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REVIEW

Genetic and epigenetic regulation of cardiomyocytes in development, regeneration and disease

Miao Cui, Zhaoning Wang, Rhonda Bassel-Duby and Eric N. Olson*

ABSTRACT

Embryonic and postnatal life depend on the uninterrupted function of cardiac muscle cells. These cells, termed cardiomyocytes, display many fascinating behaviors, including complex morphogenic movements, interactions with other cell types of the heart, persistent contractility and quiescence after birth. Each of these behaviors depends on complex interactions between both cardiac-restricted and widely expressed transcription factors, as well as on epigenetic modifications. Here, we review recent advances in our understanding of the genetic and epigenetic control of cardiomyocyte differentiation and proliferation during heart development, regeneration and disease. We focus on those regulators that are required for both heart development and disease, and highlight the regenerative principles that might be manipulated to restore function to the injured adult heart.

KEY WORDS: Cardiac disease remodeling, Cardiac transcription factors, Cardiogenesis, Epigenetic regulator, Heart regeneration

Introduction

Heart disease, the leading cause of death worldwide, arises primarily from loss of or damage to cardiomyocytes – the muscle cells that generate the contractile force of the heart. Cardiomyocyte loss can occur in response to myocardial infarction (MI), toxic insults or genetic mutations. Over the past two decades, significant effort has been invested in devising new approaches for cardiac repair, including drug development, improvement of medical devices, and development of cell-based therapies such as injection of cardiac progenitor cells into the heart, reprogramming of non-muscle cells into cardiomyocytes, and stimulation of cardiomyocyte proliferation (Cahill et al., 2017; Hashimoto et al., 2018; Tzahor and Poss, 2017). Despite these many new strategies, a clinically effective approach for therapeutically repairing an injured adult human heart does not yet exist, underscoring the need for further insight into the fundamental mechanisms of cardiomyocyte differentiation, proliferation and function.

It is generally accepted that cardiomyocyte proliferation is an essential element of heart regeneration (Senyo et al., 2014; Xin et al., 2013a, b). For instance, although the proliferative capacity of adult mammalian cardiomyocytes is minimal, cardiomyocytes from various other species, including zebrafish, amphibians, and reptiles, can replicate throughout life, thus allowing heart regeneration in response to injury (Jopling et al., 2010; Oberpriller and Oberpriller, 1974). In addition, cardiomyocytes of the neonatal mammalian heart retain a transient capacity to proliferate and can drive heart regeneration following injury, although this capacity is lost soon after birth (Xin et al., 2013a, b; Porrello et al., 2011; Eschenhagen et al., 2017). Thus, defining the molecular mechanisms that govern the proliferative capacity of cardiomyocytes, during both development and regeneration, is a central goal in cardiac biology.

In recent years, significant advances in our understanding of heart development and regeneration have been provided by studies in different animal models, including mice, birds, amphibians and fish. Moreover, recent technological advances in transcriptomic and epigenomic profiling have provided global views of the dynamic molecular control of cardiomyocyte gene expression during cardiac development, repair and regeneration. These studies have highlighted key factors that can influence the behavior of cardiomyocytes in normal and disease contexts. In this Review, we discuss the roles of cardiac transcription factors (TFs), histone modifications, and chromatin organization in cardiac development, regeneration and remodeling during disease. We highlight analogous mechanisms used to regulate the expression of genes associated with both cardiac development and repair. We also discuss the mechanisms involved in postnatal cardiomyocyte cell-cycle arrest and highlight recent efforts to modulate cardiomyocyte proliferation as a strategy for cardiac regeneration.

An overview of cardiac development

Heart development requires the precise specification, proliferation, differentiation and maturation of cardiomyocytes, and their seamless integration with the many other cell types of the heart. These processes begin early in embryogenesis and continue into postnatal life. A wealth of studies on cardiac development have revealed the molecular and cellular signatures of the developing heart. We now know many of the signaling pathways and TFs that play pivotal functions during these developmental processes. Below, we provide an overview of the molecular and morphological changes associated with cardiac development and highlight the cardiac TFs with key roles in these processes. Although the scope of this Review focuses on cardiomyocytes, it should be noted that the heart is composed of numerous other cell types, including fibroblasts, endothelial cells and smooth muscle cells, which are also important for heart development, as reviewed elsewhere (Xin et al., 2013a; Meilhac and Buckingham, 2018; Meilhac et al., 2014).

The embryonic and postnatal development of cardiomyocytes

The heart is the first organ to form and function during embryogenesis. Cardiogenesis begins around embryonic day (E) 6.5 in mice when cardiac progenitor cells derived from the anterior region of the primitive streak migrate to the heart-forming region and form the cardiac crescent, in which cardiac specification markers are first detected. The cardiac crescent contains two distinct progenitor cell populations, referred to as the first and second heart fields (FHF and SHF, respectively), that contribute to different

Department of Molecular Biology, Hamon Center for Regenerative Science and Medicine, and Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA.

*Author for correspondence (eric.olson@utsouthwestern.edu)

E.N.O., 0000-0003-1151-8262

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regions of the developing heart (Brade et al., 2013; Buckingham et al., 2005) (Fig. 1A). Cells in the FHF give rise primarily to the left ventricle with a small contribution to the atria, whereas the SHF gives rise to the right ventricle, outflow tract and atria. By E8, a beating heart tube is formed and subsequent differentiation of the FHF gives rise to an interior layer of endocardial cells and an exterior layer of myocardial cells. The SHF contributes to the subsequent extension of the heart tube, adding cells to the anterior arterial pole and posterior venous pole at E8.5, resulting in rapid elongation and rightward looping of the heart tube, which then leads to the formation of primitive ventricles and atria. Cells in both the FHF and the SHF express the early mesoderm TF Mesp1. MESP1 expression starts at the early gastrulation stage (E6) and is the earliest known marker of cardiac specification in the developing embryo (Bondue et al., 2008; Devine et al., 2014). Both lineage-tracing and single-cell analyses have shown that MESP1-expressing cardiac progenitors at the early gastrulation stage are committed to different cardiovascular lineages and regions of the heart (Devine et al., 2014; Lescroart et al., 2018), highlighting that cardiac lineage specification precedes the establishment of anatomically distinct cardiac structures. Elucidating how early signaling events and regulation by TFs control this early specification of cardiac lineages will be important for understanding the basis of congenital heart defects.

FHF-derived cardiomyocytes that make up the linear heart tube proliferate slowly and display weak contractility (Soufan et al., 2006; Spater et al., 2014; Paige et al., 2015). These characteristics are retained in the cardiomyocytes residing in the outflow tract, inner curvature, atrioventricular canal and sinus horns of the developing heart. In contrast, ventricular and atrial cardiomyocytes undergo rapid proliferation and differentiation, and as such are primary contributors to the growth of the developing heart (Auman et al., 2007; Dietrich et al., 2014). These cells display fast conduction velocity, strong contractility and enhanced sarcomere structure to cope with the mechanical stress from the blood flow. However, whereas these cardiomyocytes are highly proliferative during embryonic development, they are quiescent in the adult heart, exhibiting a turnover rate of <1% per year (Bergmann et al., 2009). The loss of cell-cycle activity in postnatal cardiomyocytes occurs gradually in mice (Alkass et al., 2015; Naqvi et al., 2014) coinciding with an increase in multinucleation and polyploidy. Indeed, cardiomyocytes exhibit three phases: (1) the proliferative phase, which is restricted to embryogenesis and the first week after birth; (2) the multinucleation phase, which occurs in the second postnatal week; and (3) the polyploidization phase, which takes place mainly in the second and third postnatal weeks (Alkass et al., 2015). As a result, the majority (>80%) of murine adult cardiomyocytes are tetraploid and contain two nuclei (Naqvi et al., 2014).

Cardiomyocyte maturation, which begins after birth and continues until adulthood, is crucial for efficient contractility and calcium handling, as well as for meeting the metabolic demands of the adult mammalian heart. During the maturation process, cardiomyocytes elongate and develop highly aligned, uniformly distributed sarcomeres and dense myofibrillar structures. A hallmark of cardiomyocyte maturation is isoform switching of contractile genes from the fetal to the adult state (Ames et al., 2013; Taegtmeyer et al., 2010). For example, in mice, the predominant isoform of myosin heavy chain (MHC) protein switches from the beta (β-MHC or MYH7) to the adult alpha (α-MHC or MYH6) isoform. In addition, mature cardiomyocytes express higher levels of cardiac troponin T 3 (cTnT3) in comparison with the fetal cTnT1 and cTnT2 isoforms. The troponin I isoform also changes from slow skeletal troponin I (TNNI1), which predominates in fetal and neonatal cardiomyocytes, to cardiac troponin I (cTni; TNNI3), found primarily in adult cardiomyocytes. These changes in troponin isoforms allow the adult heart to achieve higher contractility to meet increased hemodynamic demands. Concomitant with the

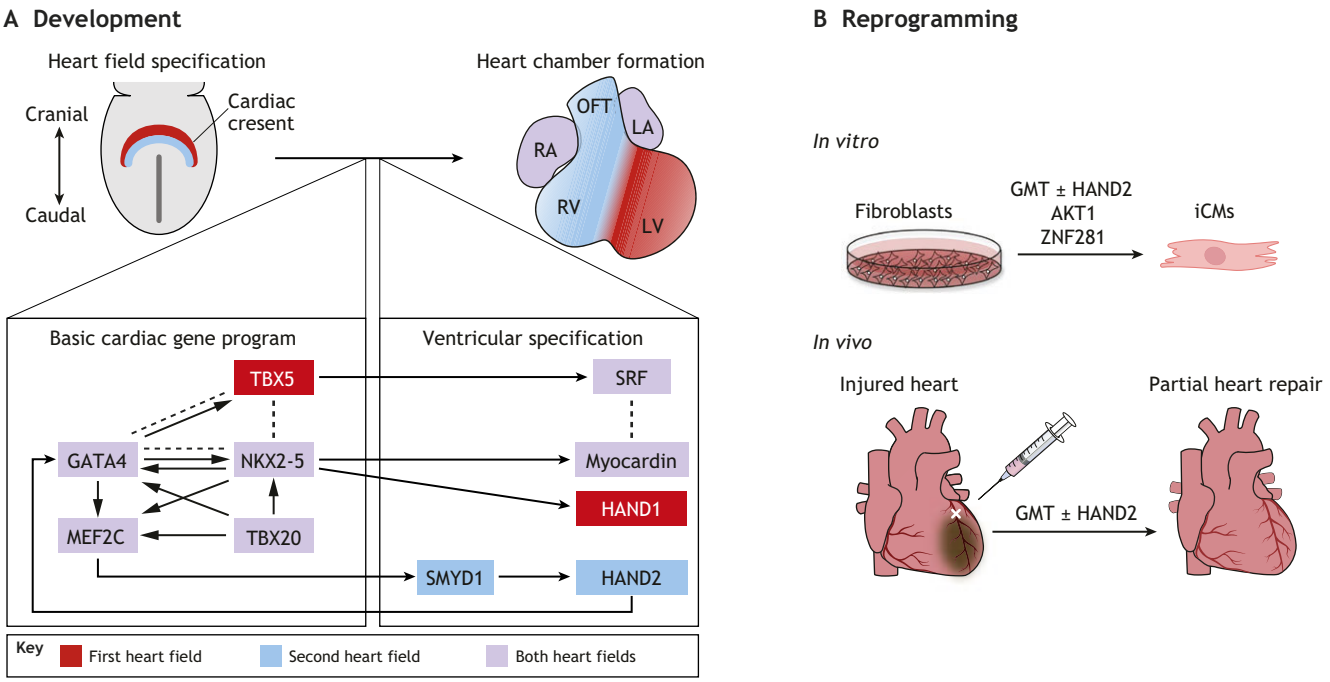

Fig. 1. Gene networks underlying cardiac development and reprogramming. (A) A brief overview of a subset of transcription factor interactions that drive the specification of the first heart field (red), second heart field (blue) and ventricular chamber. Transcription factors colored in purple represent regulators of both heart fields. Arrows indicate transcriptional activation; dashed lines indicate functional interdependency. LA, left atrium; LV, left ventricle; OFT, outflow tract; RA, right atrium; RV, right ventricle. (B) Fibroblasts with forced expression of GATA4, MEF2C and TBX5 (GMT) with or without HAND2 are reprogrammed into induced cardiomyocytes (iCMs) both *in vitro* and *in vivo*. Addition of AKT1 and ZNF281 to the reprogramming cocktail further increases reprogramming efficiency.

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irreversible withdrawal from the cell cycle and isoform switching of structural genes, maturing cardiomyocytes also undergo a metabolic switch (from glycolytic to oxidative), providing the adult heart with a more effective means of producing ATP (Ellen Kreipke et al., 2016). To account for these cellular alterations, extensive transcriptomic changes must occur in cardiomyocytes during postnatal maturation. In fact, the transcriptome of adult cardiomyocytes is dramatically different from that of neonatal cardiomyocytes, to the extent that they could be considered as two distinct cell types based on their transcriptome differences (Quaife-Ryan et al., 2017). Therefore, reverting adult cardiomyocytes to a neonatal state may require a reprogramming approach to reset the global transcriptome and epigenome landscape.

Transcription factor networks that govern cardiac development

Cardiogenesis relies on spatially and temporally regulated signaling and the activation of a plethora of cardiac TFs (reviewed by Paige et al., 2015; Brand, 2003; Bruneau, 2013; Olson, 2006). A number of these cardiac TFs constitute an evolutionarily conserved gene regulatory network that is essential for cardiac development (Fig. 1A). The core TFs of the cardiac gene network include members of the GATA zinc-finger family (GATA4, GATA6, etc.), the T-box protein family (TBX5), the NK homeodomain family (NKX2-5), the MADS box family (MEF2, SRF), and the basic helix-loop-helix family (HAND1 and HAND2). These TFs regulate a broad cardiac developmental gene program for cardiac specification and cardiomyocyte differentiation. Studies using genetic loss-of-function mouse models have demonstrated the essential roles of these cardiac TFs in early development, as evident by embryonic lethality due to heart malformations (Bruneau et al., 2001; Firulli et al., 1998; Lin et al., 1997; Lyons et al., 1995; Riley et al., 1998; Srivastava et al., 1997; Tanaka et al., 1999; Watt et al., 2004; Zhao et al., 2008). Mutations of these cardiac TFs in humans are also often associated with congenital heart disease, highlighting their evolutionarily conserved functions in cardiac development (reviewed by McCulley and Black, 2012).

The regulatory interplay between cardiac TFs is demonstrated by their positive-feedback and feed-forward transcriptional regulation during cardiogenesis. For example, GATA4 and NKX2-5 regulate each other's expression (Gottlieb et al., 2002; Lien et al., 1999; Molkentin et al., 2000), and MEF2C is also regulated by GATA4, which in turn regulates the expression of *Hand2* by directly activating its upstream regulator SMYD1 (Gottlieb et al., 2002; Dodou et al., 2004; Phan et al., 2005). Additionally, core cardiac TFs function cooperatively to co-occupy cardiac enhancers and/or physically interact with each other. This is seen in the complex interdependency of GATA4, TBX5 and NKX2-5. It is well-documented that GATA4 and TBX5 proteins physically interact (Bruneau et al., 2001; Hiroi et al., 2001). A mutation in the *GATA4* coding region that disrupts the GATA4/TBX5 interaction and blocks GATA4/TBX5 co-binding to cardiac super-enhancers leads to an impaired cardiac gene program, causing congenital heart defects (Ang et al., 2016; Garg et al., 2003). Similarly, TBX5 directly interacts with NKX2-5 to regulate cardiac gene expression (Bruneau et al., 2001; Hiroi et al., 2001), and the genomic profiling of DNA-binding events for GATA4, TBX5 and NKX2-5 reveals significant genome-wide co-occupancy of these cardiac TFs during cardiomyocyte differentiation (Luna-Zurita et al., 2016). The co-occupancy of cardiac TFs likely requires co-occurrence of TF binding sites in the genome as well as protein-protein interactions among various combinations of factors. Indeed, co-crystallization of an NKX2-5/TBX5 fusion protein bound to the cardiac-specific *Nppa* promoter uncovered a conserved NKX2-5-TBX5 protein-binding interface (Luna-Zurita et al., 2016). This interaction suggests co-evolution of protein-protein interactions between core cardiac TFs and their DNA-binding motifs on the genome.

The genetic regulatory circuits formed by cardiac TFs are likely evolutionarily advantageous to ensure robust activation of the cardiogenic program and, hence, to safeguard the formation of the heart. Furthermore, the combinatorial effects of these cardiac TFs may enable complex fine-tuning and multi-layer regulation of cardiac gene expression as the heart undergoes extensive morphological changes during development. The timing of cardiac development is also likely attributable to this co-operative feature of cardiac TFs. Cardiac development in the mouse occurs at the same time or shortly after the formation of the different germ layers (E6-E7.5). Similar to the specification of the germ layers, cardiac development is initiated by signaling inputs and TFs that have broad expression patterns across multiple embryonic domains. Moreover, the combinatorial regulation of these TFs subdivides the cardiac regions into more defined structures.

Finally, it should be noted that, although previous studies in skeletal muscle have identified the transcription factor MYOD (MYOD1) as a master regulator of skeletal muscle differentiation (Davis et al., 1987; Tapscott et al., 1988), no single TF has been shown to induce cardiac muscle fate by itself; rather, combinations of core cardiac TFs are required to direct the lineage reprogramming of fibroblasts to cardiomyocytes (Fig. 1B). GATA4, MEF2C and TBX5 (GMT) were first identified as a minimum set of TFs sufficient to induce cardiac differentiation from fibroblasts (Qian et al., 2012; Ieda et al., 2010). Adding HAND2 to the GMT reprogramming cocktail (to give a GHMT cocktail) significantly enhanced reprogramming efficiency *in vitro*, and greatly improved cardiac function after MI within the adult heart *in vivo* (Song et al., 2012). AKT1 and the zinc-finger protein ZNF281 were later shown to further boost reprogramming efficiency on top of the effect of GHMT (Zhou et al., 2015; Zhou et al., 2017). Although extensive work using both screen-based and genomics-based approaches has identified additional activators, repressors and pathways that can modulate direct cardiac reprogramming, the key to reprogramming appears to be the combinatorial actions of the core cardiac TFs (Abad et al., 2017; Ifkovits et al., 2014; Liu et al., 2017; Mohamed et al., 2017; Muraoka et al., 2014; Zhao et al., 2015). This highlights conserved mechanisms of cardiac differentiation, both in the context of embryonic development and in direct cardiac reprogramming.

From cardiac development to cardiac regeneration

Cardiomyocytes are essential for proper cardiac contraction and function. Following a heart attack, there is dramatic and acute loss of cardiomyocytes, resulting in impaired cardiac function. The regeneration of cardiomyocytes is therefore an effective way to repair an injured heart. This type of regeneration is seen in lower vertebrates as well as in neonatal mammals, but is absent in adult mammals (Senyo et al., 2014; Xin et al., 2013a, b). Over the past several years, especially since the discovery of the transient regenerative capacity of the neonatal mouse heart in 2011 (Porrello et al., 2011), studies have focused on the proliferation and differentiation of cardiomyocytes during heart regeneration using various animal models. These studies have led to the discovery of many factors (TFs, cell-cycle regulators and growth factors) that can promote cardiomyocyte proliferation and have unveiled mechanisms that might underlie the blockade of regeneration in adult mammalian hearts.

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Animal models of cardiac regeneration

In contrast to mammals, lower vertebrates, including fish and salamanders, possess cardiac regenerative capacity. The zebrafish heart, for example, can regenerate lost muscle after ventricle resection, cryoinjury, or genetic ablation of cardiomyocytes (Tzahor and Poss, 2017; Jopling et al., 2010; Karra and Poss, 2017). Likewise, the salamander heart regenerates after amputation of the ventricle apex as well as after cryoinjury (Becker et al., 1974; Godwin et al., 2017). Importantly, lineage-mapping studies of adult zebrafish heart regeneration have shown that newly regenerated cardiomyocytes are derived from existing cardiomyocytes rather than from of a pool of cardiac progenitors (Jopling et al., 2010). This has shifted the focus of the cardiac regeneration field from identifying cardiac progenitors to activating the cell cycle of endogenous postnatal cardiomyocytes.

Although the adult mammalian heart lacks regenerative capability, the neonatal heart of mice is fully capable of regeneration after apical resection, as well as after MI, within the first week after birth (Porrello et al., 2011; Porrello et al., 2013). However, whereas the regenerative injury response in zebrafish is associated with cardiomyocyte dedifferentiation, characterized by the disassembly of sarcomeres and the reactivation of a GATA4-dependent embryonic gene program (Jopling et al., 2010; Malek Mohammadi et al., 2017; Nistri et al., 2012), global induction of the embryonic developmental program has not been observed in the neonatal mouse heart in response to injury. In fact, a recent study that profiled the transcriptome of cardiomyocytes from the neonatal regenerating mouse heart at 3 days post-MI injury detected a minimal degree of injury response compared with other cardiac cell types, i.e. fibroblasts and endothelial cells (Quaife-Ryan et al., 2017). Instead, this study showed that cardiomyocytes from neonatal hearts retained cell-cycle gene activity from embryonic development, suggesting they exist in a permissive state that allows them to continue to proliferate after injury. Thus, zebrafish heart regeneration represents an active process that involves a regeneration-responsive gene program, whereas neonatal heart regeneration in mammals may rely on a permissive embryonic developmental state that is retained transiently after birth. However, this view only considers the role of cardiomyocytes in the regenerative response; non-muscle cell types in the neonatal mouse heart may also engage in a unique injury response to facilitate cardiomyocyte proliferation, likely by modulating extracellular matrix (ECM) remodeling and promoting angiogenesis.

Why is the adult mammalian adult heart so resistant to regeneration? Did heart regeneration evolve in lower vertebrates to allow adaptation to unpredictable living environments, or is it a trait that was selected against during mammalian evolution? It seems that loss of regenerative capacity in the hearts of adult mammals is more likely a trade-off that was evolutionarily preserved in order for the adult heart to achieve greater functional efficiency. This view is supported by the inverse correlation between systolic blood pressure and cardiomyocyte cell-cycle activity (O'Tierney et al., 2010; Tiemann et al., 2003). During the first week of postnatal life in mice, the blood pressure nearly doubles and is ∼25-fold higher than that in the two-chambered zebrafish heart (Tiemann et al., 2003; Hu et al., 2001). At the same time, cardiomyocytes undergo cell-cycle exit and become multinucleated and polyploid, leading to an increase in the DNA content per cardiomyocyte. In humans, although most cardiomyocytes remain mononucleated throughout life, they usually become tetraploid during adolescence (Mollova et al., 2013). Perhaps, to achieve higher pump strength, cardiomyocytes in the adult mammalian heart are programmed to increase their DNA content to enhance expression of contractile proteins. In addition, replication of cardiomyocytes requires the disassembly and reassembly of sarcomeres, which may disrupt the essential contractility of cardiomyocytes required for heart function. Furthermore, efficient blood pumping requires synchronized contraction among billions of cardiomyocytes. By exiting the cell cycle and becoming multinucleated, cardiomyocytes may achieve synchronization and reduce the chance of lethal arrhythmias.

The links between cardiomyocyte ploidy, regeneration and function are further highlighted by recent findings showing that the percentage of mononuclear diploid cardiomyocytes in the adult heart is a genetic trait that varies substantially among different murine strains, and is positively correlated with the potential for cardiac regeneration after injury (Patterson et al., 2017). This suggests that mononuclear diploid cardiomyocytes have greater proliferative potential, and that multinucleation or polyploidization inhibits the ability of cardiomyocytes to proliferate. This observation has been tested in a recent study that directly manipulated ploidy in the zebrafish heart, in which ∼95% of cardiomyocytes are usually mononucleated and diploid (Gonzalez-Rosa et al., 2018). This study showed that polyploidization indeed creates a barrier for cardiomyocyte proliferation. Because a decrease in the abundancy of mononuclear diploid cardiomyocytes is concurrent with the loss of regenerative capacity in the mouse heart, it is tempting to speculate that mononuclear diploid cardiomyocytes represent a subpopulation of cardiomyocytes that maintain the potential to proliferate, allowing for neonatal heart regeneration. In support of this notion, a study has shown that cardiomyocytes residing in a hypoxic environment in the adult heart are more proliferative after injury and are indeed preferentially mononucleated (Kimura et al., 2015). Future studies aiming to identify the molecular signatures of mononuclear and diploid cardiomyocytes may reveal the mechanisms contributing to postnatal cardiomyocyte proliferation.

Reversing cardiomyocyte cell-cycle arrest to promote heart regeneration

As highlighted above, cardiomyocyte proliferation is a prerequisite for cardiac regeneration, and the cell-cycle arrest of cardiomyocytes proves to be a barrier to regeneration of the adult heart under conditions of stress and injury. Deactivation of the regulatory mechanisms of cardiomyocyte cell-cycle arrest could therefore provide new strategies for promoting cardiac regeneration in the adult heart.

A number of factors are now known to contribute to the cell-cycle arrest of cardiomyocytes (Fig. 2). To date, MEIS1 is the only TF shown to directly activate the expression of the cyclin-dependent kinase (CDK) inhibitors (P15, P16 and P21; also known as CDKN2B, CDKN2A and CDKN1A, respectively) and promote postnatal cell-cycle arrest in cardiomyocytes (Mahmoud et al., 2013). MEIS1 translocates into the nucleus within the first week after birth, as cardiomyocytes exit the cell cycle. Cardiac-specific deletion of *Meis1* has been reported to extend the postnatal proliferative window of cardiomyocytes and reactivate their proliferation in the adult heart (Mahmoud et al., 2013). Conversely, overexpression of MEIS1 decreases neonatal cardiomyocyte proliferation and inhibits heart regeneration (Mahmoud et al., 2013).

As cardiomyocytes exit the cell cycle during maturation, they also switch their metabolic energy source from glycolysis to fatty acid beta-oxidation (Ellen Kreipke et al., 2016; Mills et al., 2017). This inverse relationship between cell-cycle activity and oxidative metabolism suggests reciprocal regulation between these two processes. Indeed, oxidative metabolism produces reactive oxidative species (ROS), which increase postnatally and are linked

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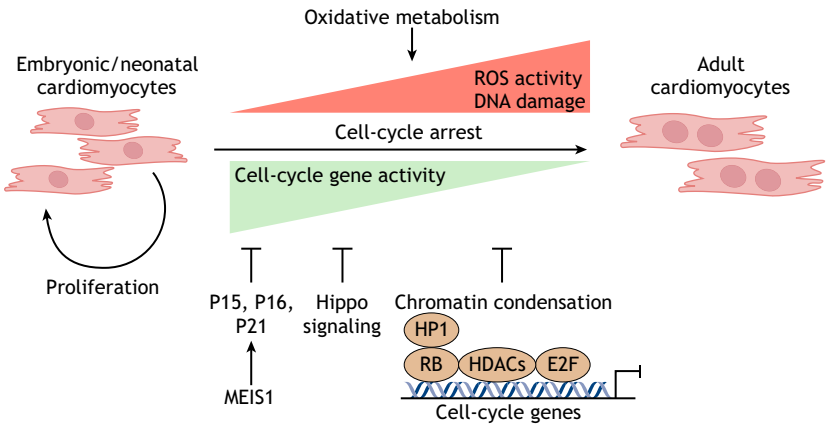

Fig. 2. Regulation of cardiomyocyte cell-cycle arrest. The cell-cycle arrest of cardiomyocytes is accompanied by reduced expression of cell-cycle genes and an increase in ROS activity. Fatty acid oxidation also contributes to cell-cycle arrest by inducing ROS activity and DNA damage. Finally, a number of factors, including MEIS1, Hippo signaling and chromatin condensation at cell-cycle gene promoters, can also directly repress the expression of cell-cycle genes.

to the cell-cycle arrest of cardiomyocytes (Puente et al., 2014). Accordingly, the pharmacological inhibition of ROS activity prolongs postnatal cardiomyocyte proliferation and improves cardiac function after injury (Puente et al., 2014), whereas increasing oxidative stress with the ROS generator diquat decreases neonatal cardiomyocyte proliferation (Puente et al., 2014). Similarly, human pluripotent stem cell-derived cardiomyocytes undergoing maturation exhibit a metabolic switch from glycolytic to oxidative metabolism, and this is accompanied by an increased DNA-damage response along with cell-cycle arrest (Mills et al., 2017). The mechanism by which this metabolic switch to fatty acid oxidation induces cell-cycle arrest remains unclear but may involve YAP1 (a terminal effector in the Hippo signaling pathway) and β-catenin (a mediator of Wnt signaling). The activation of these factors is associated with the metabolic switch, and their synergistic activation in cardiomyocytes is able to reactivate the cell cycle (Mills et al., 2017). This suggests a potential molecular link between cardiomyocyte metabolism and cell-cycle arrest by regulation of the Wnt/β-catenin and Hippo/YAP1 signaling pathways.

The stable repression of cell-cycle gene expression is also crucial to maintain cell-cycle arrest and the post-mitotic features of adult cardiomyocytes. Indeed, it has been shown that chromatin associated with the promoter regions of cell-cycle genes undergoes condensation during cardiomyocyte maturation, as observed by ATAC-seq (Quaife-Ryan et al., 2017). This chromatin condensation requires epigenetic regulation by the RB family proteins, RB (RB1), p107 (RBL1) and p130 (RBL2); deletion of both RB and p130 disrupts heterochromatin and allows adult cardiomyocytes to re-enter the cell cycle (Sdek et al., 2011). The ability of RB to repress cell-cycle gene expression is related to its capacity to recruit heterochromatin protein 1 (HP1) to the promoters of these genes to initiate the formation of heterochromatin (Sdek et al., 2011). RB also interacts with and recruits HDAC1 to E2F-regulated promoters to deacetylate and inactivate cell-cycle genes (Hille et al., 2016). Whether other chromatin regulators are involved in maintaining the post-mitotic state of cardiomyocytes, and whether any of these could be targeted to trigger cardiomyocyte proliferation, remains to be studied.

Stimulating cardiomyocyte proliferation to promote heart regeneration

Several studies have identified factors that when overexpressed in the adult mammalian heart promote cardiomyocyte proliferation (Fig. 3). Constitutive overexpression of cell-cycle regulators, such as cyclins and CDKs, can promote cell-cycle activity in adult cardiomyocytes (Hille et al., 2016; Chaudhry et al., 2004; Pasumarthi et al., 2005). For example, transgenic mice with cardiomyocyte-specific overexpression of Cyclin D2 or Cyclin A2 exhibit increased DNA synthesis in adult cardiomyocytes, resulting in a hyperplasia phenotype of the adult heart (Chaudhry et al., 2004; Pasumarthi et al., 2005). The proliferative phenotype induced by these cell-cycle regulators can be enhanced by simultaneously delivering four factors: CDK1, Cyclin B, CDK4 and Cyclin D (Mohamed et al., 2018). These four factors, administered by viral delivery, improve cardiac function in mouse hearts following MI. These findings demonstrate the potential of cell-cycle regulators to force cell-cycle activation in adult cardiomyocytes. However, because direct overexpression of cell-cycle genes could be tumorigenic, long-term effects should be carefully assessed before moving to clinical studies.

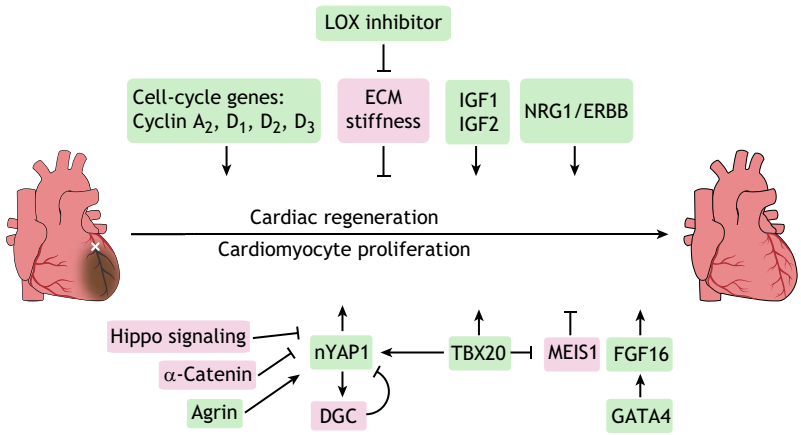

Fig. 3. Stimulating cardiomyocyte proliferation to promote cardiac regeneration. A scheme summarizing recently discovered factors that promote heart regeneration by stimulating endogenous cardiomyocyte proliferation (green boxes). Negative regulators of heart regeneration are indicated in pink boxes. nYAP1, nuclear yes-associated protein 1.

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Several growth factors, including IGF1, IGF2 and NRG1, are also capable of promoting cardiomyocyte proliferation after injury (Gemberling et al., 2015; Samarel, 2002; Huang et al., 2013). The effects of these growth factors are evolutionarily conserved in both zebrafish and mouse. In zebrafish, Igf2b is upregulated during heart regeneration, and inhibition of Igf signaling blocks cardiomyocyte proliferation (Huang et al., 2013). In mice, cardiac-specific overexpression of IGF1 increases cell-cycle activity in adult cardiomyocytes and inhibits the progression of dilated cardiomyopathy (Samarel, 2002). Nrg1, too, has been reported to be upregulated in both zebrafish and mouse hearts in response to injury (Gemberling et al., 2015; Bersell et al., 2009). Moreover, studies have shown that forced cardiac overexpression of NRG1 or its receptor ERBB2, or administration of NRG1 recombinant protein, promotes cardiomyocyte proliferation in mice and improves cardiac function after injury. However, this beneficial effect is more pronounced in young animals compared with adults, suggesting a temporal restriction to the effect of NRG1 (Gemberling et al., 2015; D'Uva et al., 2015; Polizzotti et al., 2015; Santoro and Sahara, 2015). Notably, IGF1, IGF2 and NRG1 are also required for cardiac development (Bersell et al., 2009; Li et al., 2011); therefore, their function in regulating cardiomyocyte proliferation is crucial for both heart development and regeneration. Similarly, Hedgehog (HH) signaling is another evolutionarily conserved pathway that is crucial for cardiovascular development and is also required for heart regeneration in newts, zebrafish and neonatal mice (Singh et al., 2018). These findings underscore the parallel functions of signaling pathways in cardiac development and regeneration, and highlight the potential of developmental regulators to promote cardiomyocyte proliferation and cardiac regeneration.

The Hippo/Yap signaling pathway, which regulates organ size, has also been identified as a key regulator of cardiomyocyte proliferation. In normal tissues, activation of the Hippo pathway leads to phosphorylation of its effector YAP1, which prevents its entry into the nucleus and thereby restricts its activity and, ultimately, limits organ growth during development (Juan and Hong, 2016; Zhao et al., 2011). In embryonic hearts, YAP1 is nuclear but this nuclear localization decreases after birth, as a result of increased Hippo signaling activity (Wang et al., 2018; Xin et al., 2013b). However, the overexpression of constitutively active nuclear YAP1 is sufficient to stimulate cardiomyocyte proliferation in adult hearts (Xin et al., 2013b). Similarly, inhibiting the Hippo signaling pathway, by cardiac deletion of the upstream kinases LATS1 or LATS2, and the adaptor protein Salvador (SAV1), also promotes cardiomyocyte cell-cycle re-entry and improves cardiac function after injury (Wang et al., 2018; Leach et al., 2017).

Genome-wide profiling using RNA-seq and ChIP-seq has revealed YAP1 targets in the heart are associated with cell-cycle regulation and include a subset of Wnt target genes (Morikawa et al., 2015). Intriguingly, genes related to the cytoskeleton are also dysregulated in the heart after inhibition of Hippo signaling, suggesting that cardiac regeneration involves cytoskeleton destabilization (Morikawa et al., 2015). Indeed, recent studies have shown that the dystrophin-glycoprotein complex (DGC), which links the ECM and the actin cytoskeleton, is required for heart regeneration by regulating the nuclear translocation of YAP1 (Morikawa et al., 2017). Notably, the DGC components SGCD and SNTB1 are direct targets of YAP1, revealing that Hippo signaling and the DGC form a regulatory feedback loop to control cytoskeleton formation and cell-cycle activity. Additionally, it has been shown that cytoskeletal tension influences cardiomyocyte proliferation. Loss of α-Catenin increases cytoskeleton tension, leading to nuclear accumulation of YAP1 and thereby promoting cardiomyocyte proliferation (Vite et al., 2018). Furthermore, the ECM protein Agrin was identified as an inducer of cardiomyocyte division, and its regulation is associated with disassembly of the DGC and induction of YAP- and ERK-mediated signaling (Bassat et al., 2017). These findings demonstrate that, as is seen in many other contexts (Chakraborty and Hong, 2018; Dupont et al., 2011), subcellular matrix rigidity can regulate YAP1 in response to local ECM composition and can thus regulate cell proliferation.

The ECM may broadly influence cardiac regeneration through YAP-independent mechanisms. A recent study showed that the expression of ECM genes undergoes extensive postnatal reprogramming as early as 2 days after birth, when YAP activity is still high (Notari et al., 2018). This differential gene expression is associated with a blockade to neonatal heart regeneration, suggesting an inverse regulation between ECM deposition and cardiac regeneration. Moreover, decreasing ECM stiffness using a pharmacological lysyl oxidase (LOX) inhibitor enhances cardiac regeneration, although the underlying mechanism is unclear (Notari et al., 2018). Understanding how the ECM regulates cardiomyocyte behavior could provide insights into how the heart transitions from a regenerative state to a non-regenerative state.

Cardiomyocyte proliferation also requires the cardiac TFs GATA4 and TBX20. In neonatal mice, loss of *Gata4* in cardiomyocytes impairs heart regeneration in response to cryoinjury and apical resection (Malek Mohammadi et al., 2017). Injured hearts lacking GATA4 show reduced cardiomyocyte replication and increased hypertrophy compared with injured wild-type hearts. This phenotype can be rescued by overexpressing FGF16 via viral delivery, suggesting that GATA4 is required for heart regeneration through its regulation of the FGF16 pathway. TBX20 overexpression in adult cardiomyocytes also promotes proliferation and improves cardiac repair after MI in mice (Xiang et al., 2016). The proliferative phenotype associated with TBX20 overexpression involves increased activity of several pro-proliferation pathways and factors, including YAP1, as well as direct repression of the cell-cycle inhibitors P21 and MEIS1.

In summary, many studies using different approaches have demonstrated that factors regulating cardiomyocyte proliferation during cardiac development can also promote cardiomyocyte regeneration after cardiac injury in the adult heart. This highlights the importance of elucidating the developmental regulators that are induced following injury and ascertaining their potential to promote heart regeneration in an otherwise non-regenerative adult heart.

From cardiac development to cardiac repair: reactivation of a fetal gene program

Owing to its limited regenerative capacity, the adult mammalian heart undergoes pathological remodeling to compensate for functional impairment in response to stress or injury. This remodeling, which can occur in response to a variety of deleterious stimuli, including MI, hypertension, contractile abnormalities and pressure overload, triggers the hypertrophic remodeling of cardiomyocytes. At the cellular level, hypertrophic remodeling is manifested by cell enlargement, restructuring of the contractile apparatus, and changes in energy metabolism. Molecularly, the hallmark of pathological remodeling in the adult heart is global transcriptome reprograming, resulting in a shift in gene expression towards a fetal gene program that is likely to be adaptive in the short term (Taegtmeyer et al., 2010). For example, the shift in gene expression during cardiac remodeling

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produces a switch in MHC proteins, from MYH6 to MYH7 in mice. (Nakao et al., 1997). Other characteristic biomarkers of cardiac remodeling include destrin, α-SM-actin (ACTA2), the natriuretic peptides ANP (NPPA) and BNP (NPPB), and smooth-muscle actinin (ACTN1). Transcriptome profiling of isolated adult cardiomyocytes from injured hearts at 3 days post-MI identified ∼700 upregulated genes that were associated with stress responsiveness, inflammation, and leukocyte migration (Quaife-Ryan et al., 2017). Disease and injury to cardiomyocytes also activates cardiac TFs and epigenetic regulators either transcriptionally or post-transcriptionally (Passier et al., 2000; Peterzan et al., 2017; Warren et al., 2011). Of note, many of the injury-responsive molecular regulators are known to play important developmental roles, highlighting parallel functions for these factors in cardiac development and disease remodeling (Fig. 4).

Cardiac transcription factors involved in hypertrophic remodeling

A number of cardiac TFs play important roles in cardiac remodeling under pathological conditions. GATA4, for example, is a key regulator of cardiac hypertrophy. Overexpression of GATA4 activates hypertrophic growth both *in vitro* and *in vivo* (Liang et al., 2001a, b). Multiple signaling pathways are able to trigger GATA4 phosphorylation, which enhances GATA4 regulatory activity, in response to hypertrophic stimuli (Charron et al., 2001; Liang et al., 2001a, b; Morisco et al., 2001). GATA4 is the upstream regulator of several hallmark genes during pathological remodeling, including those encoding BNP, β-MHC and angiotensin II type I receptor (AT1) (Hautala et al., 2001). Profiling of GATA4 binding in hearts subjected to pressure overload reveals a unique gene program regulated by GATA4 (in addition to a subset of the fetal gene targets) that was not activated during normal heart development (He et al., 2014). This demonstrates that GATA4 regulates different sets of genes in disease remodeling and during cardiac development, possibility by interacting with different cardiac TFs and co-factors that subsequently change its genomic binding profile. Moving forward, this notion could be further tested by analyzing the TF motifs enriched at GATA4-bound genomic regions and profiling for GATA-interacting proteins in the diseased heart.

MEF2 TF family members are also induced by hypertrophic signaling pathways. Upon pressure and volume overload, the DNA-binding activity of MEF2 increases, similar to that of GATA4 (Molkentin et al., 2000). MEF2 is an important effector of calcium signaling downstream of several key regulators of cardiac hypertrophy, including calcium, calmodulin-dependent kinases (CaMKs), calcineurin and histone deacetylases (HDACs) (Blaeser et al., 2000; Diedrichs et al., 2004; Molkentin et al., 1998; Molkentin and Markham, 1993; Olson and Williams, 2000; Zhang et al., 2002). Other cardiac TFs, including HAND2 and NKX2-5, are reported to have synergistic effects on hypertrophy marker genes, and thus have been implicated in disease remodeling in hypertrophied hearts (Shiojima et al., 1999; Thattaliyath et al., 2002). Together, these findings suggest that the cardiac TFs used during cardiogenesis get reactivated by stress stimuli during cardiac disease remodeling, causing the transcriptome to partially revert to a fetal state. Concurrently, the cardiac TFs activate stress-specific genes and together contribute to the transcriptional remodeling of the diseased heart.

Epigenetic regulation in cardiac disease remodeling

As occurs during development (Wamstad et al., 2012), the epigenetic landscape of the cardiac genome changes dramatically during hypertrophic remodeling. A genome-wide analysis of the distribution of seven different histone modifications in adult mouse cardiomyocytes undergoing hypertrophic remodeling has shown that 596 of 1109 differentially regulated genes have at least one altered histone modification at their promoter (Papait et al., 2013). This finding suggests that the epigenetic landscape is a key determinant of transcriptome reprogramming in hypertrophic cardiomyocytes. Accordingly, the genetic deletion or cardiac-specific overexpression of enzymes that catalyze the deposition or removal of epigenetic marks has highlighted functional links between epigenetic modifications and transcriptomic changes during cardiac disease remodeling. Notably, many of the histone-modifying enzymes that regulate disease remodeling also play a role in cardiac development.

BRG1 (SMARCA4), for instance, is an essential ATPase component of the SWI/SNF-like BAF chromatin remodeling complex, and is vital for both normal heart development and cardiac disease remodeling. The function of BRG1 during cardiac development is mediated through two separate mechanisms: BRG1 promotes cardiomyocyte proliferation by maintaining the expression of BMP10 (a cardiomyocyte growth factor) and suppressing the expression of CDKN1C (an inhibitor of the cell cycle), and it also preserves fetal cardiac differentiation by concurrently repressing

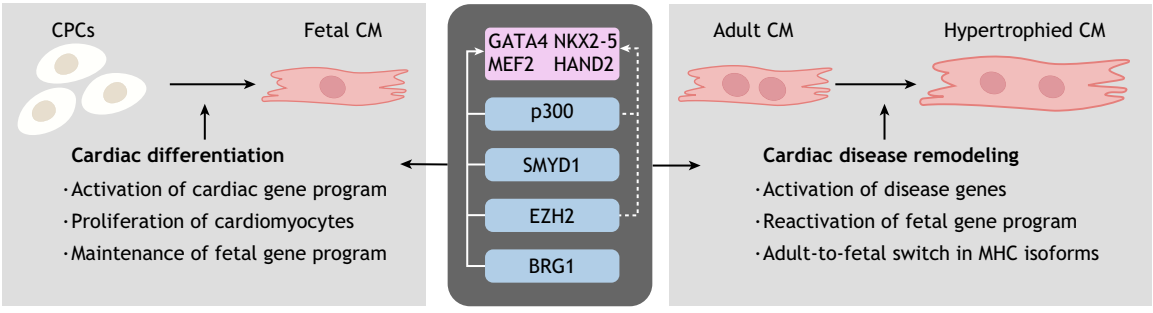

Fig. 4. Parallel molecular mechanisms underlie cardiac development and disease remodeling. Many cardiac transcription factors (TFs; pink box) and epigenetic regulators (blue boxes) play vital functions in regulating both cardiac differentiation and cardiac disease remodeling. During cardiac differentiation, TFs function cooperatively to activate a cardiac morphogenesis gene program. The expression and activity of these TFs is transcriptionally (white solid arrows) and post-transcriptionally (white dashed arrows) regulated by the indicated epigenetic modifiers. Epigenetic regulators also control cardiac development by stimulating cardiomyocyte proliferation, and by maintaining the fetal isoforms of metabolic and contractile genes. In response to cardiac disease or injury, the same cardiac TFs (MEF2, GATA4 and NKX2-5) are activated by phosphorylation and some epigenetic regulators (i.e. p300 and BRG1) that are used during development are upregulated. The transcriptomic remodeling that occurs in injured cardiomyocytes, including the activation of disease-responsive gene expression and an adult-to-fetal switch in MHC isoforms, requires reactivation of these developmental regulators. CPC, cardiac progenitor cell; CM, cardiomyocyte.

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MYH6 and activating MYH7 (Hang et al., 2010). This effect on the expression of the MHC isoforms is mediated by BRG1 interacting with both HDACs and PARP (PARP1) at the promoter region of the *Myh6* gene, and with PARP alone at the promoter of the *Myh7* gene. Additionally, BRG1 interacts with cardiac TFs, including TBX5 and GATA4, via BAF60C (Smarcd3), the cardiac-specific subunit of the BAF complex, potentiating binding of TBX5 and GATA4 to cardiac genes (Takeuchi and Bruneau, 2009). Intriguingly, TBX5 haploinsufficiency reduces the genomic occupancy of BRG1 at cardiac gene promoters, indicating that the relative levels of cardiac TFs and BAF complexes are crucial for proper cardiac development (Takeuchi et al., 2011).

Although the expression of BRG1 is downregulated postnatally, it is reactivated by cardiac stress. In the stressed adult heart, reactivated BRG1 associates with its embryonic partners, HDACs and PARP, to induce a pathological switch from MYH6 to MYH7 (Hang et al., 2010). In addition to complexing with HDAC and PARP, BRG1 recruits the histone methyltransferase G9A/GLP (Ehmt2/1) and DNMT (DNMT1) (both of which are reactivated upon stress) to the *Myh6* promoter in order to assemble a repressive chromatin scaffold (Han et al., 2016). The recruitment of G9A/GLP and DNMT is unique to the *Myh6* promoter and, consequently, only MYH6 but not MYH7 is repressed (Han et al., 2016). Together, these studies demonstrate an epigenetic mechanism by which the BRG1/BAF complex cooperates with other chromatin-modifying factors (i.e. HDACs and PARP) and cardiac TFs to control developmental and pathological gene expression.

The histone acetyltransferase (HAT) p300 (EP300) also plays a key role in both cardiac development and pathological cardiac remodeling. The chromatin binding of p300, the most widely studied HAT in the heart, and its resulting histone modification, H3K27ac, are broadly used as epigenetic markers of active chromatin regions, including promoters and enhancers of active genes. Genome-wide profiling for p300 and H3K27ac chromatin binding in mammalian hearts has identified thousands of potential cardiac enhancers that are associated with cardiac-specific genes, including *MYH2* and *MYH7*, as well as known cardiac disease genes, such as *TGFB3* and *PRKAG2* (May et al. 2011). This suggests an important function of p300 in activating gene expression during cardiac development and disease. Indeed, the function of p300 in cardiac development is manifested by its two-layered regulatory interactions with cardiac TFs. At the post-transcriptional level, p300 directly interacts with and acetylates GATA4, NKX2-5 and MEF2C to modulate their DNA-binding and -regulatory activity (Sun et al., 2010; Takaya et al., 2008). p300 also activates the transcription of these cardiac TFs through direct binding to their promoters (Sun et al., 2010; Dickel et al., 2016). Given the important function of p300 in regulating cardiac genes, it is not surprising that p300-deficient mice are embryonic lethal, owing to the failure to activate cardiac-specific gene programs required for coordinated cardiac morphogenesis and differentiation (Shikama et al., 2003).

Similar to BRG1 expression, the expression of p300 is repressed after birth, but is reactivated in response to stress. Reactivated p300 plays a key role in cardiomyocyte hypertrophy and heart failure. Forced overexpression of p300 either *in vivo* or *in vitro* leads to spontaneous hypertrophy of cardiomyocytes (Miyamoto et al., 2006). Conversely, inhibition of p300 in the pathologically stressed heart ameliorates cardiomyopathy (Wei et al., 2008). As it does during cardiac development, p300 acetylates and activates MEF2 and GATA4 during cardiac hypertrophy to reprogram gene expression (Takaya et al., 2008; Wei et al., 2008). Moreover, in hypertrophic cardiomyocytes induced by transverse aortic constriction, H3K27ac deposition changes at the chromatin regions of stress-responsive cardiac genes (Papait et al., 2013; Wei et al., 2008). This suggests that the acetyl transferase activity of p300 is important for activating pathological gene expression.

In addition to histone acetylation, histone methylation has been identified as a key epigenetic regulator of hypertrophic remodeling. Unlike histone acetylation, which generally leads to gene activation, histone methylation can both activate and repress genes, depending where on the histone it occurs. For example, the trimethylation of lysine 4 on histone 3 (H3K4me3) is associated with gene activation, whereas trimethylation of lysine 27 on the same histone (H3K27me3) leads to gene repression (Zhang and Liu, 2015). The deposition of H3K4me3 at cardiac gene promoters is necessary for normal function of adult cardiomyocytes. This is demonstrated by the severe cardiac developmental defect when the muscle-specific H3K4 methyltransferase SMYD1 (also known as BOP) is deleted (Gottlieb et al., 2002; Phan et al., 2005). Furthermore, in the context of adult heart disease, SMYD1 is upregulated to restrict hypertrophic growth and is thought to directly repress a core set of hypertrophy-associated genes, including those encoding TGFβ3 and NPPA (Franklin et al., 2016).

The level of H3K27me3, which is catalyzed by polycomb repressive complex 2 (PRC2), is also dynamically regulated to allow activation of cardiac genes and silence noncardiac gene programs in a spatial and temporal manner during development. The importance of maintaining a proper level of H3K27me3 during cardiac development is demonstrated by the genetic deletion of *Ezh1* and *Ezh2*, which are subunits of PRC2. The cardiac-specific deletion of *Ezh2* using Nkx2-5-Cre causes developmental defects due to the ectopic expression of noncardiac genes and upregulation of cell-cycle inhibitors (He et al., 2012a, b). In addition, deletion of *Ezh2* in early cardiac precursors leads to cardiac hypertrophy and heart failure (Delgado-Olguin et al., 2012). In addition to methylating histones, EZH2 directly methylates GATA4 to attenuate the transcriptional activity of GATA4 (He et al., 2012a, b). These findings suggest that EZH2 is normally required to silence non-cardiac gene programs and restrict hypertrophic gene activation in cardiomyocytes. Interestingly, although the paralog gene EZH1 seems to be dispensable for early cardiac development, it is essential for neonatal heart regeneration; moreover, the overexpression of EZH1 is sufficient to promote cardiac regeneration in 10-day-old mice, whose hearts are typically non-regenerative (Ai et al., 2017). The exact mechanism by which EZH1 promotes cardiac regeneration remains to be further explored; however, it appears to be H3K27me3 independent and may involve activation of cardiac-muscle genes.

In summary, these studies highlight that many of the TFs and histone regulators required for heart formation also contribute to cardiac pathological remodeling (Fig. 4). Indeed, many congenital heart disease-associated genes recently identified by exome sequencing are involved in the production, removal or reading of histone modifications, including the genes encoding members of the MLL, WDR and SMAD families, which regulate the H3K4 and H3K27 methylation pathways (Homsy et al., 2015; Zaidi et al., 2013). Curiously, the activation of these developmental factors in the diseased heart is not sufficient to evoke heart regeneration, despite being required for heart development. This might be due to the markedly distinct transcriptomes and epigenetic states in cardiomyocytes of adult and embryonic hearts, leading to different downstream responses to the activation of the same factors. Moreover, the simultaneous activation of stress-specific genes after injury may create additional barriers for cardiac

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regeneration. Understanding these mechanisms and pinpointing the differential responses of cardiomyocytes during heart regeneration and in disease remodeling will be essential for advances in cardiac regenerative medicine.

3D chromatin structure in cardiac disease remodeling

Little is known regarding how 3D chromatin structure (see Box 1) is shaped and reorganized as cardiomyocytes undergo differentiation and maturation. For instance, as mature cardiomyocytes become multinucleated, how chromatin folding changes and is maintained in each nucleus after the final round of karyokinesis is unknown. Additionally, it is unknown how chromatin conformations change in response to increasing mechanical force of cellular contraction. Despite these uncertainties, it is clear that the structural integrity of chromatin is important for proper cardiac development and is likely involved in pathological remodeling. For example, the deletion of *Ctcf*, which encodes a key chromatin structural protein, in cardiac progenitor cells (using Nkx2.5-cre) has been shown to result in embryonic lethality (Gomez-Velazquez et al., 2017). Indeed, of the ∼2000 genes dysregulated upon *Ctcf* deletion, half are downregulated and related to cardiac development and contraction (such as NKX2-5 and IRX4). Furthermore, ChIP-seq analysis has revealed that CTCF directly binds at these gene loci. As further evidence that CTCF regulates DNA looping, chromosome conformation capture assays in *Ctcf* mutant hearts has revealed disorganized chromatin organization at the *Irx4-Ndufs6* locus together with changes in expression of the *IrxA* cluster genes, which are known to be important for cardiac development (Gomez-Velazquez et al., 2017).

CTCF is also required for regulating pathological transcription and chromatin remodeling. CTCF deletion in adult cardiomyocytes using α-MHC MerCreMer causes cardiomyopathy with dilated ventricles and muscle hypertrophy (Rosa-Garrido et al., 2017). This phenotype is associated with decreased long-range interactions of cardiac enhancers, decreased boundary strength of topologically associating domains (TADs; see Box 1), and increased global chromatin fluidity. These findings suggest that, in cardiomyopathy, the chromatin structure of cardiomyocytes is reorganized to allow disease-associated gene expression. Indeed, in response to pressure overload, cardiomyocyte chromatin structure undergoes global reorganization, with a decrease in the total number of long-range loops, resulting in sparse compartmentalization changes that are positively correlated with gene expression (Hautala et al., 2001); notably, differential gene expression in stressed cardiomyocytes is also observed in cardiomyopathy induced by CTCF deletion. This suggests that pressure overload-induced pathological remodeling is at least partly regulated by CTCF. Interestingly, increased CTCF levels have been reported in human hearts after implantation of left ventricular assist devices that mechanically unload the heart and reverse disease remodeling (Rosa-Garrido et al., 2017).

High mobility group B protein (HMGB), which is a chromatin architectural protein that binds and bends DNA to control chromatin accessibility and thereby gene transcription, has also been implicated in cardiomyocyte chromatin organization. The levels of HMGB1 and HMGB2 are increased and decreased, respectively, in isoproterenol-induced hypertrophic cardiomyocytes (Franklin et al., 2012), suggesting their functional relevance in hypertrophic remodeling. Indeed, functional perturbation of HMGB2 (but not HMGB1) induces genomic structural remodeling associated with hypertrophic growth (Franklin et al., 2012). The function of HMGB2 in regulating pathological transcription is, in part, mediated by its reciprocal regulation with CTCF: HMGB2 inversely binds to CTCF-occupied genomic regions to promote heterochromatin spreading and attenuate CTCF binding (Monte et al., 2016).

Heterochromatin maintenance at the nuclear lamina is surmised to be crucial for regulating gene expression but also for ensuring the mechanical stability of the nucleus. This is particularly important for cardiomyocytes, as they are continuously under mechanical stress from contraction. Indeed, overexpression of the nucleosome-binding protein HMGN5, which is a member of the high mobility group N family, results in global chromatin decompaction, is accompanied by decreased sturdiness of the nucleus, and eventually leads to hypertrophy and cardiac malfunction (Furusawa et al., 2015). Given the direct contact between heterochromatin and the nuclear lamina, it is not surprising that human mutations in *LMNA*, which encodes A-type nuclear lamin, cause dilated cardiomyopathy and conduction defects (Lu et al., 2011). These observations once again emphasize that dynamic structural changes in chromatin underlie alterations in gene expression in heart disease.

Box 1. 3D chromatin structure and organization

Recent technological advances in DNA imaging (e.g. DNA FISH and chromatin paint) and chromosome conformation capturing techniques have revealed details about the 3D organization of the chromosome within a single nucleus. These studies have shown that higher-order chromatin structures include: (1) A and B compartments, which represent transcriptionally active and inactive chromatin, respectively, and are multi-Mb in size; (2) topologically associating domains (TADs) that range from 100 kb to 5 Mb and contain regions of DNA that preferentially self-interact; and (3) DNA loops that allow physical interaction between distal enhancers and gene promoters and usually involve a few kb to hundreds of kb (Matharu and Ahituv, 2015). Importantly, these studies have revealed that the correct folding of chromatin within the nucleus is crucial for appropriate gene expression during development and pathological conditions.

Conclusions and perspectives

Heart development and regeneration both rely on active cardiomyocyte proliferation. In contrast, heart repair in the absence of an efficient regeneration program (as seen in the adult heart) elicits disease remodeling of cardiomyocytes. Emerging evidence supports the premise that many of the same regulatory factors are required for cardiac development, heart regeneration and heart repair, highlighting the context-dependent functions of these factors throughout the life of cardiomyocytes. In mammals, although reactivation of the developmental gene program after injury is insufficient for adult heart regeneration, it is required for the transcriptomic remodeling of cardiomyocytes and is essential for cardiac regeneration during the neonatal period. Future studies aimed at identifying key genes and developmental gene networks involved in the neonatal regenerative response promise to uncover additional factors that may promote cardiomyocyte proliferation and repair. Delivery of these factors to the injured adult heart could provide a therapeutic approach for treating heart disease, although it will be imperative to carefully validate and monitor the cellular targeting specificity and long-term effects of such treatments. Furthermore, although cardiomyocytes are collectively seen as the functional ‘engine’ of the heart, other non-myocyte cell types also play crucial functions in heart development and remodeling, and understanding how these cells communicate with cardiomyocytes during development and regeneration may identify signaling factors that can re-ignite the proliferative activity of adult cardiomyocytes.

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Competing interests
E.N.O. is a co-founder and member of the Scientific Advisory Board of Tenaya Therapeutics and holds equity in the company.

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