Chromatin Remodeling in Cardiovascular Development and Physiology

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Abstract: Chromatin regulation provides an important means for controlling cardiac gene expression under different physiological and pathological conditions. Processes that direct the development of normal embryonic hearts and pathology of stressed adult hearts may share general mechanisms that govern cardiac gene expression by chromatin-regulating factors. These common mechanisms may provide a framework for us to investigate the interactions among diverse chromatin remodelers/modifiers and various transcription factors in the fine regulation of gene expression, essential for all aspects of cardiovascular biology. Aberrant cardiac gene expression, triggered by a variety of pathological insults, can cause heart diseases in both animals and humans. The severity of cardiomyopathy and heart failure correlates strongly with abnormal cardiac gene expression. Therefore, controlling cardiac gene expression presents a promising approach to the treatment of human cardiomyopathy. This review focuses on the roles of ATP-dependent chromatin-remodeling factors and chromatin-modifying enzymes in the control of gene expression during cardiovascular development and disease. (Circ Res. 2011;108:378-396.)

Key Words: chromatin ■ epigenetics ■ heart development ■ cardiomyopathy ■ gene expression

Forming a heart that pumps for perhaps 80 years in a human life with near-perfect fluid dynamics is a challenging task. Heart development is thus a precisely regulated process, and the required gene expression is tightly controlled. Many different genes are expressed in fetal and adult hearts to meet the distinct developmental and physiological needs. However, when adult hearts are stressed by various pathological insults such as ischemia, pressure, or volume overload, they may express or activate certain genes that are normally only expressed in fetal hearts. This reexpression of “fetal” genes in adult hearts contributes to the disease process that leads to cardiomyopathy and congestive heart failure. Therefore, it is important to understand the mechanisms that control cardiac gene expression under developmental and different pathophysiological conditions.

One important control of gene expression is through chromatin regulation. Human cells have evolved sophisticated packaging methods to compact the 1.7-m-long DNA into a nucleus approximately 6 μm in diameter. First, 147 base pairs of DNA are wrapped around an octameric core of histone proteins, composed of 2 molecules of each of the canonical H2A, H2B, H3, and H4 histones, to form a nucleosome, a basic building block of the chromatin (Figure, A). These repeating units of nucleosomes are then tightly organized into higher-order scaffolds to further condense the DNA. Within each nucleosome, the positively charged resi-
Chromatin remodeling and histone modifications play essential roles in heart development and disease by reprogramming gene expression under different pathophysiological conditions. During heart development, chromatin remodelers exert their functions in a temporal- and tissue-specific manner to direct the formation of various cardiovascular tissues. In adult hearts, chromatin regulation alters gene expression in response to pathophysiological changes. Pathological stresses trigger individual myocytes to increase in size and the whole heart to enlarge (or hypertrophy). Cardiac hypertrophy, although considered as a compensatory response to workload increased by pathological stresses, leads to a decline of muscle contractility and heart failure. Hypertrophy and heart failure are characterized by transcriptional reprogramming of gene expression and reactivation of fetal genes, many of which are known to be controlled by chromatin-regulating factors. This review summarizes the roles of major chromatin remodeling and modifying factors in the control of embryonic heart development and pathogenesis of adult heart diseases.

The ATP-Dependent Chromatin-Remodeling Complexes

There are 4 different families of SWI-like ATP-dependent chromatin-remodeling complexes: SWI/SNF (switching defective/sucrose nonfermenting), ISWI (imitation switch), CHD (chromodomain, helicase, DNA binding), and INO80 (inositol requiring 80) complexes (see elsewhere1,4 for reviews). All members of the families share an evolutionarily conserved SWI-like ATPase catalytic domain, but each has its own distinct flanking domains. The ATPase domain serves as a motor to adjust histone–DNA contacts for DNA movement and chromatin restructuring. The other domains may recognize covalently modified histones, modulate the ATPase activity, or interact with other chromatin and transcription factors. Therefore, the unique domains and their associated proteins determine the genomic targeting specificity and biological functions of each family of chromatin remodelers.

The SWI/SNF Complex in Heart Development and Disease

The SWI/SNF chromatin remodelers, initially purified from Saccharomyces cerevisiae, are composed of 8 to 14 subunits (see elsewhere1,4 for reviews). The vertebrate homolog of the yeast SWI/SNF complex is the BAF (brahma-associated factor) complex. In mammals, the BAF complex contains 12 protein components, including an ATPase subunit encoded by either Brm (brahma) or Brg1 (brahma-related gene 1). These 2 ATPase subunits, although highly homologous, exhibit...
nonredundant functions in vivo. Brg1-null mutation is implantation lethal,
whereas Brm-null mice are viable and develop normally with slight increase in body mass. Several other subunits of the BAF complex are also encoded by gene families, which give rise to a diverse composition of the BAF complex that have distinct functions in different cell types. Recent studies have demonstrated that BRG1 and individual subunits of the BAF complex are essential in heart development and disease (Table 1).

BRG1

Brg1 is known to control cardiovascular development in a time- and tissue-specific manner. Ablation of Brg1 in the endothelium causes embryonic lethality around embryonic day (E)10.5 of mouse development. The mutant embryos are anemic and display vascular defects in the yolk sac but not in the embryo proper, indicating that endothelial Brg1 is required for primitive erythropoiesis and extraembryonic yolk sac vasculogenesis. Within the developing heart, Brg1 acts in the endocardium to control myocardial trabeculation through regulating Adamts1 expression. Adamts1 is a secreted matrix metalloproteinase whose substrates include versican, a cardiac jelly component required for heart development. Adamts1 is normally repressed by Brg1 from E9.5 to E11.5 to allow the establishment of cardiac jelly for myocardial trabeculation. Later, from E12.5 to E14.5, Adamts1 is derepressed in the endocardium, releasing the protease into the cardiac jelly to degrade versican and other matrix proteins, thereby terminating myocardial trabeculation at E13.5 to E14.5 and preventing excessive trabecular growth. Mice lacking endocardial Brg1 have Adamts1 prematurely activated in the endocardium at E9.5, resulting in an early degradation of the cardiac jelly and subsequent hypotrabeculation. Conversely, mice lacking Adamts1 exhibit hypertrabeculation in the ventricles. Therefore, the dynamic control of Adamts1 and cardiac jelly composition by Brg1 provides a developmental mechanism to delimit the extent of myocardial trabeculation. Such regulation is important because inadequate or excessive trabeculation can both cause cardiomyopathy and heart failure.

Brg1 also functions in the myocardium to control cardiac gene expression, tissue growth, and differentiation. In mouse embryos, Brg1 promotes cardiomyocyte proliferation by maintaining Bmp10 and suppressing p57kip2 expression. Bmp10 is a key factor required for myocardial proliferation, whereas p57kip2 is a cyclin-dependent kinase (Cdk) inhibitor that prevents cell cycle progression. Mice lacking myocardial Brg1 die around E11.5 because of the thin compact myocardium and absent interventricular septum. These defects are caused by a failure of myocardial proliferation due to Bmp10 deficiency and ectopic p57kip2 expression. In parallel to proliferation control, Brg1 preserves fetal cardiac differentiation by interacting with histone deacetylases (HDACs) and poly(ADP-ribose) polymerases (PARPs) to repress α myosin heavy chain (α-MHC) and activate β-MHC expression in embryonic hearts. In mice, α-MHC is the primary MHC isoform expressed in adult hearts, whereas β-MHC is the major isoform in embryonic hearts. By repressing α-MHC and activating β-MHC, the Brg1/HDAC/PARP protein complex maintains a fetal state of MHC expression.
Table 1. Roles of ATP-Dependent Chromatin-Remodeling Factors in Cardiovascular Development and Disease

<table>
<thead>
<tr>
<th>Class</th>
<th>Chromatin-Remodeling Factor</th>
<th>Tissue of Gene Modification</th>
<th>Phenotype</th>
<th>Comment</th>
<th>Reference</th>
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<tbody>
<tr>
<td>SWI/SNF</td>
<td>BRG1</td>
<td>Deletion in endothelium and endocardium</td>
<td>Lethality at E10.5 to E11.5; hypotrabeculation, yolk sac vascular defects, anemia</td>
<td>Brg1 represses endocardial Adamts1 to prevent premature degradation of cardiac jelly, essential for trabeculation</td>
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<td></td>
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<td>Deletion in myocardium</td>
<td>Lethality at E11.5; thin compact myocardium and absent NS</td>
<td>Brg1 maintains Bmp10 to promote myocardial proliferation in embryos; Brg1 associates with HDACs and PARPs to repress α-MHC and activate β-MHC in embryonic hearts</td>
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<td>Inducible Brg1 deletion in adult myocardium prevents cardiac hypertrophy and fibrosis</td>
<td>Inducible Brg1 deletion in adult myocardium prevents cardiac hypertrophy and fibrosis</td>
<td>Cardiac stress activates Brg1, which then associates with HDACs and PARPs to control the pathological MHC switch, hypertrophy and fibrosis</td>
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<td>Deletion in secondary heart field</td>
<td>Lethality at E10.5; hypoplastic right ventricle and outflow tract</td>
<td>Brg1 maintains Bmp10 to promote proliferation of right ventricular and outflow tract myocardium</td>
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<td>Heterozygous germline mutation Brg1+/−</td>
<td>Variable heart defects: VSD, patent foramen ovale, cardiac dilatation, sinus node dysfunction, AV block, conduction abnormalities</td>
<td>Brg1 interacts dose-dependently with Nkx2.5, Tbx5, and Tbx20 to regulate heart development</td>
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<td>Point mutation; morpholino knockdown in zebrafish</td>
<td>Mutation of brg1 in zebrafish causes hypoplastic myocardium, abnormal shape and alignment of cardiomyocytes; knockdown causes cardiac looping defects, chamber narrowing, and contractility reduction</td>
<td>Brg1 is evolutionarily conserved in zebrafish heart development</td>
<td>34a</td>
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<td></td>
<td>BAF180</td>
<td>Germline deletion</td>
<td>Lethality at E12.5 to E15.5; thin compact myocardium, hypoplastic ventricles, VSD, coronary defects</td>
<td>Baf180 interacts with the retinoid acid pathway to regulate cardiac chamber formation; Baf180 is required for expression of genes essential for coronary vessel formation</td>
<td>38, 41</td>
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<td>BAF60c</td>
<td>Knockdown in mouse hearts</td>
<td>Lethality at E10.0 to E11.0; hypoplastic ventricles, hypoplastic, shortened outflow tract, abnormal cardiac looping</td>
<td>Baf60c regulates heart morphogenesis through Hand2, Bmp10, Ix3, and others; Baf60c controls cardiac looping by activating Notch signaling and Nodal expression</td>
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<td></td>
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<td>Knockdown in zebrafish</td>
<td>Randomized cardiac looping</td>
<td>Zebrafish and mouse Baf60c share conserved functions for heart looping regulation</td>
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<td>BAF45c</td>
<td>Knockdown in zebrafish</td>
<td>Abnormal cardiac looping, poorly defined AV boundary, reduced cardiac contractility, disarranged muscle fibers</td>
<td>Baf45c recognizes acetylated and methylated histones; this recognition may recruit BRG1/BAF to muscle-relevant gene promoters</td>
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<td>ISWI</td>
<td>SNF2H</td>
<td>Germline deletion</td>
<td>Lethality at E5.5−7.5 due to growth failure of the inner cell mass and trophoblast</td>
<td>Snf2 hours may regulate heart development through the Wstf-containing WICH complex</td>
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<td>CHD</td>
<td>CHD7</td>
<td>Heterozygous germline mutation Chd7+/−</td>
<td>Aortic arch interruptions attributable to hypoplastic 4th pharyngeal arch artery</td>
<td>Chd7 interacts with Tbx1 to regulate aortic arch formation in mice</td>
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<td></td>
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<td>Knockdown; expression of dominant-negative Chd7 in frog</td>
<td>Abnormal position of truncus arteriosus and cardiac outflow tract</td>
<td>CHD7 associates with BRG1/PAF to control Sox9, Twist, Slug, and neural crest cell activation</td>
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<td>INO80</td>
<td>Reptin and Pontin</td>
<td>Activation of Reptin or knockdown of Pontin in zebrafish</td>
<td>Cardiac muscle hyperplasia</td>
<td>Pontin and Reptin antagonistically control heart muscle growth, in part, through the β-catenin pathway</td>
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ASD indicates atrial septal defect; AV, atrioventricular; IVS, interventricular septum; VSD, ventricular septal defect.
Embryonic cardiomyocytes lacking Brg1 switch the expression of MHC from β- to α-MHC. Also, inhibition of HDAC or PARP activity triggers a premature switch from β- to α-MHC expression in embryonic hearts. Therefore, Brg1 commands distinct molecular pathways to maintain cardiomyocytes in a fetal state of proliferation and differentiation.

Brg1, although highly expressed in embryonic hearts, is downregulated in the adult cardiomyocytes, coinciding with the physiological switch from the fetal β-MHC to adult α-MHC expression. However, when the adult heart is stressed by pressure overload, Brg1 is reactivated in the cardiomyocytes and forms a complex with its embryonic partners, HDACs and PARP1, to repress α-MHC and activate β-MHC expression. This stress-dependent assembly of fetal Brg1/HDAC/PARP complex triggers the adult heart to return to a fetal state of MHC expression, a critical step in the myopathic process. Preventing such stress-induced Brg1 expression dramatically reduces cardiac hypertrophy, abolishes cardiac fibrosis, and reverses the pathological MHC switch in mice. Interestingly, Brg1 is also upregulated in some patients with hypertrophic cardiomyopathy. Its level correlates with the disease severity and pathological MHC switch, suggesting that Brg1 may play a role in human hypertrophic heart disease. Because Brg1, HDAC, and PARP are 3 classes of chromatin regulators required for cardiac hypertrophy, the assembly of these chromatin factors on the MHC promoters of stressed hearts suggests that chromatin may ultimately be where all the stress-response signals converge for the regulation of MHC genes.

Brg1 also functions in the secondary heart field to regulate right heart development in mice. Deletion of Brg1 in the secondary heart field results in hypoplastic right ventricle and shortened cardiac outflow tract at E10.5 before embryonic lethality at E11.5. These defects are caused by inadequate proliferation of the right ventricular myocardium resulting from Bmp10 deficiency and ectopic p57kip2 expression.

Brg1 is evolutionarily conserved in zebrafish heart development. Mutation of Brg1 in zebrafish yields hypoplastic myocardium with abnormal shape and alignment of cardiomyocytes. Morpholino knockdown of Brg1 in zebrafish causes cardiac looping defects, chamber narrowing, and contractility reduction.

The gene dosage of Brg1 appears crucial for heart development. Brg1+/- mice survive at a sub-Mendelian ratio with various degrees of congenital heart anomalies, including ventricular septal defects, patent foramen ovale, and cardiac dilatation. Those Brg1+/- mice that survive to adulthood display abnormal cardiac functions, including sinus node dysfunction, atrioventricular block, conduction abnormalities, and impaired cardiac relaxation. Brg1 genetically interacts with other cardiac transcription factors in a dosage-dependent manner. Compound Brg1 and Tbx5 heterozygous mice exhibit more severe cardiac defects than either mutation alone. Furthermore, when a single Brg1-null allele is introduced alongside with Nkx2.5 or Tbx20 heterozygous mutation that alone causes mild or no cardiac phenotypes, severe cardiac defects with atrial and ventricular septal defects arise, and none of the compound mutants survives beyond E14.5. Transcriptional analyses of E11.5 hearts from single or compound heterozygous mutants for Brg1, Tbx5, or Nkx2.5 demonstrate a high degree of complexity of the genetic program governed by the 3 genes, with separate and overlapping gene regulation. This complexity appears partly mediated through the dosage of Brg1 and other transcription factors on cardiac gene promoters.

BAF180

Many other subunits of the BAF complex are involved in cardiovascular development. Baf180, also known as polybromo, contains 6 bromodomains that recognize acetylated histone tails. It is expressed ubiquitously in the developing embryo and is required for heart development. Baf180-null mouse embryos die between E12.5 and E15.5 because of trophoblast placental defects and severely hypoplastic ventricles with ventricular septal defects. These defects are similar to those observed in embryos lacking RXRα, a component of the retinoic acid pathway. It is shown that Baf180 is required for the expression of a subset of retinoic acid target genes such as RARβ2 and CRABPII, suggesting that Baf180 interacts with the retinoic acid pathway to regulate cardiac chamber formation. In addition, Baf180 is necessary for the development of coronary vasculature. Baf180-null epicardium fails to properly undergo epithelial-to-mesenchymal transformation, and the mice display downregulation of many factors that promote epithelial-to-mesenchymal transformation, vasculogenesis, or angiogenesis. All of these defects may result in abnormal coronary development.

BAF60c

Baf60c, a component of the BAF complex, is expressed by the developing heart and skeletal muscle. Baf60c is required for heart morphogenesis and the differentiation of mesodermal cells into cardiomyocytes. Small interfering RNA knockdown of Baf60c in mice causes embryonic lethality around E10.0 to E11.0 with multiple cardiac defects, namely hypoplastic ventricles with reduced trabeculation and shortened outflow tract. Many genes required for heart morphogenesis are also misregulated, including Hand2, Bmp10, and Irx3, among others. These Baf60c knockdown embryos also show randomization of cardiac looping and situs, a function conserved between mouse and zebrafish. Baf60c stabilizes the interactions between activated Notch and its DNA-binding partner RBP-J to control Nodal expression and thus establishes the left/right asymmetry of embryos and the heart. Also, Baf60c interacts with transcription factors Tbx5, Nkx2.5, and Gata4 to activate cardiac-specific genes for cardiogenesis. Ectopic expression of Baf60c, Tbx5, and Gata4 in noncardiogenic mesodermal cells induces these cells to differentiate into contracting cardiomyocytes that express an early cardiac marker Actc1. Therefore, Baf60c is required for early cardiogenesis and subsequent heart morphogenesis.

BAF45c

Baf45c/DPF3 is a subunit of the BAF complex and contains 2 PHD (plant homeo domain) domains that recognize acetylated and methylated lysine residues of histone H3 and H4. BAF45c/DPF3 is upregulated in the right ventricular...
myocardium of patients with tetralogy of Fallot, and it is expressed by the developing heart and somites in mouse, chicken, and zebrafish embryos. Morpholino knockdown of dpf3 in zebrafish results in abnormal cardiac looping, poorly defined atrioventricular boundary, reduced cardiac contractility, and disarrayed muscle fibers. In muscle gene regulation, DPF3 and BRG1 highly overlap in their binding to 21 muscle-relevant gene loci that contain acetylated or methylated H3 and H4, suggesting the DPF3 may recognize those modified histones and help recruit BRG1 there. Indeed, the tandem PHD domains of DPF3, PHD1 and PHD2, act as 1 functional unit in the sequence-specific recognition of acetylated lysine 14 of H3 (H3K14ac). The binding of DPF3 to histone H3 is promoted by H3K14ac, recognized by PHD1, and inhibited by methylated lysine 4 (H3K4me3), recognized by PHD2. This regulation of PHD binding by different histone modifications produces 2 opposing effects on the transcriptional activation of Dpf3 target genes, such as Pitx2 and Mnx1. Studies support a model in which H3K14 acetylation marks a gene locus for recruiting DPF3/BAF to preinitiate gene transcription, whereas H3K4 methylation dissociates DPF3/BAF from the locus, thus allowing the entry of migratory neural crest cells, which contribute to the formation of ear, thymus, and the fourth pharyngeal arch.

**Implication of BAF in Human Congenital Diseases**

BRG1/BRM and BAF proteins have been indirectly implicated in the pathogenesis of CHARGE syndrome (coloboma, heart defects, atresia choanae, retarded growth and development, genital hypoplasia and ear abnormalities/deafness) and Williams syndrome. CHARGE syndrome is described below in the section on CHD chromatin remodelers. Williams syndrome is an autosomal dominant disorder characterized by mental and growth deficiency, dysmorphic facial features, aberrant vitamin D metabolism, and cardiovascular abnormalities. Cardiovascular defects linked to the syndrome include supravalvular aortic stenosis, pulmonary arterial stenosis, aortic coarctation, bicuspid aortic valve, and atrioventricular septal defect.

The Williams syndrome transcription factor (WSTF) is among the 28 genes lost by heterozygous deletion of 7q 11.23 in Williams syndrome. Animal studies suggest that WSTF deficiency may account for some of the cardiac defects observed in Williams syndrome patients. Wstf-null mice show perinatal lethality with hypotrabeculation, thin myocardium, atrial septal defect, and ventricular septal defects. The fourth pharyngeal arch arteries appear hypoplastic, resulting in mild coarctation of aorta between the left common carotid and subclavian arteries. These cardiovascular malformations are also present in Wstf heterozygous mice at a lower frequency, suggesting a partial haploinsufficiency of Wstf and therefore a possible role of WSTF in the pathogenesis of Williams syndrome.

WSTF proteins are present in 2 different types of chromatin-remodeling complexes: the BRG1/BRM-containing WINAC complex (WSTF-including nucleosome assembly complex) and the SNF2H-containing WICH complex (WSTF-ISWI chromatin-remodeling complex). In E9.5 mouse hearts, Wstf recruits Brg1, Baf155, and cardiac transcription factors Nkx2.5, Gata4, and Tbx5 to the promoter of connexin 40 (Cx40) or Gja5. Transcriptional studies show that Wstf interacts with Brg1, but not Snf2h, to support the activation of Cx40 promoter. These ChIP and reporter assays suggest that Wstf regulates heart development through Brg1/WINAC, but not Snf2h/WICH.

However, the role of Snf2h/WICH in heart development cannot be excluded. WINAC alone does not fully explain the phenotypes of Wstf-null mice. These mice do not have the same cardiovascular defects observed in mice lacking either components of WINAC or its associated transcription factors. Further investigations are necessary to determine the relative contributions of BRG1/WINAC and Snf2h/WICH to the phenotypes of Williams syndrome.

**ISWI Complex in Heart Development and Disease**

Three ISWI complexes, NURF (nucleosome remodeling factor), ACF (ATP-utilizing chromatin assembly and remodeling factor), and CHRAC (chromatin accessibility complex), were purified from the fruit fly *Drosophila melanogaster* with subsequent identification of NoRC (nucleosome remodeling complex) and WICH in mammals (see elsewhere for reviews). The fly complex has only 1 single type of core ATPase, whereas the mammalian complex has 2 types, SNF2H and SNF2L, which are present in different complexes and have nonoverlapping expression patterns in mice. The role of Snf2h in heart development is not defined because of an early lethality of Snf2h-null mouse embryos, which die between E5.5 and E7.5 because of growth failure of inner cell mass and trophoblast. However, Snf2h may play a role in heart development because it is present in the WICH complex that contains Wstf, whose mutation causes myocardial and septal defects in mice (details described above). A tissue-specific deletion of Snf2h in mice will help in elucidating the function of Snf2h in cardiovascular development.

**CHD in Heart Development and Disease**

The CHD family of chromatin remodelers has 9 members, CHD1 to CHD9. Haploinsufficiency of CHD7 in humans results in CHARGE syndrome. Patients with CHARGE syndrome may have a variety of heart defects, including conotruncal malformations, atrial/ventricular septal defects, and endocardial cushion anomalies. *Chd7*−/− mice recapitulate several aspects of the human disease, such as inner-ear vestibular dysfunction and heart defects. These *Chd7*−/− mice display aortic arch interruption because of hypoplastic pharyngeal arch arteries. Formation of these arch arteries requires *Chd7* and *Tbx1* in the pharyngeal ectoderm. Analysis of *Tbx1*−/−;*Chd7*−/− double heterozygous mice suggest that *Chd7* and *Tbx1* synergistically regulate the development of ear, thymus, and the fourth pharyngeal arch.

CHD7 is essential for the development of multipotent migratory neural crest cells, which contribute to the formation
of many tissues affected in CHARGE syndrome. In frog embryos, knockdown of Chd7 or overexpression of dominant-negative form of Chd7 recapitulates major features of CHARGE syndrome: otolith defects, coloboma, craniofacial malformation, and heart defects. The heart defects involve abnormal positioning of the truncus arteriosus and cardiac outflow tract. All of these anomalies are consistent with neural crest cell defects. Indeed, Chd7 is required in the frog for the expression of Sox9, Twist, and Slug (Snail2), genes essential for early neural crest cell migration and specification. Studies using frog embryos and human neural crest-like cells further suggest that CHD7 associates with BRG1/PBAF to control Sox9, Twist, and neural crest cell activation. Therefore, CHD7 and BRG1/PBAF interactions may underlie the pathogenesis of CHARGE syndrome.

INO80 in Heart Development

The INO80 complexes, conserved from the yeast to vertebrate, include the INO80, SRCAP (SNF2-related CREB-activator protein) and p400 in humans (see elsewhere1,4,63 for reviews). These INO80 complexes contain DNA helicases: Pontin (alias Rvb1, Tip49, Tip49a) and Reptin (alias Rvb2, Tip48, Tip49b). The absence of Pontin or Reptin activity causes disruption of chromatin remodeling activity of the INO80 complexes, suggesting essential roles of those 2 proteins in chromatin and transcriptional regulation.64–67 Interestingly, Pontin and Reptin are known to regulate myocardial growth.68 Morpholino knockdown of pontin in zebrafish causes cardiac hyperplasia, whereas an insertionional mutation of reptin that activates the ATPase activity of reptin stimulates cardiomyocyte proliferation in zebrafish.69 Studies suggest that Pontin and Reptin antagonistically regulate heart muscle growth, at least in part, through their opposing effects on the β-catenin pathway.58 These functions of Pontin and Reptin suggest the involvement of INO80 complexes in heart development. Unfortunately, studies of INO80 subunits in mouse heart development are not yet available. It remains to be investigated how INO80 regulates mammalian heart development.

The Histone Deacetylases

Histone deacetylases (HDACs) are a class of chromatin-remodeling factors that usually repress gene expression (see elsewhere3,69 for reviews). These enzymes remove acetyl group from conserved lysine residues within the N-terminal tails of histone H3 and H4, thereby increasing histone–histone and histone–DNA contacts and causing condensation of chromatin. Histone deacetylation by HDACs is countered by a class of antagonistic enzymes, histone acetyltransferases (HATs), which add acetyl group to the lysine residues. Thus, dynamic balance in the state of histone acetylation and deacetylation provides flexibility and reversibility in gene expression control.

HDACs are known to function in a genome-wide manner and contribute to an extensive range of biological functions from development to physiology.3,69 The mammalian genome encodes 11 HDAC proteins that contain a highly conserved deacetylase domain. The HDACs are classified into 4 subfamilies (class I, IIa, IIb, and IV) based on their protein structure, enzymatic activity, subcellular localization, and expression pattern. In addition, the mammalian genome encodes another group of deacetylases (class III HDACs) or sirtuins, which contribute to heterochromatinization through their NAD+-dependent histone deacetylase activity.70 Class IV HDAC has only 1 known member, HDAC11,71 whose function in the heart remains elusive. Among HDAC proteins, class I and IIa are the most extensively studied and are regarded as classic HDACs. Class I HDACs display high enzymatic activity toward histone substrates, whereas class IIa HDACs possess only weak enzymatic activity72,73 because of a tyrosine-to-histidine change in the catalytic pocket of these class IIa proteins.74,75 The intrinsic catalytic activity is not required for class IIa HDACs to repress gene expression. Gene repression by class IIa HDACs is mediated by their interactions with class I HDACs and other transcriptional repressors such as nuclear receptor corepressor (N-CoR) and silencing mediator of retinoic acid and thyroid hormone receptors (SMRT), heterochromatin protein 1 (HP-1), and C-terminal-binding protein (CtBP).76–79 Recent studies of HDAC knockout mice have demonstrated critical functions of HDACs in cardiovascular development and disease (Table 2).

Class I HDACs in Heart Development and Disease

Class I HDAC proteins, consisting of HDAC1, 2, 3, and 8, are expressed ubiquitously and localized predominantly to the nucleus of the cell. HDAC1 and HDAC2 are generally found together in repressive complexes such as Swi-independent 3 (Sin3), NuRD (nucleosome remodeling and deacetylase), CoREST (corepressor of RE1-silencing transcription factor), and PRC2 (polycomb repressive complex 2) complexes.5,69,80 HDAC3, on the other hand, associates with 2 homologous transcriptional corepressors, N-CoR and SMRT, which stimulate its deacetylase activity.76 No complex has been described for HDAC8.69

HDAC1

Hdac1-null mice die in utero before E9.5 with general growth retardation.184 These embryos exhibit reduced cell proliferation and increased expression of Cdk inhibitor, including p21 and p27. The p21 and p27 promoters of the Hdac1-null embryonic stem cells show modest hyperacetylation of histone 3 and 4, suggesting that Hdac1 may normally deacetylate histones on these promoters to repress p21 and p27 expression, thereby promoting cell proliferation. However, tissue-specific deletion of Hdac1 in the myocardium of mice results in no apparent phenotype,181 suggesting that Hdac1 may function redundantly with other Hdac proteins, such as Hdac2, to regulate heart development.

HDAC2

Hdac2 is expressed in the hearts of mouse embryos.31 Germline deletion of Hdac2 in mice results in a variety of cardiac defects, and these Hdac2-null mice die within the first 24 hours after birth.31 In contrast, another line of Hdac2-deficient mice, generated from a gene-trap embryonic stem cell line, exhibits partial perinatal lethality; the surviving mutant mice, when examined at 2 months of age, appear grossly normal.31 Although the exact cause of their difference is not clear, both mouse lines provide insights into the roles of
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<tr>
<th>Class</th>
<th>Chromatin-Modifying Factor</th>
<th>Tissue of Gene Modification</th>
<th>Phenotype</th>
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<tr>
<td><strong>HDAC1</strong></td>
<td>Germline deletion</td>
<td>Lethality before E9.5; general growth retardation</td>
<td>Hdac1 suppresses Cdk inhibitor p21 and p27 to promote cell proliferation</td>
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<tr>
<td></td>
<td>Deletion in myocardium</td>
<td>No apparent cardiac defects</td>
<td>Hdac1 functions redundantly with Hdac2 in the myocardium</td>
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<td><strong>HDAC2</strong></td>
<td>Germline deletion</td>
<td>Lethality at birth; thickened myocardium, thickened IVS, hypertrophobulation</td>
<td>Hdac2-Hop inhibits cardiomyocyte proliferation by suppressing SRF- and Gata4-dependent gene expression</td>
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<td>Surviving adult mice are resistant to hypertrophy</td>
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<td>Hdac2 suppresses Inpp5f, which inhibits the prohypertrophic Akt/Gsk3β pathway</td>
<td>31, 93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overexpression in myocardium</td>
<td>Cardiac hypertrophy</td>
<td>Hdac2 overexpression activates the Akt/Gsk3β pathway</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deletion in myocardium</td>
<td>No apparent cardiac defects</td>
<td>Hdac2 functions redundantly with Hdac1 in the myocardium</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deletion of Hdac1 and Hdac2 in myocardium</td>
<td>Lethality within 2 weeks after birth; arrhythmias, dilated cardiomyopathy</td>
<td>Hdac1 and Hdac2 interact with REST to repress fetal genes involved in calcium handling and contractility</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td><strong>HDAC3</strong></td>
<td>Germline deletion</td>
<td>Lethality at E9.5 attributable to gastrulation defects</td>
<td>Hdac3 is required for gastrulation</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overexpression</td>
<td>Cardiac hyperplasia without hypertrophy</td>
<td>Hdac3 suppresses several Cdk inhibitors to promote cardiomyocyte proliferation</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deletion in myocardium</td>
<td>Lethality at 3–4 months after birth; cardiac hypertrophy, fibrosis, abnormal fatty acid metabolism, lipid accumulation in heart muscle</td>
<td>Hdac3 suppresses PPARα activity on the promoters of genes involved in metabolic regulation</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td><strong>Class II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HDAC5 and HDAC9</strong></td>
<td>Germline deletion</td>
<td>Single Hdac5- or Hdac9-null mutation causes no apparent cardiac phenotype; double Hdac5 and Hdac9 mutations cause lethality starting at E15.5, frequent VSD, and occasional thin myocardium</td>
<td>Hdac5/9 may suppress the transcriptional activity of Mef2, SRF, or myocardin involved in cardiac growth regulation</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hdac5- or Hdac9-null mice show enhanced hypertrophic response to cardiac stress and display protection of female hearts from ischemia injury</td>
<td></td>
<td>97, 98, 99, 104</td>
<td></td>
</tr>
<tr>
<td><strong>HDAC7</strong></td>
<td>Germline deletion</td>
<td>Lethality at E11.5, severe hemorrhage from leaky and dilated blood vessels</td>
<td>Hdac7 suppresses Mmp10 in the endothelium to maintain the integrity of vessel wall</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td><strong>Class III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SIRT1</strong></td>
<td>Germline deletion</td>
<td>Lethality at birth in an inbred genetic background, ASD, VSD, heart valve defects</td>
<td>Sirt1 deacetylates p53, preventing p53 from triggering cell apoptosis after DNA damage and stress</td>
<td>110, 111, 113</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overexpression in myocardium</td>
<td>Low/moderate Sirt1 overexpression reduces cardiac hypertrophy and apoptosis; high Sirt1 overexpression triggers cardiac hypertrophy and apoptosis</td>
<td>Sirt1 expression is activated by cardiac stress, and it regulates the stress response in a dose-dependent manner</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td><strong>SIRT3</strong></td>
<td>Germline deletion</td>
<td>Cardiac hypertrophy and interstitial fibrosis at 2 months of age</td>
<td>Sirt3 inhibits apoptosis through activating Ku70-Bax interaction and inhibiting the proapoptotic activity of Bax</td>
<td>118, 120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overexpression in myocardium</td>
<td>Resistant to stress-induced cardiac hypertrophy</td>
<td>Sirt3 activates Foxo3a-dependent antioxidant genes and attenuates the pro-hypertrophic RAS, MAPK/ERK, and PI3K/Akt pathways</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td><strong>SIRT7</strong></td>
<td>Germline deletion</td>
<td>Shortened lifespan, extensive cardiac fibrosis, hypertrophy, and inflammatory cardiomyopathy</td>
<td>Sirt7 deacetylates p53 and protects cardiomyocytes from stress-induced apoptosis</td>
<td>122</td>
<td></td>
</tr>
</tbody>
</table>

IVS indicates interventricular septum; ASD, atrial septal defect; VSD, ventricular septal defect.
Hdac2 in heart development and disease. Cardiac malformations in these mice include occlusion of the right ventricle, thickened interventricular septum, thickened compact myocardi-um, and reduced ventricular trabeculation. These defects are possibly caused by a combination of regional increase of cardiomyocyte proliferation and apoptosis. Hdac2-mediated cardiomyocyte growth may require the presence of a homeodomain-only protein Hop, a regulator of cardiomyocyte proliferation. Disruption of Hop in mice results in hyperproliferation of the developing cardiomyocytes. Hop associates with Hdac2 to inhibit the serum response factor–dependent gene expression involved in cardiomyocyte proliferation and differentiation. Also, this Hop-Hdac2 complex deacetylates Gata4, reduces Gata4 transcrip-tional activity, and downregulates Gata4-controlled cell cycle genes, thereby inhibiting cardiomyocyte proliferation. Mice with Hop and Hdac2 double mutations exhibit perinatal lethality with hyperacetylation of Gata4, upregulation of Gata4 target genes, and a marked increase in cardiomyocyte proliferation. These studies support that Hdac2 interacts with Hop to suppress cardiomyocyte growth.

Within the myocardium, Hdac2 functions redundantly with Hdac1 to regulate cardiac gene expression and cardiomyocyte differentiation. Mice lacking either Hdac1 or Hdac2 in the myocardium have no apparent cardiac defects and survive to adulthood. However, mice lacking both Hdac genes in the myocardium die within 2 weeks after birth because of cardiac arrhythmias and dilated cardiomyopathy. These abnormalities are accompanied by an upregulation of genes that encode fetal calcium channels and skeletal muscle–specific contractile proteins. These fetal genes, including hyperpolarization-activated nonselective cation current (Ih), the T-type Ca2+ current (Ica,T), and α-skeletal actin (α-SA), are transcriptionally repressed in the normal adult myocardium by the RE1-silencing transcription factor (REST) (also known as the neuron-restrictive silencer factor [NRSF]) through the re-recruitment of class I and class IIa HDACs. Similar to mice lacking myocardial Hdac1 and Hdac2, mice that overexpress a dominant-negative form of REST develop dilated cardiomyopathy, ventricular arrhythmias, and sudden cardiac death. Therefore, the combined loss of Hdac1 and Hdac2 may result in the failure of REST to repress fetal genes involved in calcium handling and contractility, thereby causing cardiac arrhythmias and cardiomyopathy. Those Hdac2-deficient mice that survive to adulthood without cardiomyopathy are resistant to hypertrophic stimulation. Conversely, mice overexpressing Hdac2 display augmented cardiac hypertrophy. In the mouse heart, Hdac2 suppresses the expression of Inpp5f (inositol polyphosphate-5-phosphatase f), a negative regulator of the prohypertrophic PI3K-Akt-Gsk3β pathway. By reducing phosphorylation of the Akt kinase and glycogen synthase kinase 3β (Gsk3β), Inpp5f activates Gsk3β to suppress several prohypertrophic pathways, thereby inhibiting cardiac hypertrophy. Without Inpp5f, mice are susceptible to stress-induced cardiac hypertrophy. Therefore, the resistance of Hdac2-deficient mice to hypertrophy can be explained by an increase of Inpp5f and Gsk3β activity in these mice. Furthermore, unlike Hdac2, overexpression of Hdac1 or Hdac3 in mice do not cause cardiac hypertrophy, suggesting that among class I proteins, Hdac2 is the major Hdac that modulates Gsk3β activity to regulate cardiac hypertrophy.

HDAC3
Hdac3 overexpression in cardiomyocytes produces cardiac hyperplasia without hypertrophy, resulting in thickening of ventricular myocardium and obstruction of ventricular cavities in newborn mice. Hdac3 suppresses the expression of several Cdk inhibitors, such as Cdkn1 and Cdkn2, to promote cardiomyocyte proliferation. Therefore, Hdac3 is a regulator of cardiomyocyte growth during development.

Germline deletion of Hdac3 in mice results in embryonic lethality by E9.5 because of defects in gastrulation. In contrast, mice with cardiomyocyte-restricted deletion of Hdac3 survive until 3 to 4 months of age, at which point, they develop massive cardiac hypertrophy and fibrosis. These pathological changes are accompanied by an upregulation of genes that control fatty acid metabolism and by a downregulation of genes that govern glucose utilization. The gene changes result in lipid accumulation in the heart muscle. ChIP analyses of rat myocytes indicate that Hdac3 and the nuclear hormone receptor PPARα form a protein complex on the promoters of many genes involved in metabolic regulation. The absence of Hdac3 counterbalance in Hdac3-null hearts may thus cause an excessive PPARα activity on those gene promoters, leading to metabolic derangements and lipid accumulation in the heart. Interestingly, mice with deletion of Hdac1, Hdac2, or other Hdac genes do not display such metabolic abnormalities, indicating an unique role of Hdac3 in myocardial energy metabolism.

Class II HDACs in Heart Development and Disease
Class II HDACs are subdivided into class IIa (HDAC4, 5, 7, and 9) and class IIb (HDAC6 and 10) proteins. Class IIa HDACs contain large N-terminal domains with conserved binding sites for Mef2 (transcription factor) and 14-3-3 (chaperone protein). Although most class I HDACs are subunits of multiprotein nuclear complexes crucial for gene repression and epigenetic landscaping, class IIa HDACs regulate cytoplasmic processes or transduce signals between the cytoplasm and the nucleus. Furthermore, unlike class I HDACs, class II HDACs have relatively restricted pattern of expression. HDAC5 and HDAC9 are highly expressed in muscle, brain, and heart, while HDAC7 is present in thymocytes and endothelial cells.

HDAC7
Germline deletion of Hdac7 in mice causes embryonic lethality at E11.5, and embryos die from severe hemorrhage caused by vascular defects. The dorsal aortae and cardinal veins of Hdac7-null mice are dilated, leaky, and surrounded by fewer smooth muscle cells. Hdac7 normally interacts with Mef2 in the endothelium to repress Mmp10 expression. When Hdac7 is absent, Mmp10 is derepressed in the endothelium, releasing this proteinase to damage the blood vessel wall. Concurrent with Mmp10 upregulation, the tissue inhibitor of metalloproteinase 1 (Timp1) is downregulated in Hdac7-null endothelial cells, further enhancing Mmp10 ac-
tivity and exacerbating vascular destruction. Therefore, Hdac7 is essential for maintaining the integrity of vessel wall during development.

**HDAC5 and HDAC9**
Hdac5 and Hdac9 are functionally redundant during heart development. Single Hdac5 or Hdac9 knockout mice survive to adulthood without apparent cardiac defects. In contrast, double Hdac5 and Hdac9 mutations cause lethality starting at E15.5 with multifocal hemorrhages in embryos. Most of the double mutants exhibit ventricular septal defects; some have thin myocardium. Only a few double-mutant mice survive to postnatal day 7 or adulthood. These cardiac defects may result from the removal of Hdac suppression of Mef2, serum response factor, myocardin, or CAMTA2 transcriptional activity that regulates cardiac growth.

Hdac5- and Hdac9-null mice display enhanced hypertrophic response to calcineurin activation and pressure overload. Cardiac stress triggers the phosphorylation of class IIa HDACs by calcium/calmodulin-dependent protein kinase (CaMK) and protein kinase D (PKD). Phosphorylated HDACs bind 14-3-3 and translocate from the nucleus to the cytoplasm. This process dissociates HDACs from Mef2c to a transcriptional activator and thus enabling Mef2c to activate prohypertrophic genes. Furthermore, the absence of nuclear HDACs removes the suppression of calmodulin-binding transcription activator 2 (CAMTA2), whose hyperactivity triggers cardiac hypertrophy. Therefore, by removing the suppression of Mef2 and CAMTA2, the loss of Hdac5 or H dac9 augments the stress-induced cardiac hypertrophy.

Deletion of Hdac5 or Hdac9 reduces the maladaptive remodeling after myocardial infarction in female mice. This protective effect is attributed in part to an enhanced expression of vascular endothelial growth factor-a (Vegf-a), which promotes neoangiogenesis with consequent reduction of myocardial infarction. Hdac proteins normally suppress the transcriptional activity of Mef2 and estrogen receptor α (ERα). The loss of H dac5 or Hdac9 triggers Mef2 to activate its target gene ERα. In the presence of estrogen (E2), ERα then activates Vegf-a expression. Therefore, this ERα-dependent transcriptional cascade, released by the loss of H dac5 or H dac9, may account for the gender-specific cardiac protection.

**HDAC4, HDAC6, and HDAC10**
HDAC4 seems unnecessary for cardiovascular development. HDAC4 controls chondrocyte hypertrophy through interacting with Runx2 (Runt related transcription factor 2) and Mef2c during the formation of the skeleton. No cardiac abnormalities have been reported. HDAC6 also seems dispensable for cardiovascular development as H dac6-null animals develop normally and grow to adulthood, although with some minor immunologic response differences. Little is known about the functions of HDAC10.

**Class III HDACs in Heart Development and Disease**
Members of the class III HDACs are homologous to the yeast transcriptional repressor Sir2p and have no sequence homology to class I and II HDACs. Class III HDACs, also called sirtuins, require NAD+ (nicotinamide adenine dinucleotide) for deacetylation. To date, 7 mammalian homologs have been identified, SIRT1 to -7. Mammalian sirtuins have diverse cellular locations: SIRT1 and SIRT2 are present in both the nucleus and the cytoplasm, whereas SIRT3, SIRT4, and SIRT5 are localized in the mitochondria, and SIRT6 and SIRT7 are nuclear proteins. A key function of the class III HDACs is their regulation of transcriptional repression.

**SIRT1**
Sir1 knockout mice, when generated in an inbred genetic background, die perinatally with neurological and cardiac malformations, which include atrial septal, ventricular septal, and heart valve defects. However, in outbred backgrounds, Sir1 deletion produces viable mice with diverse phenotypes, such as imperfect gametogenesis and autoimmune disease. Sir1 is known to deacetylate p53, preventing p53 from triggering cellular senescence and apoptosis in response to DNA damage and stress. Sir1-deficient cells exhibit hyperacetylation of p53 and increased cell death after DNA damage. Furthermore, Sir1 induction by sibulin protects rat neonatal cardiomyocytes from isoproterenol-induced injury and cell death. In adult mouse hearts, Sir1 increases by 4- to 8-fold in response to pressure overload and oxidative stress. Cardiomyocyte-specific overexpression of Sir1 in mice shows that a low-to-moderate increase of Sir1 (2.5- to 7.5-fold) protects mice from age-dependent cardiac hypertrophy, apoptosis, and fibrosis. In contrast, a higher level of Sir1 (≈13-fold) induces cardiac hypertrophy and apoptosis, ultimately leading to cardiomyopathy. These studies suggest a narrow window of optimal Sir1 activity in the regulation of cardiac responses to stress.

**SIRT2**
Sirt2 is a negative regulator of stress tolerance; knockdown or inhibition of Sirt2 enhances the survival of cells under stress. Sirt2 knockdown reduces anoxia–reoxygenation injury in H9c2 rat cardiac cells. Its knockdown induces expression of the chaperone protein 14-3-3 ζ, which sequesters the proapoptotic Bad (Bcl2 antagonist of cell death) protein in the cytoplasm and thus reduces mitochondrial Bad. This prevents Bad proteins from inhibiting the antiapoptotic activities of Bcl-XL or Bcl-2 in the mitochondria. Therefore, Sirt2 may control 14-3-3 ζ and Bad to regulate cardiac stress response.

**SIRT3**
Sirt3 is a stress-responsive deacetylase in cardiomyocytes. In cultured cardiomyocytes, Sirt3 is induced by genotoxic or oxidative stress, and it protects myocytes from cell death caused by such stress. Induced Sirt3 deacetylates Ku70 and enhances the binding of Ku70 to the proapoptotic Bax (Bcl2-associated X protein), thereby preventing the entry of Bax proteins into the mitochondria to trigger apoptosis. Although Sirt3-deficient mice are born grossly normal, they develop cardiac hypertrophy and interstitial fibrosis at 8 weeks of age. In addition, when exposed to hypertrophic stimuli, these Sirt3-deficient mice develop severe cardiac hypertrophy, whereas mice overexpressing Sirt3 in the myo-
cardiomyocytes are resistant to hypertrophy from similar stimuli. Therefore, Sirt3 possibly protects cardiomyocytes from stress-induced damage by activating the Foxo3a-dependent expression of antioxidant genes and by attenuating the RAS, MAPK/ERK, and PI3K-Akt pathways.

**SIRT4, SIRT5, and SIRT6**

Sirt4 and Sirt5-deficient mice are born at a Mendelian ratio and appear grossly healthy. No cardiac defects have been reported. Sirt6-deficient mice appear runted with lymphopenia, loss of subcutaneous fat, lordokyphosis, and severe metabolic defects. These mice die at ∼4 weeks, but no cardiac defects have been reported.

**SIRT7**

Sirt7 deletion in mice leads to various pathological changes in adult hearts. Sirt7-deficient animals have shortened lifespan and develop cardiac hypertrophy, extensive cardiac fibrosis, and inflammatory cardiomyopathy. Sirt7 interacts with p53 and efficiently deacetylates p53 in neonatal rat cardiomyocytes, and Sirt7-null hearts exhibit p53 hyperacetylation with increased cell apoptosis. Also, Sirt7-deficient primary cardiomyocytes show increased apoptosis after oxidative or genotoxic stress. These results suggest a critical role of Sirt7 in the regulation of cardiac stress responses and cell death.

**The Histone Acetyltransferases**

Actions by histone deacetylases are counteracted by histone acetyltransferases (HATs), which add acetyl groups to histone tails, resulting in decrease of histone–histone and histone–DNA interaction, thereby loosening the chromatin and increasing transcriptional activation. Both HDACs and HATs are known to regulate heart development and cardiac hypertrophy, suggesting that the balance of acetylation and deacetylation of histones is essential for the heart to respond to developmental and hypertrophic cues (Table 3).

One well-defined member of the HATs, p300, is required for heart muscle development and hypertrophy. p300 is broadly expressed by the developing embryo by E7.5, and p300-null embryos die between E9.5 and E11.5 with defects in neural and heart development. Embryos lacking p300 display poorly vascularized yolk sac and severe pericardial effusion. The mutant heart has thin myocardium and diminished trabeculation at E10.5 and expresses lower levels of /H9251 actinin. The thinning of myocardium is possibly caused by diminished myocardial cell proliferation because p300-null embryos have generalized reduction in cell proliferation, and p300-null embryonic fibroblasts stop dividing after 3 to 4 cell generations. Another mouse model of p300 mutation, in which a single amino acid mutation ablates acetyltransferase activity of p300, shows multiple defects in heart development, although the embryos die a little later, between E12.5 and E15.5. These embryos display a variety of cardiac malformations, including thinning of the compact myocardium, ventricular and atrial septal defects, and reduced coronary vessels.

In rat neonatal cardiomyocytes, the expression of p300 is induced by phenylephrine, and its acetyltransferase activity is required for the phenylephrine-induced cardiac hypertrophy. p300 acts as a coactivator of the prohypertrophic transcription factor Gata4. It acetylates Gata4 and enhances the DNA binding activity of Gata4. Transgenic overexpression of p300 in the heart results in acetylation of Gata4 and increased expression of Gata4-dependent cardiac genes such as endothelin-1. These p300-overexpressing mice develop left ventricular (LV) hypertrophy, dilatation, and systolic dysfunction. They also have more severe progression of LV dilation and reduction of systolic function after myocardial infarction in adult mice. In contrast, mice overexpressing p300 that lacks the acetyltransferase activity do not have such severe progression of LV dysfunction after infarction. Pharmacological inhibition of the acetyltransferase activity of p300/CBP by curcumin also prevents cardiac hypertrophy and LV deterioration in rodent models of hypertrophy and infarction.

**Histone Methylation and Heart Development**

**JUMONJI**

Methylation of histones at arginine and lysine residues offers another avenue of epigenetic control. Junonji (jmj) (or Jarid2) belongs to a family of histone demethylases containing the conserved Jumonji C (JmjC) domain (see elsewhere for a review). Mice heterozygous of jmj mutation are normal, whereas homozygous knockouts have various degrees of lethality and embryonic defects according to the genetic background. In 1 strain, the jmj homozygous mutant animals survive to postnatal day 0, with ventricular septal defects, ventricular noncompaction, and double outlet of the right ventricle. On the other hand, in the C3H/He background, embryos die between E11.5 and E12.5 and display defects in neurulation with abnormalities in the right heart and hyperplasia of the trabecular myocardium, resulting in obstruction of the ventricle. The hyperproliferation is apparently attributed to overexpression of cyclin D1, which is normally repressed by Jmj. Although Jmj contains the JmjC domain, it lacks conserved residues for binding iron cofactors, suggesting that Jmj may not have demethylase activity. Instead, the Jmj complex has methyltransferase activity; it recruits G9a and GLP methyltransferases to methylate H3K9 and repress cyclin D1 promoter. Also, Jmj recruits PRC2 (polycomb repressive complex 2) to many target genes, where PRC2 represses transcription by methylating histone 3 lysine 27 (H3K27) through the Ezh2 methyltransferase. Besides cyclin D1, Jmj is known to repress several other genes. Jmj associates with Gata4 and Nkx2.5 to repress Nppa (atrial natriuretic factor), inhibits MeF2a to repress α-MHC, and interacts with retinoblastoma (Rb) proteins to repress gene expression. It is possible that Jmj also silences these genes by recruiting histone methyltransferases. These studies suggest that
Jumonji may associate with other histone-modifying factors to regulate gene expression essential for heart development.

**SMYD**

SMYD proteins belong to a family of histone methyltransferases that contain conserved SET domains necessary for methyltransferase activity and MYND domain required for transcriptional repression by interaction with HDAC. At least 2 members of the Smyd family are studied in heart development. Smyd1 is expressed in adult cardiac and skeletal muscle, as well as in embryonic hearts. Targeted deletion of Smyd1 in mice yields embryonic lethality caused by cardiac enlargement. Smyd1-null animals have hypoplastic right ventricle with little trabeculation. Mutant cardiomyocytes downregulate Hand2, a gene essential for right ventricle development.

**Wolf–Hirschhorn Syndrome Candidate 1 (WHSC1)**

Patients with Wolf–Hirschhorn syndrome (WHS) display a range of defects, including mental retardation, epilepsy, craniofacial abnormalities, and cardiac septal defects. WHS patients have regional deletion of the short arm of chromosome 4 (4p16.3), termed Wolf–Hirschhorn critical region.

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### Table 3. Roles of HAT, Histone Methylation, and PARP in Cardiovascular Development and Disease

<table>
<thead>
<tr>
<th>Class</th>
<th>Chromatin-Modifying Factor</th>
<th>Tissue of Gene Modification</th>
<th>Phenotype</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAT</td>
<td>p300</td>
<td>Germline deletion</td>
<td>Lethality at E9.5 to E11.5; thin myocardium and diminished trabeculation</td>
<td>p300-null embryos have generalized reduction in cell proliferation</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td>Point mutation that ablates p300 acetyltransferase activity</td>
<td>Lethality at E12.5 to E15.5; thin compact myocardium, ASD, VSD reduced coronary vessels</td>
<td>The acetyltransferase activity of p300 is required for heart development</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overexpression in myocardium</td>
<td>Cardiac hypertrophy, dilatation, and systolic dysfunction</td>
<td>p300 is a coactivator of the prohypertrophic Gata4; it acetylates Gata4 to enhance the DNA binding of Gata4 and transcriptional activity</td>
<td>125, 127</td>
<td></td>
</tr>
<tr>
<td>Histone Methylation</td>
<td>JUMONJI</td>
<td>Germline deletion</td>
<td>Variable defects: VSD, noncompaction, double outlets of right ventricle, trabecular hyperplasia</td>
<td>Jumonji represses cyclin D1 to inhibit myocardial proliferation</td>
<td>132, 133</td>
</tr>
<tr>
<td></td>
<td>SMYD1</td>
<td>Germline deletion</td>
<td>Malformed right ventricle with little trabeculation</td>
<td>Smyd1 regulates Hand2, which is essential for right ventricle development</td>
<td>146, 147</td>
</tr>
<tr>
<td></td>
<td>Knockdown in zebrafish</td>
<td>Disrupted myofibril formation, absent cardiac contraction</td>
<td>Smyd1 is required for myofibril organization</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WHSC1</td>
<td>Germline deletion</td>
<td>Lethality within 10 days after birth, growth retardation, defective bone formation, ASD, VSD</td>
<td>Whsc1 associates with Nkx2.5 to repress gene transcription and to regulate cardiac septal formation</td>
<td>154</td>
</tr>
<tr>
<td>PARP</td>
<td>PARP-1 and PARP-2</td>
<td>Germline deletion</td>
<td>Parp-1–null and Parp-2–null mice are viable; Parp-1 and Parp-2 double knockout mice die at E7 to E8</td>
<td>PARP activity is required for Brg1 to repress α-MHC and activate β-MHC in embryonic hearts</td>
<td>17, 166</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Parp-1–null mutation or inhibition of PARP activity in mice reduces stress-induced cardiac hypertrophy, heart failure, ischemia/reperfusion injury, and infarction</td>
<td>PARP-1 interacts with HDACs and Brg1 to control cardiac hypertrophy and the pathological MHC switch in hypertrophic hearts; PARP also regulates stress-responsive signaling pathways</td>
<td>17, 28, 33, 167–174</td>
</tr>
</tbody>
</table>

ASD indicates atrial septal defect; VSD, ventricular septal defect.
The Poly(ADP-ribose) Polymerases
The PARPs catalyze the transfer of ADP-ribose units from NAD$^+$ to the carboxyl group of glutamic acid, aspartic acid, or lysine residues of acceptor proteins (see elsewhere for a review). PARP-1, the most extensively studied of the 17-member PARP family, is a nuclear enzyme known to respond to DNA damage and facilitate DNA repair. Once activated, PARP-1 transfers 50 to 200 molecules of ADP-ribose to a variety of nuclear proteins, including PARP-1 itself and histone H1 and H2B tails.156–157 This poly(ADP-ribosylation) (PAR) results in the release of PARP-1 from DNA, modification of histones, and relaxation of the chromatin superstructure.158–160 These local chromatin changes facilitate the recruitment and access of repair machinery to the site of DNA damage. Besides DNA repair, PARP-1 modulates chromatin to control the transcriptional machinery in response to various stimuli. Signals, such as steroids or heat shock, induce PARP-1 activation and the PAR-dependent stripping of histones from chromatin, thereby favoring the opening of chromatin to allow transcriptional regulation.160,161 PARP-1 is present at transcriptionally repressed chromatin regions in the fruit fly,161 and biochemical studies have shown that its activation and subsequent dissociation from the chromatin is a prerequisite for transcriptional activity to occur.160

Although PARP activity is required for the maintenance of genome integrity,162–165 Parp-1–null mice are viable without apparent cardiovascular defects; Parp-1 and Parp-2 double knockout mice die at early embryogenesis before E7 to E8 (Table 3).166 These phenotypes suggest redundant functions of Parp-1 and Parp-2 in early mouse embryogenesis.166 A recent study shows that PARP activity is essential for maintaining fetal MHC expression in embryonic hearts.17 Mouse embryos treated with a chemical inhibitor that blocks PARP activity exhibit increased $\alpha$-MHC and reduced $\beta$-MHC expression. In embryos, Parp-1 associates with Brd1 and/or Hdac proteins on the cardiac MHC promoters to repress $\alpha$-MHC and activate $\beta$-MHC expression.17 This fetal mechanism of MHC control, although silenced in normal adult hearts, is reactivated by cardiac stress to trigger the pathological switch from adult $\alpha$-MHC to fetal $\beta$-MHC, a crucial step in the myopathic process of stressed adult hearts.17,22

PARP-1 is activated in cardiac hypertrophy, and its activity increases further in the failing hearts of mice and humans.33,167,168 Null mutation of Parp-1 in mice or pharmacological inhibition of PARP activity reduces cardiac hypertrophy stimulated by angiotensin II33 or pressure overload,167,169 delays the progression from hypertensive cardiomyopathy to heart failure,26 diminishes myocardial ischemia/reperfusion injury,170,171 and decreases cell death and heart failure after myocardial infarction.172,173

Besides MHC regulation, PARP-1 contributes to cardiac hypertrophy and failure in a NAD$^+$– and Sirt1-dependent manner.33,169 The PARP-mediated cell death in stressed hearts appears to be caspase 3–independent168 and caused at least in part by a depletion of NAD$^+$169 and mitochondria-to-nucleus translocation of an AIF (apoptosis-inducing factor).167,173,174 The PARP-induced depletion of NAD$^+$ and accumulation of nicotinamide reduce Sirt1 deacetylase activity,169,175 which may lead to hyperacetylation and activation of the proapoptotic factor p53,110,113 and cell death. Also, the newly translocated AIF fragments the chromatin and condenses the nucleus, resulting in cell death. Experiments using chemical inhibitors of PARP suggest that PARP regulates several stress-responsive signaling pathways, including ERK1/2, p38 MAP kinase, JNK, and PI3K-Akt-Gsk3$\beta$ pathways, to control cardiac responses to hypertrophic stimuli, ischemia/reperfusion, and infarction.28,172,176,177 Furthermore, through its interaction with TEF1, Brd1, and HDACs,17,178,179 PARP-1 controls the expression of cardiac-specific genes, including troponin T and MHC, thereby contributing to pathological gene expression in stressed hearts. Interestingly, under inflammatory stimuli, PARP-1 proteins are acetylated and deacylated by p300/CBP and class-I HDACs,180 respectively, raising the possibility that p300/CBP and HDACs may also contribute to the PARP-1–mediated cardiac hypertrophy and failure.

Discussion
Target Specificities of Chromatin Regulating Factors
This review summarizes the cardiovascular functions of BRG1/BAF, ISWI, CHD, INO80, HDAC, SIRT, HAT, histone methyltransferase, and PARP. These families of chromatin regulators can form large protein complexes with one another to modify chromatin structure and interact with various transcription factors to control gene expression (Figure, B). For example, BRG1/BAF forms a complex with HDAC and PARP proteins to regulate MHC in both developing and hypertrophic hearts. BRG1 associates with CHD7 to regulate Twist and Slug in neural crest cells, essential for cardiac outflow tract development. Given the large families
of BAF, HDAC, PARP, and CHD, a combinatorial assembly of epigenetic complexes may provide a unique protein composition that enables specific targeting of cardiac gene loci under different pathophysiological conditions. Furthermore, the stoichiometry of chromatin regulators may modulate transcriptional activities, adding another layer of gene expression control. Brg1 interacts dose-dependently with Nkx2.5, Tbx5, and Tbx20 to control heart development. The gene dosage of Chd7 and Tbx1 is critical during aortic arch formation. Wtsc1 synergizes with Nkx2.5 to direct cardiac septal formation. Therefore, both the quantity and composition of chromatin-regulating complexes are critical for cardiac gene expression.

How the genomic loci are marked and present themselves to chromatin regulators is not fully understood. Histone modifications may represent one means to mark the epigenetic landscape and form “histone codes” on the genome (reviewed elsewhere181). A large number of enzymes can covalently add or remove chemical groups at specific residues of histone tails. These marks can act as beacons or docking sites recognized by proteins such as transcription factors or other chromatin remodelers, thereby introducing target gene specificity and additional regulatory control. Modification of specific residues of a histone tail can change histone–DNA contacts, modulate modification of other residues on the same or neighboring histone tail, or alter the binding affinity of other modified histone residues to chromatin remodelers.46,182,183 These combined effects may create a specific epigenetic scene or histone code for chromatin remodelers to recognize. On the other hand, chromatin remodelers may change the conformation and position of nucleosomes to produce an epigenetic architecture for histone-modifying enzymes to identify and modify specific histone residues. Further investigations are needed to fully define how chromatin regulators interact with each other and with transcription factors to target to specific genomic loci.

Chromatin Link Between Fetal Heart Development and Adult Heart Disease

The epigenetic landscape of cardiomyocytes and other cell types of the heart undergoes profound changes as the heart goes through development, maturation, and disease. Chromatin regulation provides a mechanism underlying the transcriptional reprogramming and fetal gene activation in diseased hearts. Studies have suggested that many chromatin regulators are required to suppress fetal gene expression in postnatal hearts. For instance, Hdac1 and Hdac2 suppress fetal calcium channel and contractile protein gene expression as cardiomyocytes mature in neonatal mice. Loss of Hdac1 and Hdac2 causes an ectopic expression of those fetal genes, leading to severe cardiac arrhythmias and dilated cardiomyopathy. On the other hand, chromatin remodelers can be induced by cardiac stress and promote reactivation of fetal genes in a diseased adult heart. For example, stressed adult hearts reactivate the expression of a fetal gene Brg1, which then associates with its embryonic partners, HDAC and PARP, to form an epigenetic complex that triggers fetal MHC expression, cardiac hypertrophy, and fibrosis. Prevention of such stress-induced Brg1 activation reverses pathological MHC expression, diminishes hypertrophy, and abolishes cardiac fibrosis. These studies thus suggest a link at the chromatin level between fetal hearts and myopathic hearts. Therefore, targeting chromatin regulation may provide a promising approach to prevent and possibly reverse the myopathic process. To realize that goal, further studies are necessary to determine the dynamic changes of chromatin architecture, genomic specificities of chromatin regulators, and interactions among chromatin regulators under different pathophysiological conditions of the heart.

Chromatin Regulation and Extracellular Matrix Control

Besides cell-autonomously regulating cardiac growth and differentiation, chromatin regulators can indirectly control cardiovascular development by modulating the extracellular matrix environment. Within the blood vessel, endothelial Hdac7 suppresses a matrix metalloproteinase, Mmp10, to maintain the matrix environment and integrity of subjacent vessel wall, thereby preventing aneurysmal dilation and leakage of developing vessels. In the heart, endocardial Brg1 represses Adams1, which encodes a secreted metalloproteinase that degrades cardiac jelly matrix. This repression of Adams1 allows the establishment of cardiac jelly that promotes myocardial trabeculation. Therefore, chromatin regulators can coordinate the development of adjacent cardiovascular tissues through extracellular matrix. Whether chromatin regulation can also modulate extracellular matrix in adult hearts to synchronize the responses of different cardiac tissues to pathological stress remains to be elucidated.

Nonchromatin Functions of Histone-Modifying Enzymes

Histone modifiers may control cardiac pathophysiology through functions not directly related to their chromatin activities. For instance, by acetylating the transcription factor Gata4, p300 enhances DNA-binding affinity of Gata4 and promotes Gata4-dependent prohypertrophic cardiac gene expression. This aspect of the contribution of p300 to cardiac hypertrophy does not require histone modification by p300. On the other hand, Hdac2 deacetylates Gata4 and downregulates Gata4-activated cell cycle genes, thereby inhibiting cardiomyocyte proliferation. This function of Hdac2 requires its interaction with Hop but not its chromatin activities. Furthermore, Sirt1 and Sirt7 deacetylase p53 to suppress apoptosis and prevent cardiomyopathy. It remains to be determined how such nonchromatin functions interplay with chromatin activities of these histone regulators to control cardiac pathophysiology.

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References


61. Hurd EA, Capers PL, Blauw kump MN, Adams ME, Raphael Y, Poucher Y, Han et al Chromatin in Heart Development and Disease


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