consisting of a guide strand and a passenger strand (designated as miRNA and miRNA*), each approximately 22 nucleotides in length.

The mature miRNA possesses, within its 5' region (from nucleotides 2 to 8), a seed sequence, which determines the specificity of the miRNA for its target mRNAs. The guide strand forms part of the RNA-induced silencing complex (RISC) and incompletely base pairs with the target mRNA. The result is that the target mRNA is either silenced or is targeted for degradation. While the passenger strand is typically degraded, in some instances this strand can also enter into a RISC complex to regulate other mRNAs. An important consequence of the incomplete base pairing between the miRNA and mRNA strands is that a single miRNA can target multiple target mRNAs. Accurate prediction of the biologically relevant targets of miRNAs remains technically challenging, since it remains unclear exactly how other sequences outside of the seed affect target selection.

The genes encoding miRNAs are themselves highly regulated. miRNA genes frequently exist within the introns of genes, some of which are even targeted by the miRNA. In many cases, cis-acting DNA elements governing expression of the host gene may also control expression of the miRNA. However, miRNA genes, particularly when found outside of mRNA-encoding genes, may also possess their own enhancer elements. For example, expression of the miR-1/miR-133 miRNA cluster is regulated by a variety of muscle-enriched transcription factors such as *Nkx2-5*, *Mef2c* and *MyoD* [110].

2.4.2 General Requirement of miRNAs for Cardiogenesis

The pioneering work of the Srivastava lab first demonstrated that specific miRNAs exhibit cardiac-specific expression, and are required for normal cardiac development [111, 112]. While a variety of studies have now implicated specific miRNAs in regulating cardiac specification and development (see below), the broader approach of genetic deletion of components of the miRNA processing pathway has revealed a general requirement for miRNAs at various stages of cardiogenesis. Since Dicer is required for maturation of all pre-miRNAs to miRNAs, deletion of the Dicer gene effectively eliminates miRNA-mediated mRNA silencing and degradation. These studies exploit the Cre-lox conditional gene deletion system by driving Cre expression in mice using cardiac-specific, temporally-activated promoters, and permit the development of a general timeline for when miRNAs are important for particular events or processes.

Zhao et al. [113] deleted Dicer early in cardiogenesis using the *Nkx2-5*-Cre driver mouse line, which resulted in gene excision by embryonic day E8.5. The resulting embryos died by E12.5 with pericardial edema, indicating that heart failure may have occurred. The ventricular myocardium exhibited thin walls, which suggested that the hearts were hypoplastic. While several early markers of cardiac differentiation and patterning showed normal expression, including *Tbx5* and *Hand2*, the endoderm marker α -fetoprotein was upregulated. This result, combined with the observed hypoplasia, suggests the possibility that cardiac progenitor fate had been altered. This alteration is likely due to the roles of several

key miRNAs in governing progenitor differentiation and proliferation (see below) as well as a demonstrated critical role for Dicer in maintaining stem cell survival during development [114]. In related cell types, Dicer may or may not play a critical role in cell survival. Neural crest cells migrate to form parts of both the cardiac outflow tract as well as craniofacial structures. Deletion of Dicer in these cells using a *Wnt1*-Cre driver demonstrated that Dicer was not required for survival of cells fated to form the outflow tract, although formation of this structure was significantly impaired due to altered neural crest cell migration and patterning [115]. In contrast, neural crest cells that contributed to craniofacial structures failed to survive, resulting in dramatic defects resembling human congenital malformations.

In contrast to early deletion of Dicer, deletion later in development using α MHC-Cre resulted in viable pups and normal Mendelian ratios of offspring at birth [116]. However, Dicer-null animals failed to thrive, and all died by four days after birth with signs of dilated cardiomyopathy including reduced sarcomere number and length, and signs of increased apoptosis. Proliferation of cardiomyocytes was normal in the Dicer-null pups, thus later deletion of Dicer in the heart exhibits quite distinct consequences in comparison to early deletion, reflecting a shift in the role of miRNAs at different stages of cardiac development and maturation. However, it is important to remember that deletion of Dicer globally impacts the effects of all miRNAs. Since miRNA expression is dynamically regulated and constantly changing, the true role of miRNAs in cardiogenesis can only be resolved by deletion of specific miRNAs or miRNA clusters.

2.4.3 *The Roles of Specific miRNAs in Cardiogenesis*

Recent experiments have elegantly delineated the role of individual key miRNAs in cardiogenesis. While a complete description of all miRNAs that have been demonstrated to impact heart development is beyond the scope of this review, we present here an overview of several critical miRNAs in this process.

2.4.4 *miR-1 and miR-133*

miR-1 is highly expressed in the developing myocardium including the heart tube in the chick and was one of the first miRNAs to be studied for its role in cardiogenesis [113, 117]. miR-1 is bicistronic with miR-133, and there are two copies of this cluster: miR-1-1 and miR-133a-2 on human chromosome 2, and miR-1-2 and miR-133a-1 on chromosome 18. These miRNAs are expressed at relatively high levels in the myocardium, and along with other miRNAs such as miR-208 and miR-499, have been termed “MyomiRs” since they are specific to or enriched in muscle cell types. Another bicistronic cluster on chromosome 1 contains miR-206

and miR-133b, which contain the same seed sequences as miR-1 and miR-133a respectively, but are expressed primarily in skeletal muscle [118].

Cardiac-specific deletion of miR-1-2 (without deletion of the associated miR-133a-1) results in ventricular septal defects and dilated cardiomyopathy, leading to reduced numbers of null animals at birth and the death of approximately 50 % of mouse pups by weaning [113]. This phenotype shows interesting and intermediate similarities to those observed for cardiac-specific Dicer deletion, suggesting that miR-1 may be of particular importance among all miRNAs during cardiac development [113, 116]. Overexpression of an miR-1 transgene under control of the β MHC promoter arrests growth at E13.5 [112]. The hearts of these animals exhibit signs of failure, with hypoplasia and reduced numbers of proliferating cells without increased apoptosis, suggesting that the defects may be due to early exit of cardiomyocytes from the cell cycle. miR-1 is highly upregulated during differentiation of cardiomyocyte progenitor cells, and adenoviral delivery of miR-1 to these progenitors reduced proliferation while inducing differentiation to cardiomyocytes [119]. miR-1 may thus function to drive cardiomyocyte progenitor cells to a cardiac muscle fate. Consistent with this model, one of the gene targets of miR-1 is the Notch ligand *Delta-like 1*, which must be down-regulated to permit muscle differentiation to progress [120]. This result parallels a previous report in *Drosophila* demonstrating that the fly homolog dmiR-1 targets *Delta* [111].

Genetic deletion of either miR-133a-1 or miR-133a-2 results in offspring with hearts that are largely normal, suggesting that these miRNAs may compensate for each another [121]. Deletion of both miR-133a-1 and miR-133a-2, however, results in dramatic cardiac defects similar to those observed with miR-1-2 deletion. Such defects include ventricular septal defects, right ventricular wall thinning, and dilation and signs of impaired pump function, resulting in significant perinatal lethality. Both proliferation and apoptosis of cardiomyocytes were increased in double knock-out mice. Overexpression of miR-133a under control of the β MHC promoter resulted in a very similar phenotype to that observed in β MHC-miR-1 mice: embryonic lethality, enlarged atria, ventricular septal defects, and reduced proliferation of cardiomyocytes [121].

Superficially, these results suggest that miR-1 and miR-133 may serve similar functions in vivo, including preventing progenitor cells from adopting non-cardiac fates. It is noteworthy that in the miR-133a double knockout mice, increased smooth muscle marker gene expression was observed compared to wild type littermates [121]. However, in contrast to miR-1, miR-133 may maintain the cardiomyocyte progenitor pool and promote progenitor cell proliferation rather than differentiation [112, 120, 122]. Thus, these two miRNAs may serve distinct but overlapping roles in balancing factors that promote progenitor proliferation versus differentiation, such that disruption of either role leads to cardiac dysgenesis.

2.4.5 *miR-208 and miR-499*

miR-208a and 208b have identical seed sequences, and miR-499 is closely related to both. The genes encoding these miRNAs are found within introns in genes encoding α -myosin heavy chain, β -myosin heavy chain and β -myosin heavy chain 7B, respectively [123–125]. An intricate interplay between these factors and thyroid hormone-mediated cell signaling governs myosin isoform switching during maturation of the heart, as well as re-initiation of fetal β -myosin gene expression during cardiac remodeling. While cardiac-specific overexpression of miR-208a resulted in cardiac hypertrophy in adult transgenic mice, genetic deletion resulted in normal hearts [123]. However, alteration of miR-208a expression resulted in cardiac conduction changes, including prolonged PR intervals in both null and transgenic lines, partially penetrant second degree AV block in transgenics, and possible atrial fibrillation in the nulls. miR-208a may thus be important for proper development of the cardiac conduction system, although it is not critical for survival.

Despite its close relationship to miR-208a and 208b, miR-499 appears to play a significantly different role in the developing myocardium, including defining cardiac cell fate. Like miR-1, miR-499 expression increases during cardiac progenitor differentiation, and overexpression of miR-499 enhances differentiation and reduces proliferation of progenitor cells via regulation of *Sox6*, although the reduction of proliferation was not to the same degree as miR-1 [119, 126]. Fu et al. [127] demonstrated that lentiviral-mediated overexpression of miR-499 in human embryonic stem cells increased the yield of stem cell-derived ventricular cardiomyocytes by nearly 50 % over controls. In an exciting proof-of-concept experiment, Dzau's laboratory has reported evidence of fibroblast-to-cardiomyocyte conversion in situ following direct injection of miRNAs 1, 133, 208 and 499 into the murine myocardium following infarction [128]. Thus, developmentally important miRNAs may be useful for therapeutic strategies to repopulate the damaged myocardium, although the mechanism of this approach remains to be determined.

2.4.6 *Other miRNAs*

While the above mentioned MyomiRs have attracted significant attention recently, it is likely that numerous other miRNAs remain to be discovered that can exert significant effects on cardiogenesis. For example, the miR-17 ~ 92 cluster comprises six individual miRNAs expressed from a common locus, and is found in the secondary heart field and cardiac outflow tracts [129]. Deletion of miR-17 ~ 92 results in ventricular septal defects, as well as perinatal lethality due to severe lung hypoplasia [130]. miR-17 and miR-20a repress the cardiac progenitor genes *Isl1* and *Tbx1* downstream of BMP signaling, suggesting that the miR-17 ~ 92 cluster enhances differentiation of myocardial progenitors to a cardiac fate [129]. MiR-17 ~ 92 and the unrelated miR-130a have both been demonstrated

to target Friend-of-Gata-2 (FOG2), and overexpression of each leads to inhibition of proliferation of mouse embryonic cardiomyocytes *in vitro* and ventricular hypoplasia with septal defects *in vivo*, respectively [131, 132].

An important aspect of postnatal cardiac maturation is the withdrawal of cardiomyocytes from the cell cycle. Work from Olson and colleagues demonstrates that the miR-15 family member miR-195 is upregulated from postnatal day 1–10, a time period that overlaps with cell cycle withdrawal [133]. MiR-195 regulates expression of the checkpoint kinase Chek1, and overexpression of miR-195 under control of the β MHC promoter gave rise to ventricular hypoplasia and ventricular septal defects, consistent with early withdrawal of cardiomyocytes from the cell cycle. Conversely, antagonism of the miR-15 family using a locked nucleic acid anti-miR approach caused an increase in cardiomyocyte proliferation.

2.5 Conclusions

Studies of cardiac transcriptional control over the past 15 years have been instrumental in our understanding of the processes of cardiac development. New roles for transcription factors in controlling the differentiation of cardiac cells are still being discovered. The emerging field of cardiac miRNAs has tremendous potential to further extend our knowledge, and has already had a dramatic impact on our understanding of cardiogenesis. The study of these developmental signaling cascades is also clinically relevant as many of them are frequently reutilized in the adult following stresses such as myocardial infarctions and have furthermore been implicated in congenital defects in humans. Despite the remarkable depth of knowledge that we now have in understanding how the heart is specified and forms, a number of outstanding questions remain. For example, the very earliest signals that induce cardiac progenitor specification are unknown. The factors that are redundant with *Nkx2-5* also remain unclear. A mechanism governing development of the cardiac extracellular matrix is also lacking. Finally, our understanding of the reasons for reactivation of the fetal gene program in response to many forms of cardiac stress remains elusive. Clearly, further research is required to elucidate the complex interplay between cardiogenic factors and the pathways which they activate, during both embryonic development and the onset of cardiovascular disease.

References

1. Hoffman JI (1995) Incidence of congenital heart disease: II. Prenatal incidence. *Pediatr Cardiol* 16:155–165
2. Harvey RP (2002) Patterning the vertebrate heart. *Nat Rev Genet* 3:544–556
3. Zaffran S, Frasch M (2002) Early signals in cardiac development. *Circ Res* 91:457–469

4. Wu X, Golden K, Bodmer R (1995) Heart development in *Drosophila* requires the segment polarity gene *wingless*. *Dev Biol* 169:619–628
5. Kwon C, Arnold J, Hsiao EC et al (2007) Canonical *Wnt* signaling is a positive regulator of mammalian cardiac progenitors. *Proc Natl Acad Sci USA* 104:10894–10899
6. Marvin MJ, Di Rocco G, Gardiner A, Bush SM, Lassar AB (2001) Inhibition of *Wnt* activity induces heart formation from posterior mesoderm. *Genes Dev* 15:316–327
7. Pandur P, Lasche M, Eisenberg LM, Kuhl M (2002) *Wnt*-11 activation of a non-canonical *Wnt* signalling pathway is required for cardiogenesis. *Nature* 418:636–641
8. Schneider VA, Mercola M (2001) *Wnt* antagonism initiates cardiogenesis in *Xenopus laevis*. *Genes Dev* 15:304–315
9. Tzahor E, Lassar AB (2001) *Wnt* signals from the neural tube block ectopic cardiogenesis. *Genes Dev* 15:255–260
10. Bodmer R (1993) The gene *tinman* is required for specification of the heart and visceral muscles in *Drosophila*. *Development* 118:719–729
11. Lyons I, Parsons LM, Hartley L et al (1995) Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene *Nk2-5*. *Genes Dev* 9:1654–1666
12. Tanaka M, Chen Z, Bartunkova S, Yamasaki N, Izumo S (1999) The cardiac homeobox gene *Csx/Nkx2.5* lies genetically upstream of multiple genes essential for heart development. *Development* 126:1269–1280
13. Schott JJ, Benson DW, Basson CT et al (1998) Congenital heart disease caused by mutations in the transcription factor *NKX2-5*. *Science* 281:108–111
14. Reamon-Buetner SM, Borlak J (2010) *NKX2-5*: an update on this hypermutable homeodomain protein and its role in human congenital heart disease (CHD). *Hum Mutat* 31:1185–1194
15. Nguyen HT, Bodmer R, Abmayr SM, McDermott JC, Spoerel NA (1994) *D-mef2*: a *Drosophila* mesoderm-specific MADS box-containing gene with a biphasic expression profile during embryogenesis. *Proc Natl Acad Sci USA* 91:7520–7524
16. Edmondson DG, Lyons GE, Martin JF, Olson EN (1994) *Mef2* gene expression marks the cardiac and skeletal muscle lineages during mouse embryogenesis. *Development* 120:1251–1263
17. Lin Q, Schwarz J, Bucana C, Olson EN (1997) Control of mouse cardiac morphogenesis and myogenesis by transcription factor *MEF2C*. *Science* 276:1404–1407
18. Kolodziejczyk SM, Wang L, Balazsi K et al (1999) *MEF2* is upregulated during cardiac hypertrophy and is required for normal post-natal growth of the myocardium. *Curr Biol* 9:1203–1206
19. Bi W, Drake CJ, Schwarz JJ (1999) The transcription factor *MEF2C*-null mouse exhibits complex vascular malformations and reduced cardiac expression of angiopoietin 1 and VEGF. *Dev Biol* 211:255–267
20. Lin Q, Lu J, Yanagisawa H, Webb R et al (1998) Requirement of the MADS-box transcription factor *MEF2C* for vascular development. *Development* 125:4565–4574
21. Kodo K, Nishizawa T, Furutani M, et al (2012) Genetic analysis of essential cardiac transcription factors in 256 patients with non-syndromic congenital heart defects. *Circ J* 76:1703–1711
22. Ieda M, Fu JD, Delgado-Olguin P et al (2010) Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 142:375–386
23. Qian L, Huang Y, Spencer CI, Foley A et al (2012) In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature* 485:593–598
24. Biben C, Harvey RP (1997) Homeodomain factor *Nk2-5* controls left/right asymmetric expression of *bHLH* gene *eHand* during murine heart development. *Genes Dev* 11:1357–1369
25. Srivastava D, Thomas T, Lin Q et al (1997) Regulation of cardiac mesodermal and neural crest development by the *bHLH* transcription factor, *dHAND*. *Nat Genet* 16:154–160

26. Firulli AB, McFadden DG, Lin Q, Srivastava D, Olson EN (1998) Heart and extra-embryonic mesodermal defects in mouse embryos lacking the bHLH transcription factor Hand1. *Nat Genet* 18:266–270
27. Riley P, Anson-Cartwright L, Cross JC (1998) The Hand1 bHLH transcription factor is essential for placentation and cardiac morphogenesis. *Nat Genet* 18:271–275
28. Firulli BA, McConville DP, Byers JS et al (2010) Analysis of a Hand1 hypomorphic allele reveals a critical threshold for embryonic viability. *Dev Dyn* 239:2748–2760 (An official publication of the American Association of Anatomists)
29. Risebro CA, Smart N, Dupays L et al (2006) Hand1 regulates cardiomyocyte proliferation versus differentiation in the developing heart. *Development* 133:4595–4606
30. Yamagishi H, Yamagishi C, Nakagawa O et al (2001) The combinatorial activities of Nkx2.5 and dHAND are essential for cardiac ventricle formation. *Dev Biol* 239:190–203
31. Firulli BA, Hadzic DB, McDaid JR, Firulli AB (2000) The basic helix-loop-helix transcription factors dHAND and eHAND exhibit dimerization characteristics that suggest complex regulation of function. *J Biol Chem* 275:33567–33573
32. Barnes RM, Firulli BA, VanDusen NJ et al (2011) Hand2 loss-of-function in Hand1-expressing cells reveals distinct roles in epicardial and coronary vessel development. *Circ Res* 108:940–949
33. Holler KL, Hendershot TJ, Troy SE et al (2010) Targeted deletion of Hand2 in cardiac neural crest-derived cells influences cardiac gene expression and outflow tract development. *Dev Biol* 341:291–304
34. Shen L, Li XF, Shen AD et al (2010) Transcription factor HAND2 mutations in sporadic Chinese patients with congenital heart disease. *Chin Med J (Engl)* 123:1623–1627
35. Cheng Z, Lib L, Li Z et al (2012) Two novel HAND1 mutations in Chinese patients with ventricular septal defect. *Clin Chim Acta* 413:675–677
36. Esposito G, Butler TL, Blue GM, Cole AD et al (2011) Somatic mutations in NKX2-5, GATA4, and HAND1 are not a common cause of tetralogy of Fallot or hypoplastic left heart. *Am J Med Genet A* 155A:2416–2421
37. Wang J, Lu Y, Chen H et al (2011) Investigation of somatic NKX2-5, GATA4 and HAND1 mutations in patients with tetralogy of Fallot. *Pathology* 43:322–326
38. Basson CT, Bachinsky DR, Lin RC et al (1997) Mutations in human TBX5 [corrected] cause limb and cardiac malformation in Holt-Oram syndrome. *Nat Genet* 15:30–35
39. Bruneau BG, Logan M, Davis N et al (1999) Chamber-specific cardiac expression of Tbx5 and heart defects in Holt-Oram syndrome. *Dev Biol* 211:100–108
40. Bruneau BG, Nemer G, Schmitt JP et al (2001) A murine model of Holt-Oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease. *Cell* 106:709–721
41. Liberatore CM, Searcy-Schrick RD, Yutzey KE (2000) Ventricular expression of tbx5 inhibits normal heart chamber development. *Dev Biol* 223:169–180
42. Hiroi Y, Kudoh S, Monzen K et al (2001) Tbx5 associates with Nk2–5 and synergistically promotes cardiomyocyte differentiation. *Nat Genet* 28:276–280
43. Herrmann F, Bundschu K, Kuhl SJ, Kuhl M (2011) Tbx5 overexpression favors a first heart field lineage in murine embryonic stem cells and in *Xenopus laevis* embryos. *Dev Dyn* 240:2634–2645 (An official publication of the American Association of Anatomists)
44. Brown DD, Martz SN, Binder O et al (2005) Tbx5 and Tbx20 act synergistically to control vertebrate heart morphogenesis. *Development* 132:553–563
45. Ghosh TK, Song FF, Packham EA et al (2009) Physical interaction between TBX5 and MEF2C is required for early heart development. *Mol Cell Biol* 29:2205–2218
46. Maitra M, Schluterman MK, Nichols HA et al (2009) Interaction of Gata4 and Gata6 with Tbx5 is critical for normal cardiac development. *Dev Biol* 326:368–377
47. Liang Q, Molkenin JD (2002) Divergent signaling pathways converge on GATA4 to regulate cardiac hypertrophic gene expression. *J Mol Cell Cardiol* 34:611–616

48. Molkenkin JD (2000) The zinc finger-containing transcription factors GATA-4, -5, and -6. Ubiquitously expressed regulators of tissue-specific gene expression. *J Biol Chem* 275:38949–38952
49. Zhou P, He A, Pu WT (2012) Regulation of GATA4 transcriptional activity in cardiovascular development and disease. *Curr Top Dev Biol* 100:143–169
50. van Berlo JH, Elrod JW, van den Hoogenhof MM et al (2010) The transcription factor GATA-6 regulates pathological cardiac hypertrophy. *Circ Res* 107:1032–1040
51. Arceci RJ, King AA, Simon MC et al (1993) Mouse GATA-4: a retinoic acid-inducible GATA-binding transcription factor expressed in endodermally derived tissues and heart. *Mol Cell Biol* 13:2235–2246
52. Heikinheimo M, Scandrett JM, Wilson DB (1994) Localization of transcription factor GATA-4 to regions of the mouse embryo involved in cardiac development. *Dev Biol* 164:361–373
53. Kuo CT, Morrissey EE, Anandappa R et al (1997) GATA4 transcription factor is required for ventral morphogenesis and heart tube formation. *Genes Dev* 11:1048–1060
54. Molkenkin JD, Lin Q, Duncan SA, Olson EN (1997) Requirement of the transcription factor GATA4 for heart tube formation and ventral morphogenesis. *Genes Dev* 11:1061–1072
55. Charron F, Nemer M (1999) GATA transcription factors and cardiac development. *Semin Cell Dev Biol* 10:85–89
56. Watt AJ, Battle MA, Li J, Duncan SA (2004) GATA4 is essential for formation of the proepicardium and regulates cardiogenesis. *Proc Natl Acad Sci USA* 101:12573–12578
57. Zeisberg EM, Ma Q, Juraszek AL et al (2005) Morphogenesis of the right ventricle requires myocardial expression of Gata4. *J Clin Invest* 115:1522–1531
58. Belaguli NS, Sepulveda JL, Nigam V et al (2000) Cardiac tissue enriched factors serum response factor and GATA-4 are mutual coregulators. *Mol Cell Biol* 20:7550–7558
59. Sepulveda JL, Belaguli N, Nigam V et al (1998) GATA-4 and Nkx-2.5 coactivate Nkx-2 DNA binding targets: role for regulating early cardiac gene expression. *Mol Cell Biol* 18:3405–3415
60. Molkenkin JD, Antos C, Mercer B et al (2000) Direct activation of a GATA6 cardiac enhancer by Nkx2.5: evidence for a reinforcing regulatory network of Nkx2.5 and GATA transcription factors in the developing heart. *Dev Biol* 217:301–309
61. Schwartz RJ, Olson EN (1999) Building the heart piece by piece: modularity of cis-elements regulating Nk2-5 transcription. *Development* 126:4187–4192
62. Rajagopal SK, Ma Q, Obler D et al (2007) Spectrum of heart disease associated with murine and human GATA4 mutation. *J Mol Cell Cardiol* 43:677–685
63. Buckingham M, Meilhac S, Zaffran S (2005) Building the mammalian heart from two sources of myocardial cells. *Nat Rev Genet* 6:826–835
64. Mjaatvedt CH, Nakaoka T, Moreno-Rodriguez R et al (2001) The outflow tract of the heart is recruited from a novel heart-forming field. *Dev Biol* 238:97–109
65. Waldo KL, Kumiski DH, Wallis KT et al (2001) Conotruncal myocardium arises from a secondary heart field. *Development* 128:3179–3188
66. Kelly RG, Brown NA, Buckingham ME (2001) The arterial pole of the mouse heart forms from Fgf10-expressing cells in pharyngeal mesoderm. *Dev Cell* 1:435–440
67. Meilhac SM, Esner M, Kelly RG, Nicolas JF, Buckingham ME (2004) The clonal origin of myocardial cells in different regions of the embryonic mouse heart. *Dev Cell* 6:685–698
68. Zaffran S, Kelly RG, Meilhac SM, Buckingham ME, Brown NA (2004) Right ventricular myocardium derives from the anterior heart field. *Circ Res* 95:261–268
69. Tanizawa Y, Riggs AC, Dagogo-Jack S et al (1994) Isolation of the human LIM/homeodomain gene islet-1 and identification of a simple sequence repeat polymorphism [corrected]. *Diabetes* 43:935–941
70. Cai CL, Liang X, Shi Y, Chu PH et al (2003) Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Dev Cell* 5:877–889

71. Kang J, Nathan E, Xu SM, Tzahor E, Black BL (2009) Isl1 is a direct transcriptional target of Forkhead transcription factors in second-heart-field-derived mesoderm. *Dev Biol* 334:513–522
72. Lin L, Cui L, Zhou W, Dufort D et al (2007) Beta-catenin directly regulates Islet1 expression in cardiovascular progenitors and is required for multiple aspects of cardiogenesis. *Proc Natl Acad Sci USA* 104:9313–9318
73. Golzio C, Havis E, Daubas P, Nuel G et al (2012) ISL1 directly regulates FGF10 transcription during human cardiac outflow formation. *PLoS One* 7:e30677
74. Engleka KA, Manderfield LJ, Brust RD et al (2012) Islet1 derivatives in the heart are of both neural crest and second heart field origin. *Circ Res* 110:922–926
75. Laugwitz KL, Moretti A, Lam J et al (2005) Postnatal isl1 + cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature* 433:647–653
76. Bu L, Jiang X, Martin-Puig S, Caron L et al (2009) Human ISL1 heart progenitors generate diverse multipotent cardiovascular cell lineages. *Nature* 460:113–117
77. Kwon C, Qian L, Cheng P et al (2009) A regulatory pathway involving Notch1/beta-catenin/Is11 determines cardiac progenitor cell fate. *Nat Cell Biol* 11:951–957
78. Jerome LA, Papaioannou VE (2001) DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1. *Nat Genet* 27:286–291
79. Lindsay EA, Vitelli F, Su H et al (2001) Tbx1 haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature* 410:97–101
80. Merscher S, Funke B, Epstein JA et al (2001) TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell* 104:619–629
81. Xu H, Morishima M, Wylie JN et al (2004) Tbx1 has a dual role in the morphogenesis of the cardiac outflow tract. *Development* 131:3217–3227
82. Hu T, Yamagishi H, Maeda J et al (2004) Tbx1 regulates fibroblast growth factors in the anterior heart field through a reinforcing autoregulatory loop involving forkhead transcription factors. *Development* 131:5491–5502
83. Chen L, Fulcoli FG, Ferrentino R et al (2012) Transcriptional control in cardiac progenitors: Tbx1 interacts with the BAF chromatin remodeling complex and regulates Wnt5a. *PLoS Genet* 8:e1002571
84. Zhang Z, Huynh T, Baldini A (2006) Mesodermal expression of Tbx1 is necessary and sufficient for pharyngeal arch and cardiac outflow tract development. *Development* 133:3587–3595
85. Huynh T, Chen L, Terrell P, Baldini A (2007) A fate map of Tbx1 expressing cells reveals heterogeneity in the second cardiac field. *Genesis* 45:470–475
86. Chen L, Fulcoli FG, Tang S, Baldini A (2009) Tbx1 regulates proliferation and differentiation of multipotent heart progenitors. *Circ Res* 105:842–851
87. Tsuchihashi T, Maeda J, Shin CH et al (2011) Hand2 function in second heart field progenitors is essential for cardiogenesis. *Dev Biol* 351:62–69
88. Hiruma T, Hirakow R (1989) Epicardial formation in embryonic chick heart: computer-aided reconstruction, scanning, and transmission electron microscopic studies. *Am J Anat* 184:129–138
89. Manner J (1992) The development of pericardial villi in the chick embryo. *Anat Embryol* 186:379–385
90. Nahirney PC, Mikawa T, Fischman DA (2003) Evidence for an extracellular matrix bridge guiding proepicardial cell migration to the myocardium of chick embryos. *Dev Dyn* 227:511–523 (An official publication of the American Association of Anatomists)
91. Ishii Y, Garriock RJ, Navetta AM, Coughlin LE, Mikawa T (2010) BMP signals promote proepicardial protrusion necessary for recruitment of coronary vessel and epicardial progenitors to the heart. *Dev Cell* 19:307–316
92. Mikawa T, Gourdie RG (1996) Pericardial mesoderm generates a population of coronary smooth muscle cells migrating into the heart along with ingrowth of the epicardial organ. *Dev Biol* 174:221–232

93. Gittenberger-de Groot AC, Vrancken Peeters MP, Mentink MM, Gourdie RG, Poelmann RE (1998) Epicardium-derived cells contribute a novel population to the myocardial wall and the atrioventricular cushions. *Circ Res* 82:1043–1052
94. Kraus F, Haenig B, Kispert A (2001) Cloning and expression analysis of the mouse T-box gene *Tbx18*. *Mech Dev* 100:83–86
95. Christoffels VM, Mommersteeg MT, Trowe MO et al (2006) Formation of the venous pole of the heart from an *Nk2-5*-negative precursor population requires *Tbx18*. *Circ Res* 98:1555–1563
96. Wiese C, Grieskamp T, Airik R et al (2009) Formation of the sinus node head and differentiation of sinus node myocardium are independently regulated by *Tbx18* and *Tbx3*. *Circ Res* 104:388–397
97. Wills AA, Holdway JE, Major RJ, Poss KD (2008) Regulated addition of new myocardial and epicardial cells fosters homeostatic cardiac growth and maintenance in adult zebrafish. *Development* 135:183–192
98. Cai CL, Martin JC, Sun Y et al (2008) A myocardial lineage derives from *Tbx18* epicardial cells. *Nature* 454:104–108
99. Christoffels VM, Grieskamp T, Norden J et al (2009) *Tbx18* and the fate of epicardial progenitors. *Nature* 458: E8-9; discussion E9-10
100. Moore AW, McInnes L, Kreidberg J, Hastie ND, Schedl A (1999) YAC complementation shows a requirement for *Wt1* in the development of epicardium, adrenal gland and throughout nephrogenesis. *Development* 126:1845–1857
101. Wagner N, Wagner KD, Theres H et al (2005) Coronary vessel development requires activation of the *TrkB* neurotrophin receptor by the Wilms' tumor transcription factor *Wt1*. *Genes Dev* 19:2631–2642
102. Zhou B, Ma Q, Rajagopal S, Wu SM et al (2008) Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. *Nature* 454:109–113
103. Martinez-Estrada OM, Lettice LA, Essafi A et al (2010) *Wt1* is required for cardiovascular progenitor cell formation through transcriptional control of *Snail* and *E-cadherin*. *Nat Genet* 42:89–93
104. Smart N, Bollini S, Dube KN et al (2011) De novo cardiomyocytes from within the activated adult heart after injury. *Nature* 474:640–644
105. Katz TC, Singh MK, Degenhardt K et al (2012) Distinct compartments of the proepicardial organ give rise to coronary vascular endothelial cells. *Dev Cell* 22:639–650
106. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T (2001) Identification of novel genes coding for small expressed RNAs. *Science* 294:853–858
107. Lau NC, Lim LP, Weinstein EG, Bartel DP (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* 294:858–862
108. Lee RC, Ambros V (2001) An extensive class of small RNAs in *caenorhabditis elegans*. *Science* 294:862–864
109. Boettger T, Braun T (2012) A new level of complexity: the role of MicroRNAs in cardiovascular development. *Circ Res* 110:1000–1013
110. Espinoza-Lewis RA, Wang DZ (2012) MicroRNAs in heart development. *Curr Top Dev Biol* 100:279–317
111. Kwon C, Han Z, Olson EN, Srivastava D (2005) MicroRNA1 influences cardiac differentiation in *drosophila* and regulates notch signaling. *Proc Natl Acad Sci USA* 102:18986–18991
112. Zhao Y, Samal E, Srivastava D (2005) Serum response factor regulates a muscle-specific microRNA that targets *Hand2* during cardiogenesis. *Nature* 436:214–220
113. Zhao Y, Ransom JF, Li A et al (2007) Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking *miRNA-1-2*. *Cell* 129:303–317
114. Bernstein E, Kim SY, Carmell MA et al (2003) *Dicer* is essential for mouse development. *Nat Genet* 35:215–217

115. Huang ZP, Chen JF, Regan JN et al (2010) Loss of microRNAs in neural crest leads to cardiovascular syndromes resembling human congenital heart defects. *Arterioscler Thromb Vasc Biol* 30:2575–2586
116. Chen JF, Murchison EP, Tang R et al (2008) Targeted deletion of *dicer* in the heart leads to dilated cardiomyopathy and heart failure. *Proc Natl Acad Sci USA* 105:2111–2116
117. Darnell DK, Kaur S, Stanislaw S et al (2006) MicroRNA expression during chick embryo development. *Developmental dynamics: an official publication of the American association of anatomists* 235:3156–3165
118. Sempere LF, Freemantle S, Pitha-Rowe I et al (2004) Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. *Genome Biol* 5:R13
119. Sluijter JP, van Mil A, van Vliet P et al (2010) MicroRNA-1 and -499 regulate differentiation and proliferation in human-derived cardiomyocyte progenitor cells. *Arterioscler Thromb Vasc Biol* 30:859–868
120. Ivey KN, Muth A, Arnold J et al (2008) MicroRNA regulation of cell lineages in mouse and human embryonic stem cells. *Cell Stem Cell* 2:219–229
121. Liu N, Bezprozvannaya S, Williams AH et al (2008) microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. *Genes Dev* 22:3242–3254
122. Chen JF, Mandel EM, Thomson JM et al (2006) The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 38:228–233
123. Callis TE, Pandya K, Seok HY et al (2009) MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice. *J Clin Invest* 119:2772–27786
124. van Rooij E, Quiat D, Johnson BA et al (2009) A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. *Dev Cell* 17:662–673
125. van Rooij E, Sutherland LB, Qi X et al (2007) Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science* 316:575–579
126. Cohen-Barak O, Yi Z, Hagiwara N et al (2003) Sox6 regulation of cardiac myocyte development. *Nucleic Acids Res* 31:5941–5948
127. Fu JD, Rushing SN, Lieu DK et al (2011) Distinct roles of microRNA-1 and -499 in ventricular specification and functional maturation of human embryonic stem cell-derived cardiomyocytes. *PLoS One* 6:e27417
128. Jayawardena TM, Egemnazarov B, Finch EA et al (2012) MicroRNA-mediated in vitro and in vivo direct reprogramming of cardiac fibroblasts to cardiomyocytes. *Circ Res* 110:1465–1473
129. Wang J, Greene SB, Bonilla-Claudio M et al (2010) Bmp signaling regulates myocardial differentiation from cardiac progenitors through a MicroRNA-mediated mechanism. *Dev Cell* 19:903–912
130. Ventura A, Young AG, Winslow MM et al (2008) Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. *Cell* 132:875–886
131. Kim GH, Samant SA, Earley JU, Svensson EC (2009) Translational control of FOG-2 expression in cardiomyocytes by microRNA-130a. *PLoS One* 4:e6161
132. Xiang R, Lei H, Chen M et al (2012) The miR-17-92 cluster regulates FOG-2 expression and inhibits proliferation of mouse embryonic cardiomyocytes. *Braz J Med Biol Res* 45:131–138
133. Porrello ER, Johnson BA, Aurora AB et al (2011) MiR-15 family regulates postnatal mitotic arrest of cardiomyocytes. *Circ Res* 109:670–679

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