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Review

Epigenetic mechanisms in cardiac development and disease

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During mammalian development, cardiac specification and ultimately lineage commitment to a specific cardiac cell type is accomplished by the action of specific transcription factors (TFs) and their meticulous control on an epigenetic level. In this review, we detail how cardiac-specific TFs function in concert with nucleosome remodeling and histone-modifying enzymes to regulate a diverse network of genes required for processes such as cell growth and proliferation, or epithelial to mesenchymal transition (EMT), for instance. We provide examples of how several cardiac TFs, such as Nkx2.5, WHSC1, Tbx5, and Tbx1, which are associated with developmental and congenital heart defects, are required for the recruitment of histone modifiers, such as Jarid2, p300, and Ash2l, and components of ATP-dependent remodeling enzymes like Brg1, Baf60c, and Baf180. Binding of these TFs to their respective sites at cardiac genes coincides with a distinct pattern of histone marks, indicating that the precise regulation of cardiac gene networks is orchestrated by interactions between TFs and epigenetic modifiers. Furthermore, we speculate that an epigenetic signature, comprised of TF occupancy, histone modifications, and overall chromatin organization, is an underlying mechanism that governs cardiac morphogenesis and disease.

Keywords epigenetics; histone modifications; DNA methylation; congenital heart disease

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Introduction

When the famous French biologist and evolutionist Jean-Baptiste Lamarck published his *Philosophie Zoologique* in 1809 [1], one part of his theory about how different species developed was that individuals possessed the potential to pass on acquired characteristics to their scions and that they themselves can lose attributes which they no longer use. In 1940, Waddington first coined the term epigenetics by combining the words epigenesis and genetics, which was defined as the study of how heritable changes in a gene programme may occur without inherently altering the genetic material [2]. In recent years, this idea has emerged as the field of epigenetics. During mammalian development, the organism must faithfully and precisely orchestrate the expression of various gene programmes in a temporally and spatially accurate manner [3–8]. Similarly, the precise growth of the mammalian heart during embryonic development and terminal differentiation in the adult is dependent on the accurate activation of a gene programme that involves enzymes controlling nucleosome remodeling, histone modification, and DNA methylation [9–14]. Failure to properly orchestrate these mechanisms results in deleterious phenotypes like septal defects, atrioventricular malformations, or developmental absence of right or the left heart, if not lethality during embryogenesis at all [15,16]. A deeper understanding of the mechanisms involved in these epigenetic regulatory pathways during development and in differentiated stages could facilitate our capacity to diagnose and treat genetic diseases and human suffering.

In this review, we will discuss the epigenetic regulation of embryonic cardiac development and their implications and contribution to congenital heart diseases. We will discuss how epigenetic regulators in cells of the cardiac lineage affect cell fate determination and orchestrate complex gene transcription events. The abnormalities in epigenetic regulation during development may be responsible for the progression of cardiac diseases.

Overview of Epigenetic Mechanisms

In eukaryotes, 147 bp of DNA is wound around the histone octamer, which consists of two H2A, H2B, H3, and H4 core histone proteins [17–19]. This allows the entire genome to be packaged tightly into the nucleus.

Chromatin-modifying enzymes use the energy derived from ATP hydrolysis to alter the chromatin structure [20–22] between either a permissive, euchromatic state, which allows the binding of transcription factors and co-activators to the DNA, and a non-permissive heterochromatic state, which precludes active transcription of genes [23,24]. This is thought to be accomplished by nucleosome sliding, displacement, or histone exchange [25–28] (**Fig. 1**). These properties of chromatin-modifying enzymes are expected to play essential roles in embryonic developmental and terminal differentiation [29,30].

Similarly, histone-modifying enzymes involved in epigenetic modification are also able to dynamically remodel the nucleosome [31–33]. Histone methylation, such as H3K4me3 and H3K27me3, has been linked to the regulation of developmental genes and the maintenance of pluripotency in embryonic stem cell populations [34,35]. The presence of both H3K4me3 and H3K27me3 within the same chromatin region (a.k.a. bivalent domains) is associated with a 'poised' transcriptional state, demarcating genes that have the potential to be activated or repressed. On the other hand, acetylation of lysine residues on histones is generally associated with gene activation [36,37], and the balance between histone acetyltransferases (HATs) and deacetylases (HDACs) is a potent regulator of transcription during skeletal and cardiac myocyte differentiation in addition to disease states in these tissue compartments [38,39]. In addition, the DNA itself is modified by DNA methyltransferases, enzymes that convert cytosine to 5-methylcytosine [13,14]. Together, an intricate inter-play exists between histone modifications, nucleosome positioning, and DNA methylation to direct tissue-specific gene expression [40–42].

Overview of Cardiac Development

The heart is one of the first organs to develop during embryogenesis. A high degree of conservation is observed during early-stage heart development in zebrafish, chick, frog, mouse, and human [4]. The visceral part of the splanchnic mesoderm (splanchnopleura) during gastrulation is the source for the first-recognized cardiac precursors. The migration of cells from the anterior part of the primitive streak forms the cell population that eventually gives

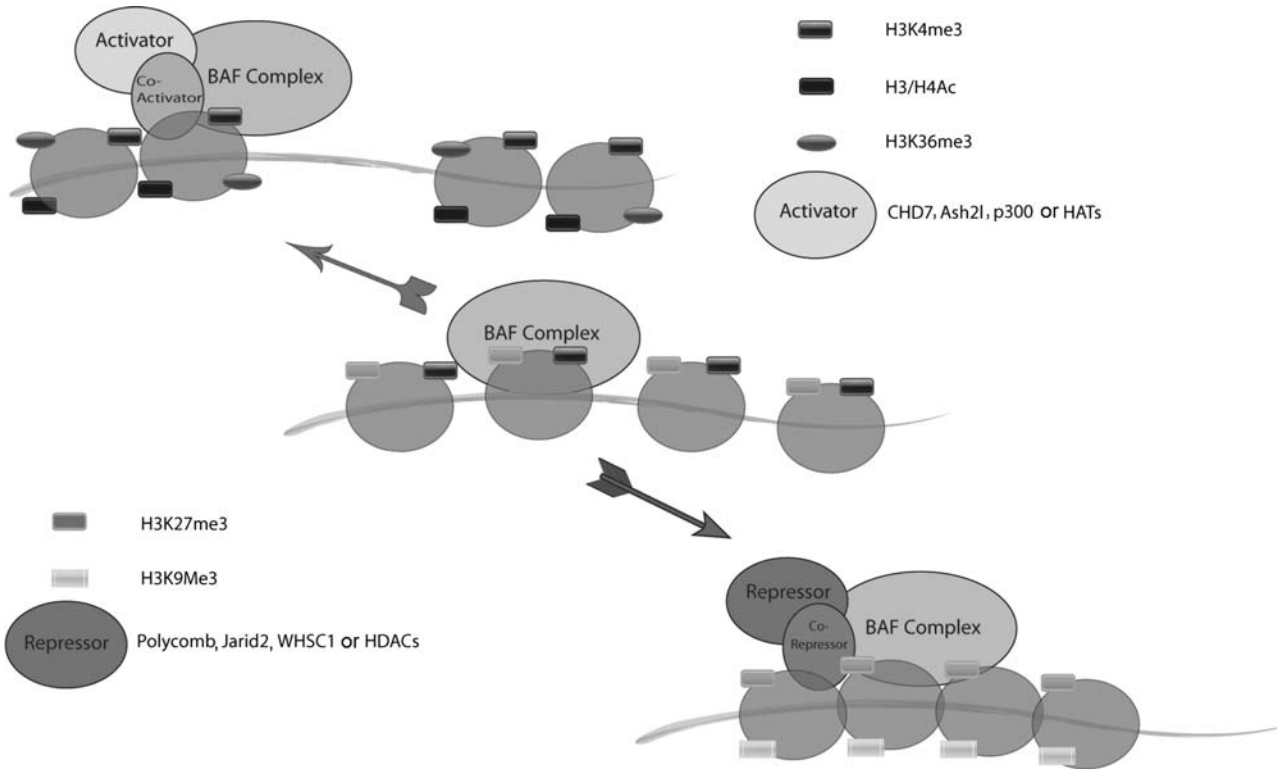

Figure 1 Developmental and adult differentiation genes are marked by H3K4 and H3K27 methylation known as a bivalent domain Additional modifications such as histone acetylation can alter histone DNA contacts allowing the binding of activator and co-activator proteins in concert with BAF complexes. BAF complexes utilize ATP to eject nucleosomes and remodel the chromatin structure to a permissive state, thereby allowing transcription to take place. Alternatively, binding of repressor and co-repressor complexes may favor repressive histone modifications. This would alter histone-DNA interactions in a way that results in nucleosomal compaction thereby precluding the binding by the transcriptional activators resulting in transcriptional silencing. (Hypo- or hyper-methylation of DNA by DNA methyltransferases can activate or repress transcription of genes, respectively, and regulate deposition of specific histone modifications. These functions are not discussed in detail as it is not pertinent for this review.)

rise to the first and second heart field (FHF and SHF, respectively). It is proposed that a common progenitor for these two heart fields, marked by the expression of MesP1 and MesP2, may exist [43]. At later stages of development, the two heart fields can be recognized as distinct and separable populations of cells. The FHF contributes to both atria, the left ventricle, and part of the inter-ventricular septum while the SHF forms the right ventricle, the outflow tract, the remainder of the inter-ventricular septum, and parts of both atria. Albeit a unique marker for the FHF is currently lacking, early Nkx2.5 positive cells are found largely within the FHF [3]. Although Isl1 expression demarcates structures of the SHF [5,44,45], studies using various recombinase-based reporters indicate that the ISL1 fate map is much broader [46]. Subsequent to the rotation and caudal folding of the mouse embryo, the developing heart tube, which contracts spontaneously as early as carnegie stage 9–10, begins to acquire an epicardium from the nearby pro-epicardial organ. This thin layer of epithelial cells envelops the myocardium and the endocardium and contributes to the formation of coronary smooth muscle cells, interstitial fibroblasts, and rarely, cardiomyocytes [47–52].

Role of Chromatin-Modifying Enzymes in Cardiac Development

Chromatin-modifying enzymes are divided into two groups: ATP-dependent remodellers and histone-modifying enzymes [9–13]. Histone-modifying enzymes mediate the addition or removal of specific chemical groups to amino acids of histones at various sites. Several types of histone modifications have been observed, and include acetylation, deacetylation, methylation, demethylation, deimination, phosphorylation, ubiquitination, ADP-ribosylation, and sumoylation. These post-translational modifications can affect the charge on histone residues, thereby strengthening or neutralizing the affinity of DNA to the histone, rendering the DNA more accessible to binding by transcription factors and co-regulatory proteins. Alternatively, deposition of certain histone marks provides a substrate for binding by regulatory complexes [9–13].

ATP-dependent nucleosome remodeling enzymes are multi-subunit enzymes that can be divided into four subgroups: ISWI, SWI/SNF, CHD, and INO80, based on the sequence and structure of the ATPase subunit contained within the complex. These complexes direct the repositioning or removal of nucleosomes in conjunction with the presence of co-activators or co-repressors to allow or prevent transcription. Moreover, these remodelers may regulate long range inter- and intra-chromosomal interactions as well as organize the nuclear architecture of chromatin in accordance with specific gene programmes [30,53,54].

Vertebrate SWI/SNF complexes are known as Brg1/Brahma-associated factor (BAF) complexes, and several studies indicate that the composition of these complexes changes as a cell progresses from a pluripotent stem cell to a multi-potent progenitor and ultimately into a terminally differentiated state [55–58]. The importance of Brg1, which functions as the ATPase subunit of SWI/SNF complexes during cardiac development, is supported by the fact that Brg1 null mouse embryos die shortly after e11.5 day post-conception when cardiomyocyte expansion and maturation begins [9]. Cardiac defects observed in these embryos include thinning of the compact myocardium and failure to form an inter-ventricular septum. These defects were concordant with a loss of Bmp10 expression and up-regulation of p57, reflecting a decrease in cardiac myocyte proliferation [9,27]. Furthermore, alpha-actinin staining was present in striated patterns in Brg1 null embryos and increased expression of adult sarcomeric gene alpha-myosin heavy chain was observed, indicating that mutant embryos undergo premature maturation, reflecting the need for Brg1 to sustain an embryonic-like phenotype.

In support of the embryonic role of Brg1/BAF complexes, Brg1 also participates in the maintenance of pluripotent state as a member of the embryonic stem cell (ES cell) BAF complexes (ESC-BAF) [55,56]. The ESC-BAF complex containing Brg1 and BAF155 has been shown to co-localize significantly with Oct4, Sox2, and Nanog to maintain the pluripotent cell transcriptional network [55,56]. In addition to their roles in maintaining self-renewal and pluripotency of mouse ESC, ESC-BAF complexes are essential for the commitment of cells to specific lineages as well. For example, in the mouse embryo, Smarcd3/Baf60c is selectively expressed in the heart and somites, and RNAi-mediated knockdown results in defects in anterior heart field development as well as aberrations in cardiac and skeletal muscle differentiation [59]. Furthermore, expression of cardiac-specific transcription factors Gata4, and Tbx5 in combination with Baf60c is sufficient to trans-differentiate cells derived from non-cardiogenic mouse mesoderm into differentiated cardiomyocytes [60], indicating a key role for this BAF complex component during cardiogenesis.

Polybromo-associated BAF (PBAF) is unique since it contains the BAF180 subunit, which facilitates the ligand-dependent transcription of nuclear receptor genes such as RXRα, VDR, and PPARγ *in vitro*. BAF180 knockout mice are lethal at approximately embryonic day 15 post-conception and exhibit severe hypoplastic ventricular development. Histologically, hearts of BAF180 knockout mice exhibit ventricular wall thinning with ventricular septal defect [61]. Interestingly, the atrioventricular valves

and neural crest-derived aortic and pulmonary artery outflow tracts and their valves are unperturbed [62,63]. Notably, the phenotype observed in BAF180-deficient mice is very similar to the phenotype of RxRα knockout (KO) mice. DNA microarray analysis revealed that in KO animals, the expression of genes involved in metabolism, cell growth and differentiation, and structure is perturbed. These results support the hypothesis that the heart defects observed in BAF180−/− animals are due to growth arrest and not cell death. Confirmatory studies demonstrate that BAF180 regulates many retinoic acid receptor-dependent genes [61,62].

In addition to the requirement for proper chamber maturation, BAF180 is essential for coronary vessel formation. BAF180 is expressed abundantly in the heart during embryogenesis, specifically in the pro-epicardium and epicardium [61]. In mutant hearts, the subepicardial space is underdeveloped, and contains a reduced number of migratory epicardial cells. This was further supported by their loss of the ability to undergo epithelial−mesenchymal transformation (EMT) on collagen gel assays using epicardial explants. Consequently, the density of epicardial as well as intra-myocardial coronary vessels was reduced. Hence, BAF180 appears to function in epicardial cell EMT and coronary vasculogenesis addition to its role in myocardial development. It will be of interest to determine whether there are separable roles of BAF180 in myocardial development independent of its requirement in epicardial cell EMT and coronary vessel formation by examining the phenotype of conditional BAF180 deletion using specific cell lineage Cre mice.

DPF3, a component of the BAF complex, was first identified in a genome-wide expression study of congenital malformed hearts [64]. DPF3 is up-regulated in the right ventricle of tetralogy of Fallot patients, and is selectively expressed in skeletal muscle and adult human heart [65]. The protein contains double plant homeodomains, which are found in proteins whose substrates are nucleosomes. Two splice variants of DPF3, Dpf3a, and Dpf3b, exist in mice and humans. The onset of DPF3 expression in mouse heart is at E7.5 within myocardial precursors. From E9.0 through adulthood, DPF3 is expressed throughout the whole heart. Knockdown of DPF3 in zebrafish results in significant heart and skeletal muscle defects, characterized by a thin elongated heart tube, reduced looping, and a poorly defined atrioventricular boundary [66]. Consequently, the contractility of the myocardium is much weaker as well. Mechanistically, the loss of DPF3 in zebrafish results in dysregulation of genes involved in transcription, nucleosome assembly, metabolic processes, and ion transport [66]. Using tandem affinity purification (TAP) and mass spectrometry (MS), the nuclear binding partners of DPF3 were identified [66]. Interestingly, nearly all core components of the BAF complex associate with both isoforms of DPF3. Reverse-TAP and MS confirm DPF3 binding to Baf60c [66]. In addition to binding BAF complex proteins, DPF3 binds histones H3 and H4 directly through interaction with methylated and acetylated residues on H3 and H4 peptides [64]. Genome-wide target analysis using ChIP-chip showed that DPF3 directly interacts with regulatory regions of genes required for cardiovascular development and cytoskeletal organization [66]. Interestingly, a high degree of overlap between DPF3 and Brg1 targets was found, suggesting that DPF3 may serve as an anchor between BAF complexes and modified histones [66].

Epigenetic Regulation and Congenital Heart Disease

A-V septum and conduction defects due to Nkx2.5 mutations

As mentioned earlier, Nkx2.5 is key transcription factor regulating the development of myocardial precursors during early-stage cardiac development. Transcriptional activation of Nkx2.5 is mediated by the polycomb-group gene Rae28 [67]. Null mutations in Rae28 mice cause cardiac anomalies similar to those found in human congenital heart diseases associated with Nkx2.5 mutations. Interestingly, Nkx2.5 itself may also participate in epigenetic regulation by modulating the expression of Jarid2 (Jarid2/Jmj), a component of a protein complex that contains histone demethylase and methyltransferases activities, in the SHF [68]. In Jmj-deficient mutant embryos, cell proliferation and cyclin D1 expression are enhanced in cardiac myocytes. Overexpression of Jmj represses cyclin D1 and rescues hyperproliferation. Therefore, Jmj regulates cardiomyocyte proliferation and cardiac morphogenesis by regulating cell cycle components such as cyclin D1 [69]. Beyond cell cycle regulation, Jmj also regulates the expression of atrial natriuretic factor (ANF), an early marker for cardiac differentiation and for cardiac hypertrophy. It represses ANF gene expression by antagonizing transactivation by Nkx2.5 and GATA4 through protein−protein interaction [70]. Hence, multiple layers of interactions between Nkx2.5 and epigenetic factors such as histone demethylase/methyltransferases are present to appropriately regulate cardiac gene expression. Any defect in Nkx2.5 itself or epigenetic factors that interact with Nkx2.5 may likely result in congenital heart disease.

Indeed, human mutations in Nkx2.5 have been associated with various septation and atrioventricular conduction defects [71,72]. Analysis of Nkx2.5 mutations in patients with congenital heart disease frequently show reduced DNA binding and transcriptional activation by mutant Nkx2.5 compared with wild type [73,74]. In mice with loss

of Nkx2.5, the expression of ANF, brain natriuretic peptide, MLC2V, MEF2C, HAND1, Msx2, and Irx4 was significantly perturbed [75]. A ventricular-restricted knockout of Nkx2.5 using MLCv-Cre resulted in aberrant expression of atrial and conduction system genes in adult ventricular myocardium [76]. The regulation of some, if not a significant number, of genes that are altered in Nkx2.5 mutant heart may be mediated by Bmp/Smad signaling pathway [77].

Wolf–Hirschhorn syndrome

Wolf–Hirschhorn syndrome is characterized by craniofacial malformations, growth retardation, learning disability, and heart defects. Deletion of a critical region of human chromosome 4q16.3 is associated with the disease and contains the gene encoding Wolf–Hirschhorn Syndrome Candidate 1 (WHSC1). WHSC1 possesses SET (Suppressor of variegation 3-9, Enhancer of zeste, Trithorax) domains that are highly conserved with yeast H3K36 methyltransferase Set2. WHSC1 mediates mono-, di-, and tri-methylation of H3K36 on nucleosomes from Hela cells. In support of this, WHSC1 also methylates nucleosome substrates that are composed of recombinant core histones. Whsc1$^{-/-}$mESCs exhibit significantly reduced trimethylation of H3K36 and increased activation of Whsc1 target genes. Restoration of WHSC1 rescues the defect in H3K36 methylation. WHSC1 associates with various chromatin-remodeling enzymes and transcription factors such as Sall4 and Nanog. Whsc1$^{-/-}$mice are observed in Mendelian ratios until birth, but die within 10 days after birth and exhibit marked growth retardation. WHSC1 is expressed throughout the embryonic heart but not the endocardial cushion, and Whsc1$^{-/-}$mice display a variety of atrial and ventricular septal defects. In embryonic hearts, WHSC1 is able to interact with Nkx2.5 to regulate genes such as Pdgfra and natriuretic precursor peptide A (Nppa). Consequently, Pdgfra and Nppa are aberrantly expressed in Whsc1$^{-/-}$mutant hearts. Furthermore, Nkx2.5 and WHSC1 both associate with the transcription start site of the Pdgfra gene and loss of Whsc1 leads to a considerable reduction in H3K36me3 at the Pdgfra locus. Trans-activation assays indicate that WHSC1 functions to repress Nkx2.5 target gene expression by facilitating H3K36me3 [78].

CHARGE syndrome

CHARGE syndrome is characterized by coloboma of the iris or retina, heart defects, atresia of the choanae, retardation of growth and/or development, genital, and ear abnormalities [79]. Seventy-one percent of children clinically diagnosed with CHARGE have genetic mutations in the CHD7 gene, a member of the chromodomain helicase DNA binding family of ATP-dependent chromatin modifiers [79]. ChIP-chip analysis of CHD7 direct gene targets showed that CHD7 binds to its target sites in a cell-type-specific fashion and is associated with all three forms of methylated H3K4, but most robustly with the mono-methylated (H3K4me1) and di-methylated (H3K4me2) sites [80]. CHD7 binding sites were found to be localized away from transcriptional start sites and overlap with DNase I hypersensitivity sites, indicating that CHD7 may bind to and regulate putative enhancer regions or insulator elements for the deposition of epigenetic marks that define lineage specification [80,81]. Inactive genes that are marked by H3K4 mono- and di-methylation undergo a round of acetylation and de-acetylation thereby precluding the binding of Pol II for gene transcription; however, these genes are maintained in a poised state for future activation. It appears that CHD7 recruits the binding of other histone-modifying enzymes such as HATs and HDACs to mediate histone methylation and acetylation/deacetylation to effect the transcriptional machinery [82]. Although no studies currently exist that directly link the association of CHD7 to methylated H3K4 to aberrant expression of cardiac genes, haploinsufficiency of CHD7 in mice does result in hypoplasia of the pharyngeal arch artery (PAA), a phenotype that is also seen in Tbx1 heterozygous mice [83]. In support of a genetic interaction between these two proteins, CHD7$^{+/-}$; Tbx1$^{+/-}$ compound heterozygotes showed greater defect in PAA morphogenesis than either CHD7 or Tbx1 heterozygote alone. Apparently, biallelic expression of these two proteins is required for normal PAA development [84,85].

Holt–Oram syndrome

Holt–Oram syndrome is caused by haploinsufficiency of Tbx5, a member of the T-box family of transcription factors that regulates mammalian limb and heart development [86]. The manifestation of this syndrome includes malformations of the upper limbs, cardiac septation defects such as atrial septal defect (ASD) and ventricular septal defect (VSD), and disruption of normal cardiac conduction system development [87,88]. The distribution of Tbx5 expression in atrial and ventricular septum and the left ventricle correlates well with the atrial and ventricular septal defects and the left-sided malformations found in Holt–Oram syndrome [89,90]. Examination of various mutations of *TBX5* found in patients with Holt–Oram syndrome showed that these mutations reduce the efficiency of Tbx5 binding to its DNA targets [91]. Mutations at distinct coding and non-coding regions within *TBX5* correlate with congenital defects in either limb or heart, indicating that Tbx5 regulate limb and heart development via separable binding domains and with tissue-specific cis-elements [92].

The ablation of Tbx5 in mice resulted in marked hypoplasia of posterior structures and significant loss of cardiac

gene expression [93]. Tbx5 haploinsufficiency resulted in reduced levels of ANF and connexin 40 (cx40) expression, while *in vitro* transactivation studies indicate that Nkx2.5 co-operates with Tbx5 to induce expression of these genes [93]. Further studies revealed that Nkx2.5 and Tbx5 physically interact to transactivate Nppa [94]. One potential role of Tbx5 may be to promote cardiac differentiation by suppressing the proliferation of embryonic cardiomyocytes and modulate cardiac growth [95]. Beyond its direct role in transcriptional regulation of cardiac genes, a recent report indicates that Tbx5 is also a key player during pre-mRNA splicing [96].

The link between Tbx5 and epigenetic regulation has recently been explored in greater detail. Ectopic expression of GATA4, Tbx5, and Baf60c was found to induce transdifferentiation of mouse mesoderm to beating cardiac cells [60]. It appears that chromatin targeting by Baf60c enables GATA4 to access enhancer regions that are critical for cardiac gene expression. Subsequently, Tbx5 is recruited to these enhancer sites to promote transcriptional activation. While the exact mechanism that Tbx5 utilizes to activate gene expression remains to be identified, it is known that Tbx5 interacts with a WW-domain-containing transcriptional regulator, TAZ, to stimulate the activation of Tbx5-dependent promoters [97]. TAZ serves to co-activate downstream Tbx5 target genes by interacting with HATs such as p300 and pCAF [97]. As p300 has been shown to bind enhancer regions, the interaction between Tbx5, TAZ, and p300 or pCAF is a potential mechanism whereby cardiac genes are regulated [98]. Tbx5 may function by binding to enhancer elements, in co-operation with other cardiac-specific transcription factors and chromatin-modifying enzymes such as HATs in order to modulate the expression of key developmental genes during cardiac morphogenesis.

DiGeorge syndrome

DiGeorge syndrome (a.k.a. velo-cardio-facial syndrome) is characterized by interruption of the aortic arch, VSDs, persistent truncus arteriosus, and tetralogy of Fallot and is one of the diseases associated with deletion of human chromosome 22q11 [99–101]. DiGeorge syndrome is also associated with a constellation of congenital malformations such as hypoplasia of the thymus and parathyroid glands, abnormal facial structures, vertebrae, and cleft palate. Gene targeting studies in mice support a key role for Tbx1 in the pathogenesis of DiGeorge syndrome [102]. Consistent with the fact that Tbx1 promotes the transcription of FgF10 in the SHF, Tbx1 deletion results in the loss of cell proliferation in the SHF.

The regulation of cardiac gene expression by Tbx1 involves co-operative interaction with other cardiac transcription factors and chromatin-modifying enzymes. Tbx1 binds co-operatively with Nkx2.5 to regulate an enhancer of Pitx2, a bicoid-like homeodomain transcription factor involved in left–right asymmetry. In addition, a physical and functional interaction exists between Tbx1 and Ash2l, the mammalian homologs of drosophila Ash2 (absent small homeotic 2). Ash2l is a core component of a multimeric histone methyltransferases subunit which is responsible for epigenetically regulating transcription by methylating histone N-terminal tails and co-activates Tbx1 target genes [103].

Summary and Future Perspective on Epigenetic Regulation of Cardiac Development

During cardiac morphogenesis, chromatin organization profoundly influences the activity of the cardiac transcription network. Epigenetic mechanisms such as DNA methylation, covalent histone modifications, and ATP-dependent nucleosome remodeling undergo dynamic changes to orchestrate a proper sequence of gene expression. A co-operative and synergistic relationship exists between tissue-specific transcription factors and global epigenetic modifiers during this process to specify cell fate and promote terminal differentiation (**Table 1**).

The deposition of histone modifications can alter histone–DNA interactions and permit or restrict the binding of co-activator/co-repressor complexes, and therefore affect transcription. The diverse combinations of histone modifications and DNA methylation changes available provide the requisite flexibility for recruitment and association of chromatin-modifying enzymes. For example, DPF3 can interact with histone H3, H4, Brg1, and Baf60c, and is thought to act as an anchor between modified histones and BAF complexes. Furthermore, CHD7 concurrently occupies sites of H3K4 methylation and p300 occupancy, providing evidence that chromatin-modifying complexes may be recruited to target genes by a diverse combination of histone modifications. The requirement for proper epigenetic regulation during cardiogenesis is underscored by the profound defects observed in animals with experimentally induced loss of function of these genes. For example, the loss of Brg1 in mice results in embryonic lethality at E11.5 with loss of the inter-ventricular septum. Null mutations of Baf180, a unique component of PBAF, result in a severely hypoplastic ventricle and VSD.

The interaction between cardiac-specific transcription factors and histone modifiers provides a mechanism for target specificity. A complex feedback loop that exists between Jarid2 and Nkx2.5 is necessary to regulate genes such as cyclin D1 and ANF that are involved in cardiac differentiation, cell proliferation, and hypertrophy. Interaction between Nkx2.5 and WHSC1, a H3K36

Table 1 Chromatin-remodeling proteins and associated cardiac defects

Transcription factors or chromatin remodelers	Animal or disease models	Defects	Molecular mechanisms	Reference no.
Brg1	Knockout mice	Thinning of compact myocardium and failure to form intra-ventricular septum; abberant down-regulation of Bmp10 and up-regulation of p57; decreased cardiac myocyte proliferation and premature differentiation	Perturbed transcriptional regulation	9, 27, 55–57
Smacd3/Baf60c	RNAi knockdown	Defects in anterior heart field development; aberrant cardiac and skeletal muscle differentiation	Perturbed transcriptional regulation	59, 60
PBAF complex (containing BAF180)	Knockout mice	Hypoplastic ventricle development and wall thinning; ventricle septal defects similar to RxRα knockout mice	Defects in growth arrest	61–63
		Underdeveloped subepicardial space; reduced number of migratory epicardial cells	Failure to undergo EMT	
DPF3b	Tetralogy of Fallot; knockdown in zebrafish	Thin-elongated heart tube with reduced looping and poorly defined atrioventricular boundary; weak myocardial contractility; dysregulation of genes involved in transcription, nucleosome assembly, metabolic processes, and ion transport	DPF3b binds Baf60c and methylated or acetylated histones H3 and H4 at cardiac target genes; DPF3b functions as anchor between BAF complexes and modified histones H3 and H4	64–66
Jarid2(Jmj)	Knockout mice	Aberrant increase in cell proliferation in cardiac myocytes; represses ANF by inhibiting protein: protein interactions between Nkx2.5 and GATA4	Jarid2 is a component of a complex containing histone methyltransferase and demethylase activity and represses cyclin D1 transcription	68–70
	Ventricle specific knockout	Perturbed expression of ANF, BNP, Mlc2V, Mef2c, Hand1, Msx2, Irx4	Reduced DNA binding and transcription activation by mutant Nkx2.5	
and Nkx2.5	Mutant human Nkx2.5 in patients	Septation and atrioventricular conduction system defects		67,70–76
WHSC1	Wolf–Hirschhorn syndrome	Craniofacial malformations, growth retardation, learning disabilities, heart defects		
	Knockout mice	Lethal at P10; growth retardation, atrial and ventricle septal defects; misregulation of platelet derived growth factor receptor alpha (Pdgfra) and Nppa	WHSC1 and Nkx2.5 interact at transcriptional start sites of Pdgfra and coincide with H3K36me3 at these sites	78

CHD7	CHARGE syndrome	Coloboma of iris or retina, heart defects, atresia of choanae, retardation of growth, developmental, genital or ear abnormalities		79–85
	Haploinsufficient mice	Hypoplasia of pharyngeal arch artery which is also observed in Tbx heterozygote mice	CHD7 binds methylated H3K4, but prefers H3K4me1, -me2 at sites distal to TSSs and CHD7 binding overlaps with DNAse hypersensitivity sites, possibly at enhancers or insulators of cardiac genes	
Tbx5	Holt—Oram syndrome; mutant human Tbx5 in patients	Upper limb malformation, cardiac septation defects, atrial and ventricle septal defects, disruption of cardiac conduction system development	Reduced efficiency of Tbx5 to bind DNA targets Mutations at coding and non-coding regions correlate with congenital defects in limb or heart	60, 86–98
	Knockout mice	Hypoplasia of posterior cardiac structures; loss of cardiac gene expression (i.e. ANF, connexin 40); suppresses proliferation of embryonic cardiac myocytes and modulates cardiac growth	Tbx5 and Nkx2.5 function to regulate cardiac gene expression; Tbx5 is also recruited by GATA4 and Baf60c to cardiac gene enhancers for transcription activation; Tbx5 interacts with WW-domain protein TAZ to stimulate activation at Tbx5-dependent promoters; TAZ interacts with p300, pCAF	
Tbx1	DiGeorge syndrome or velo-cardio-facial syndrome	Interruption of aortic arch, ventricle septal defects, persistent truncus arteriosus and tetralogy of Fallot; many congenital malformations such as hypoplasia of thymus and parathyroid glands; abnormal facial structures, vertebrae and cleft palate		99–103
	Knockout mice	Loss of proliferation in second heart field	Tbx1 interacts with Nkx2.5 and Ash2l (a component of a histone methyltransferase complex); Tbx1 required for regulation of Fgf10 in SHF	

The table summarizes the cardiac defects and diseases associated with mutations or loss of function of specific chromatin-remodeling proteins or transcription factors. A putative molecular mechanism is included for each of the proteins mentioned, delineating how these physiological anomalies may arise from faulty transcription regulation.

methylase, is required for the regulation of expression of target genes such as Pdgfra and Nppa. The absence of this interaction results in the formation of ASD and VSD in Wolf−Hirschorn syndrome. The interaction between Nkx2.5, Tbx1, and Ash2l, a component of a histone methyltransferases complex, is required to regulate the expression cardiac genes such as Pitx2 and Fgf10. The loss of such interaction is associated with branchial arch defects in DiGeorge syndrome, highlighting the need for a co-operative relationship between transcription factors and epigenetic modifiers.

Given that specific epigenetic states, as defined by a pattern of cardiac transcription factor occupancy, histone modifications, and overall chromatin organization, may be required for proper gene expression in cardiac progenitor and differentiated cells, identification of the precise pattern of these epigenetic changes during cardiac development and differentiation would be highly informative. Recent advances in high-throughput DNA sequencing technology have enabled the search for the binding sites of cardiac transcription factor, histone, and DNA modifier on a genome-wide level by ChIP-sequencing. Furthermore, the identification of directly interacting complexes between cardiac transcription factors and histone and DNA modifiers by MALDI-TOF MS may reveal novel protein–protein interactions. We may find that at each loci of cardiac gene, the epigenetic signature is distinct between embryonic vs. adult cells. The aberrant modification or significant loss of epigenetic marks at these gene loci may account for the development of congenital heart disease. Understanding these changes at the epigenetic level will facilitate the accurate diagnosis of disease and uncover novel therapeutic targets.

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