

Epigenetic Mechanisms in Heart Failure Pathogenesis

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Although neurohormonal antagonist and device therapies have improved outcomes in patients with systolic heart failure (HF), residual morbidity and mortality remain high.^{1,2} Novel HF therapeutic approaches thus remain an unmet clinical need of pressing urgency. Such approaches depend, in turn, on keener understanding of the molecular pathways underlying HF pathogenesis. Although a number of novel myocardial signaling effectors have been implicated as drivers of HF pathogenesis,^{1,3} translating these findings into human therapies has remained extremely challenging.

Gene expression profiling studies in animal HF models⁴ and in human failing hearts⁵ consistently demonstrate aberrant gene control in HF. The term epigenetics—a fusion of epigenesis and genetics—was coined ≥ 50 years ago to describe the process of cell fate commitment during development.⁶ Today, the epigenome denotes the totality of sequence-independent processes that modulate cell-state-specific gene expression (eg, post-translational histone or DNA modifications and noncoding RNA/protein complex interactions with chromatin).^{7–10} The epigenome may differ between cell types, drive local formation of higher order chromatin structures, modulate transcription factor (TF) access to DNA, and preserve memory of past transcriptional activities.¹⁰

This review focuses on the chromatin-specific epigenetic regulatory mechanisms that may inform novel therapeutic targets in HF. We specifically highlight examples of chromatin remodeling, biochemical modifications to histones, and integrated features of chromatin-dependent signal transduction that are pertinent to cardiac biology. Other epigenetic pathways in HF, including miRNAs, have been extensively reviewed elsewhere.^{11,12}

Current Concepts in Eukaryotic Gene Control

Eukaryotic cell identity or more broadly, cellular state, is largely governed by precise spatiotemporal coordination of gene expression.⁹ Pathological transformation from a normal to a diseased cardiomyocyte (eg, hypertrophied and hypocontractile) represents a cell state transition driven by defined transcriptional events. Dynamic interplay among accessible DNA sequences, chromatin-binding TFs, and associated DNA/RNA-binding proteins alter local chromatin structure to

orchestrate gene expression programs. To provide a necessary framework for this review, we first briefly summarize some fundamental features of eukaryotic gene regulation^{9,13} (Table I in the Data Supplement).

Chromatin

In the nucleus of every cell, >1 m of linear DNA is densely compacted into chromatin,¹⁴ a dynamic macromolecular complex of DNA, RNA, and diverse proteins (Figure 1). The fundamental primary unit of chromatin is the nucleosome core particle, composed of ≈ 147 bp of double-stranded DNA wrapped in 1.67 left-handed superhelical turns around a histone octamer consisting of 2 copies each of the core histones H2A, H2B, H3, and H4.¹⁵ The histones within nucleosomes can be dynamically modified and exchanged with variants to confer plasticity to primary chromatin structure.¹⁶ Primary chromatin, in turn, is arrayed into dynamic, 3-dimensional, higher order configurations to permit state-specific accessibility of the genome and to allow for efficient DNA recombination and DNA repair.^{17–19} By vastly expanding the signaling repertoire of the primary DNA template, higher order chromatin structures endow metazoans the remarkable ability to generate diverse, highly specialized cell types from a single genome.^{9,20}

TFs and *cis*-Regulatory Elements

As convergence points for multiple pathological signals in the myocardium, DNA-binding TFs have been the subject of intense interest. Accumulating evidence implicates a defined set of master TFs as capable of controlling the selective transcription of genes by Pol II, thereby governing any given cell state.⁹ Gene targeting and transgenic models have clearly demonstrated that activation of specific DNA-binding TFs (eg, nuclear factor of activated T cells, myocyte enhancer factor-2 [MEF2], nuclear factor κ -light-chain-enhancer of activated B cells, GATA binding protein 4, a TF with DNA GATA binding domain [GATA4], and myelocytomatosis viral oncogene, a TF) is critical for pathological cardiac remodeling *in vivo*.^{3,21–25} In addition, several TFs hyperactivated in HF are also key determinants of cardiomyocyte lineage (eg, GATA4 and MEF2).^{26–28}

Received August 22, 2013; accepted May 5, 2014.

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The Data Supplement is available at <http://circheartfailure.ahajournals.org/lookup/suppl/doi:10.1161/CIRCHEARTFAILURE.114.001193/-DC1>.

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(*Circ Heart Fail*. 2014;7:850–863.)

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Circ Heart Fail is available at <http://circheartfailure.ahajournals.org>

DOI: 10.1161/CIRCHEARTFAILURE.114.001193

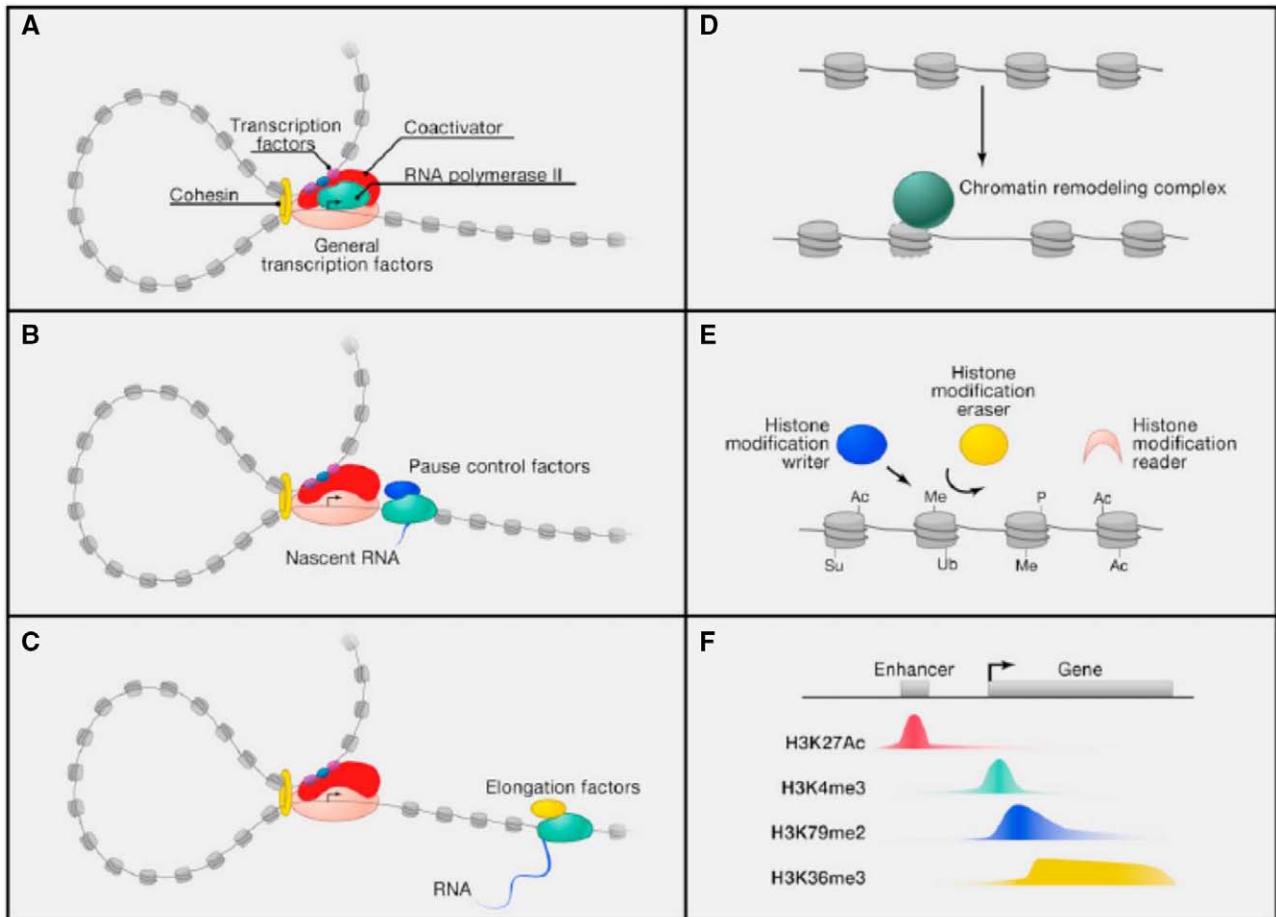


Figure 1. Transcriptional regulation and histone marks. **A**, Preinitiation complex formation. Transcription factors bind to specific DNA elements (enhancers) and to coactivators, which bind to RNA polymerase II (Pol II), which in turn binds to general transcription factors at the transcription start site (arrow). The DNA loop formed between the enhancer and the start site is stabilized by cofactors, such as the mediator complex and cohesin. **B**, Initiation and pausing by Pol II. Pol II begins transcription from the initiation site, but pause control factors cause it to stall some tens of base pairs downstream. **C**, Pause release and elongation. Various transcription factors and cofactors recruit elongation factors such as P positive transcription elongation factor b, which phosphorylate the pause release factors and Pol II, allowing elongation to proceed. **D**, Chromatin structure is regulated by ATP-dependent remodeling complexes that can mobilize the nucleosome, allowing regulators and the transcription apparatus increased access to DNA sequences. **E**, Transcriptional activity is influenced by proteins that modify and bind the histone components of nucleosomes. Some proteins add modifications (writers), some remove modifications (erasers), and other bind via these modifications (readers). Modifications include acetylation (Ac), methylation (Me), phosphorylation (P), sumoylation (Su), and ubiquitylation (Ub). **F**, Histone modifications occur in characteristic patterns associated with different transcriptional activities. Characteristic patterns at actively transcribed genes include H3K27 acetylation (H3K27Ac), H3K4 trimethylation (H3K4me3), H3K79 dimethylation (H3K79me2), and H3K36 trimethylation (H3K36me3). Reprinted from Lee and Young⁹ with permission of the publisher. Legend adapted from figure legend of Lee and Young.⁹ Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation. Copyright © 2013, Elsevier.

TFs typically regulate gene expression by binding regulatory DNA elements called enhancers, an event that recruits cofactors, the general transcriptional machinery, and Pol II complexes to target genes (Figure 1).^{29–31} An active enhancer typically binds multiple TFs in a cooperative fashion and regulates transcription from core promoters often via long-range genomic interactions that involve looping of DNA.^{32,33} In addition, TFs can bind directly to core promoter elements in proximity to transcriptional start sites to recruit transcriptional machinery and regulate gene expression.³⁴

Epigenetic Writers, Erasers, Readers, and Their Marks

A critical mechanism by which enhancer-bound TFs set the stage for gene control is via the recruitment of cofactors that

alter chromatin structure. Two major categories of cofactors are those that mobilize nucleosomes (eg, the ATP-dependent chromatin remodeling complexes [CRCs])¹⁴ and those that enzymatically modify histones (histone modifiers) via post-translational modifications (PTMs; eg, acetylation, methylation, phosphorylation, and ubiquitylation).³⁵ Modifiers that add PTMs or marks to histones (eg, acetyltransferases, methyltransferases, kinases, and ubiquitin ligases) have been dubbed epigenetic writers^{36–41} (Table 1). Conversely, histone modifiers that remove histone PTMs (eg, deacetylases, demethylases, phosphatases, and deubiquitinases) have been dubbed epigenetic erasers. Proteins harboring recognition motifs for specific histone PTMs have been dubbed epigenetic readers, which generally facilitate locus-specific protein complex formation and signal propagation.

Table 1. Common Chromatin Writers, Erasers, and Readers

	Members	Substrates	Comments
Writers* (enzymes)			
Histone acetyltransferases	5 HAT classes	Histone lysine residues; can acetylate numerous nonhistone proteins	Includes p300 and CREB-binding protein (see text)
Histone methyltransferases	KMT1-6 and other members	Histone lysine (eg, H3K9 and H3K36) and arginine residues; can deacetylate numerous nonhistone proteins	Site-dependent variable effects on transcription: H3K4me3 and H3K36me3 typically promote transcriptional activation; H3K9 and H3K27 methylations typically promote transcription repression (see text)
DNA methyltransferases	DNMT1, 3A, and 3B	Cytosine carbon 5, typically at symmetrical CG dinucleotide residues (CpG)	Critical epigenetic regulatory mechanism including expression silencing via CpG promoter and TSS methylation (see text)
Erasers (enzymes)			
Histone deacetylases	18 HDACs divided into 4 HDAC classes I to IV	Acetylated lysine residues	Appealing targets for epigenetic modulators (see text)
Histone demethylases	KDM 1–8 and other members	Histone methylated lysine residues	Site-dependent variable effects on transcription (see histone methylases above and text)
DNA demethylases	TET family dioxygenases, redox-dependent DNA 5-dehydroxymethylases	Methylated cytosine carbon 5 at symmetrical DNA CG dinucleotide residues (CpG)	Critical epigenetic regulatory mechanism including facilitating transcriptional activation via demethylating promoter and TSS (see text)
Reader (domains)			
BRDs	46 diverse types of human BRD containing chromatin-interacting proteins	Acetyl-lysine (eg, H3Kac, H4Kac, H2AKac, and H2BKac). May also function via recognizing Kac on nonhistone proteins	BRDs are found in HATs, CRCs, helicases, methyltransferases, transcriptional coactivators (TRIM/TIF1, TAF), transcriptional mediators, nuclear scaffolding proteins, BET family reader proteins
PHD finger domains	Diverse types of human chromatin regulatory proteins	Histone lysine methylation motifs (eg, H3K4me3, H3K9me2, and H3K9me3), unmodified histones (H3un)	eg, DMT2A-E subfamily contains 24 PHD fingers
CRDs	Diverse types of human chromatin regulatory proteins	Histone lysine methylation motifs (eg, H3K9me3, H3K9me2, H3K27me3, and H3K27me2)	CRDs are found in heterochromatin protein 1, polycomb repressive complexes
MBT domains	Human genes L3MBTL1–4, SCMH1–2, and SFMBT1–2 encode proteins with PHD finger domains	Histone lysine methylation motifs (eg, H3Kme1, H3Kme2, H4Kme1, and H4Kme2)	Linked to E2F/Rb- and PRC mediated repression
HTD domains	HTDs are found in Jmj family demethylases (eg, JMJD2A), p53-binding protein 1	Histone lysine methylation motifs (eg, H3K4me3, H4K20me2, H4K20me3, and H3L36me3)	HTD-containing proteins play roles in Piwi-RNA pathways, RNA metabolism, DNA damage repair, and chromatin remodeling
PWWP domains	Diverse types of human chromatin regulatory proteins	Histone lysine methylation motifs (eg, H3K36me3, H4K20me1, H4K20me2, and H3K79me3)	PWWP domain found in DMT3a

BRD indicates bromodomain; CREB, cAMP-response element binding protein; CRD, chromodomain; DNMT, DNA methyltransferase enzyme; HAT, histone acetyltransferase enzyme; H3K4me3, H3K4 trimethylation; HDAC, histone deacetylase enzyme; PRC, polycomb repressive complex; TET, ten-eleven translocation; and TSS, transcriptional start site. Data derived from Black et al,³⁶ Chi et al,³⁷ Fierz and Muir,³⁸ Filippakopoulos et al,³⁹ Lu and Wang,⁴⁰ and Musselman et al.⁴¹

*Additional histone marks include acylation, phosphorylation, ADP ribosylation, β -N-acetylglucosamine glycosylation, ubiquitylation, and sumoylation.

In aggregate, histone writers, erasers, and readers modify local chromatin structure in a stereotypic fashion¹⁶ that is associated with differential transcriptional activity (Table II in the Data Supplement).^{9,35} For example, H3K27ac marks active enhancers, H3K36me3 marks actively transcribed gene bodies, and H3K27me3 marks heterochromatic or transcriptionally

repressed regions.¹⁰ In addition to histone modifications, DNA itself can be covalently modified via methylation of cytosine, a mark generally associated with transcriptional repression.¹⁰ Collectively, interplay between TFs and dynamic alterations in local chromatin structure allows for genomic signal transduction with spatiotemporal precision.^{9,20}

Control of RNA Polymerase II Dynamics

TFs and recruited coregulatory proteins signal via chromatin to control the initiation, elongation, and termination activities of Pol II.^{42–44} Once recruited to promoters, Pol II forms an initiation complex at the transcriptional start site, transcribes a short distance generating a nascent mRNA of 20 to 50 nucleotides, and then stalls in the proximal gene body (termed promoter-proximal pausing). Physical association of Pol II with pausing factors, such as negative elongation factor and DRB sensitivity inducing factor, inhibit productive transcriptional elongation.⁴² Release of this paused state is mediated by recruitment of protein complexes, such as the positive transcription elongation factor b (P-TEFb, which consists of the kinase CDK9 and the regulatory protein Cyclin T1) and Mediator.⁴⁵ P-TEFb triggers pause release and transcriptional elongation by phosphorylating Pol II (on conserved serine residues in its C-terminal heptapeptide repeat domain) and associated pausing factors.¹³ Control of Pol II pause release and elongation dynamics is increasingly appreciated as a major epigenetic regulatory mechanism of eukaryotic gene control in physiology and disease. P-TEFb activation and Pol II pause release are critically involved in cardiac hypertrophy and HF pathogenesis,^{46,47} as will be discussed in subsequent sections.

Evolving Epigenetic Regulatory Role of Noncoding RNAs

Only 1.22% of human genome DNA encodes for protein-coding exons (ie, coding mRNA) to comprise the known 20 687 protein-coding genes.⁴⁸ Fully 80.4% of the human genome, however, is transcribed at some point in ≥ 1 cell type.⁴⁹ Hence, the majority of the human genome codes for an ever-expanding array of noncoding RNAs (ncRNAs), including ≥ 8801 small RNAs (tRNA, miRNA, snRNA, and snoRNA), 9640 long noncoding RNAs (lncRNAs), and 11 224 pseudogenes.⁵⁰ There are $\geq 399\,124$ enhancer-like regions and 70 292 promoter-like regions in the human genome that support dynamic networks of TFs and other regulatory elements, including ncRNAs. Some ncRNA regulatory mechanisms reported to date include (1) transcriptional silencing or augmentation by interaction with chromatin-associated proteins, (2) interaction with actively transcribed mRNA, (3) direct interaction with DNA itself, (4) nuclear siRNA generation via ncRNA parent fragmentation, (5) cytoplasmic miRNA decoy via acting as competing endogenous RNA, and (6) interference with cytoplasmic mRNA stability by competition for mRNA stabilization factors.⁵¹ It has been suggested that the ability of ncRNA to form complex chemical structures may confer on ncRNA, a functional repertoire exceeding that of the proteome.⁵² The ever-expanding roles of ncRNAs, particularly lncRNAs and miRNAs, are informing a more complex, finely nuanced model of epigenetic regulation.^{53,54}

ATP-Dependent Chromatin Remodeling Complexes in Cardiac Biology

CRCs harness the energy liberated by ATP hydrolysis to disrupt the DNA–nucleosome electrostatic association thereby unwrapping DNA from nucleosomes, shuttling nucleosomes along DNA and exchanging or removing nucleosomes.^{14,55}

CRCs are large macromolecular aggregates, consisting of 9 to 12 catalytic and regulatory subunits that assemble combinatorially in a cell-state-specific manner.⁵⁶ In addition to the ATPase catalytic domains, other CRC domains may read covalently modified histones, modulate ATPase activity, and interact with chromatin-associated proteins.⁵⁶ There are 4 major human CRC families (the switch/sucrose nonfermentable complex, a nucleosome remodeling complex [SWI/SNF], ISWI, CHD, and INO80), which are in turn subdivided into 24 subfamilies that can catalyze a broad range of chromatin transformations.^{55,57}

The role of the SWI/SNF family of CRCs in cardiac development and hypertrophy has been the focus of detailed investigation.^{56–61} The Brg1 (Smarca4) component of SWI/SNF promotes myocyte proliferation by suppressing p57^{kip2} expression and preserves the fetal cardiomyocyte state by interacting with histone deacetylase (HDAC) and poly-ADP ribose polymerase to repress α -MHC (adult) and activate β -MHC (fetal).⁶² Brg1 activity is suppressed in adult cardiomyocytes and reactivated during stress to trigger fetal gene induction.⁶² In selected patients with hypertrophic cardiomyopathy, Brg1 remains activated and Brg1 levels correlate with disease severity.⁶² During cardiac development, differential spatiotemporal expression of Brg1-associated subunits occurs and is required for normal heart formation.⁶³ BAF250a, a regulatory subunit of SWI/SNF, plays a key role in cardiac progenitor cell differentiation, and *Baf250a* ablation in mice results in right ventricular trabeculation defects, ventricular septal defect, persistent truncus arteriosus, reduced cardiomyocyte proliferation, and embryonic lethality.⁶⁴ De novo mutations in 4 different SWI/SNF subunits have been associated with human congenital syndromes that typically display cardiac defects.⁵⁸ As SWI/SNF components interact functionally with cardiogenic TFs, such as T-box TF 5, contains a DNA T-box binding domain, GATA4, and NKX2-5,⁵⁸ it is possible that similar interactions occur in the stressed adult myocardium and contribute to HF pathogenesis.

Histone PTMs: Lysine Acetylation

Reversible lysine acetylation was the first histone PTM discovered^{65–67} and has been the most extensively studied. Lysine side chains within the unstructured amino-terminal tail of histone proteins are sites of local N- ϵ -acetylation (Kac), and local histone hyperacetylation at regulatory regions of chromatin is generally associated with transcriptional activation.²⁰ Histone acetylation favors an open chromatin configuration, increases chromatin accessibility to TFs, promotes protein complex assembly, and facilitates downstream signal transduction to Pol II.²⁰ Dynamic positioning of Kac is mediated by lysine acetyltransferases (KATs/HATs), which function as epigenetic writers and lysine deacetylases (KDACs/HDACs), which function as epigenetic erasers. Proteins harboring acetyl-lysine recognition modules, or bromodomains, bind to acetylated histones in a context-specific manner at regions of actively transcribed euchromatin and thus serve as epigenetic readers.^{39,68} Molecular recognition of acetylated histone by reader proteins promotes assembly of macromolecular complexes that remodel chromatin and regulate transcriptional initiation and elongation.⁶⁹ In addition to histones,

lysine acetylation affects proteins of multiple classes, including mitochondrial proteins, cytoskeletal proteins, and TFs,^{70,71} and the acetylation of these nonhistone targets may also play important roles in cardiac biology.^{72,73}

EP300 Histone Acetyltransferase (Kac Writers)

Discovered as a target of the adenoviral E1A oncoprotein, the transcriptional coactivator p300⁷⁴ plays broad roles in cellular differentiation, homeostasis, and growth.⁷⁵ p300 contains a lysine acetyltransferase domain capable of acetylating histones and nonhistone proteins including TFs (eg, GATA4, MEF2, p53, and p65).⁷⁶ In cultured cardiomyocytes, p300 protein abundance and activity are elevated after neurohormonal stimulation and are required for GATA4 acetylation and cellular hypertrophy in vitro.^{77,78} Genetic studies in mice revealed that the p300 acetyltransferase domain is essential for cardiac development in vivo.⁷⁵ Mice harboring *Ep300* germline deletion die between E9 and 11.5 with multiorgan developmental defects, including cardiac abnormalities.⁷⁹ p300 abundance is increased in hypertrophied mouse hearts and in failing human LV tissue, and augmented p300 activity is both necessary and sufficient for the development of pathological cardiac hypertrophy in vivo.⁷³ Specifically, *Ep300* haploinsufficient mice that survive into adulthood are protected from pressure-overload-mediated hypertrophy, whereas mice with cardiomyocyte-specific p300 overexpression develop dose-dependent pathological hypertrophy.⁷³ Cardiac p300 acetyltransferase activity is also required for postinfarct LV remodeling in vivo.⁸⁰ Finally, the polyphenol compound curcumin, which has inhibitory activity against p300 acetyltransferase, attenuates pathological cardiac hypertrophy in vitro and in vivo.⁸¹ Recent epigenomic analyses show that p300 is enriched at cardiac enhancers genome-wide in the adult mouse heart,^{46,82} suggesting that p300 drives hypertrophic growth via its ability to hyperacetylate chromatin and nonhistone proteins locally, such as GATA4, MEF2, and CDK9.^{73,81,83,84} Taken together, these studies support that excessive activation of p300 acetyltransferase is involved in pathological cardiac hypertrophy, and inhibition of p300 might be a potential therapeutic strategy in HF. Although p300 has been the most extensively studied, other proteins with Kac writing capabilities, such as cAMP-response element binding protein and protein-binding protein-associated factor, are also emerging as important regulators of cardiac remodeling.⁸⁵

HDACs/KDACs (Kac Erasers)

There are 18 known mammalian HDACs divided into 4 major classes: class I (HDAC1, 2, 3, and 8), class IIa (HDAC4, 5, 7, and 9), class IIb (HDACs 6 and 10), class III (the Sirtuin family of NAD-dependent deacetylases Sirt1-7), and class IV (HDAC11).⁸⁶ Several of these HDACs have been studied in the context of cardiac remodeling and HF pathogenesis using genetically modified mouse models and pharmacological inhibitors^{46,85,87-111} (Table 2). Of these, members of HDAC classes I, IIa, and III have been most extensively studied, whereas little is known about class IIb and IV HDACs. Here, we will focus on class I and II HDACs in the adult heart, noting that the role of class III HDACs (Sirtuins) has been reviewed in detail elsewhere.¹¹²

Class I HDACs

This subfamily is generally thought to exert prohypertrophic effects in adult myocardium. Early insights were gleaned from pharmacological studies using the hydroxamic acid-based compounds, trichostatin-A and valproic acid, inhibitors of both class I and II HDAC acetyltransferase activity. Because class IIa HDACs are generally thought to repress MEF2 function in a deacetylase-independent manner^{113,114} and possess significantly weaker deacetylase activity,¹¹⁵ the dominant effect of such compounds is likely via inhibition of class I HDACs. Hydroxamic acid-based HDAC inhibitors attenuate pathological cardiac remodeling in response to numerous stressors, including pressure overload, neurohormonal excess, genetic abnormalities, and myocardial infarction.^{100,116-118} An important therapeutic effect of trichostatin-A may involve suppression of excess autophagic flux in the hypertrophied heart although the precise mechanisms underlying this effect are just beginning to be understood.^{91,119} The apicidin derivative, which inhibits HDACs 1 to 3 (albeit with modest in vitro activity against HDAC6),¹¹⁵ has been shown to have beneficial effects in rodent models of pressure overload.¹²⁰ Additional work using next-generation inhibitors with better target specificity will be required¹¹⁹ to elucidate the therapeutic potential of HDAC modulation in the heart^{46,85,87-111} (Table 2).

Genetically modified mouse models have provided critical insights into gene-specific effects of class I HDACs in the heart. Cardiomyocyte-specific overexpression of HDAC2 using the *Mhy6* (α MHC) promoter results in spontaneous pathological hypertrophy and decreased activity of the anti-hypertrophic kinase GSK3 β .¹²¹ Although mice harboring systemic germline deletion of *Hdac2* have perinatal death because of a spectrum of cardiac abnormalities, this phenotype is not cell-autonomous because cardiomyocyte-specific deletion of either *Hdac2* or *Hdac1* using a *Myh6*-Cre driver results in minimal developmental defects.¹²² However, cardiac-specific deletion of both *Hdac2* and *Hdac1* results in a rapid onset postnatal cardiomyopathy, suggesting functional redundancy between these 2 class I HDACs.¹²² Importantly, mice with cardiac deficiency of either *Hdac2* or *Hdac1* have comparable hypertrophic responses to pressure overload or isoproterenol as do control mice, suggesting either of these genes alone is dispensable for pathological cardiac growth in vivo.¹²² Thus, although HDAC2 overexpression is sufficient to drive pathological hypertrophy, the above genetic studies support that the net effect of hydroxamic acid-based compounds is likely because of inhibition of multiple HDACs, although off-target chemical interactions with non-HDAC proteins remain possible.

Mice with cardiac-specific deletion of *Hdac3* develop severe cardiomegaly associated with early mortality, myocardial lipid accumulation, induction of genes regulating lipid flux and excess activity of the nuclear receptor PPAR α , a central regulator of myocardial lipid metabolism.¹²² Interestingly, recent studies of HDAC3 in the liver have demonstrated that many of its metabolic effects are independent of its deacetylase activity.¹²³ Transgenic overexpression of HDAC3 in the myocardium increases cardiomyocyte hyperplasia without significant augmentation of cardiac mass.¹²⁴ Germline deletion of HDAC8 in mice leads to perinatal lethality because of

Table 2. Therapeutic Potential of Targeting Acetyl-Lysine-Dependent Pathways in Heart Failure

Potential	Comments	Direction for Future Studies Targeting Drug Potential
Inhibition of hypertrophy	Class I HDAC inhibition suppresses pathological cardiac hypertrophy in vivo; mechanism likely involves both chromatin- and non-chromatin-dependent effects ^{87–90}	Creation of more isoform specific HDAC inhibitors. Elucidating the acetylated proteins that are critical targets of various HDACs
Inhibition of autophagy	The HDAC inhibitor TSA attenuates both load- and agonist-induced hypertrophic growth in vivo and attenuates excessive autophagic flux during pathological cardiac stress ⁹¹	Defining which HDAC family members play a dominant role in regulation of autophagy. Understanding the extent to which the salutary effects of HDAC are autophagy dependent. Assessing whether HDAC inhibition affects adaptive autophagy under physiological conditions
Inhibition of apoptosis	TSA potentiates ischemic preconditioning and inhibits apoptosis in rodent infarction models. Activation of certain sirtuin family members reduces oxidative stress-induced apoptosis ^{92–97}	Understanding the role of HDACs in regulating the mitochondrial acetylome and mitochondrial function (particularly in metabolism, ROS production, and cell death/survival signals)
Inhibition of fibrosis	HDAC inhibition reduces fibrosis during pathological stress. Mechanisms include suppression of cardiac fibroblast proliferation, cardiac MF transformation, and ECM deposition ^{98–105}	The use of evolving genetic models to study the role of various HDAC genes in fibroblasts. Assessing the ability of HDAC inhibitors to halt or suppress pre-established cardiac fibrosis in vivo
Inhibition of inflammation	A subset of HDAC inhibitors has anti-inflammatory effects. Some of these effects result from inhibition of innate immune responses within the myocardium as well as effects on primary immune cells. Activation of certain Sirtuins has anti-inflammatory effects ^{92–95,106,107}	The use of genetic models to study the role of various HDAC genes in noncardiomyocytes (eg, myeloid cells) in experimental models of heart disease
Improvement in contractility	HDAC4 (class IIa HDAC) has been observed to be localized to sarcomeres where their specific role if currently not well understood. HDAC4 may decrease myofilament calcium sensitivity by promoting deacetylation of muscle LIM protein ^{85,108}	Understanding the interactions of HDACs with nonhistone proteins and the role of HDACs in regulating myofilament function
Inhibition of ischemic and reperfusion injury	HDAC inhibitors, such as TSA, decrease myocyte hypertrophy, limit collagen deposition and improve cardiac performance in animal models of myocardial infarction ^{98,109,110}	Defining the appropriate timing and dose of HDAC inhibition post ischemia/MI and whether these compounds can predispose to rupture or aneurysm formation under certain experimental circumstances
BET Bromodomain inhibition	The small-molecule JQ1, when administered early after stress, blocks pathological hypertrophy in mice during pressure overload and phenylephrine infusion ^{46,111}	Assess whether BET inhibition protects against pathological remodeling in an expanded set of clinically relevant experimental models (post-MI, established HF, HF with preserved EF, large animal models of HF). Understand the therapeutic window of BET inhibitor drugs in humans (many of which are in early phase cancer trials). Elucidate the gene-specific and tissue-specific effects of BRD2, 3, and 4 in cardiovascular biology using genetic models

BET indicates bromodomain and extra terminal; HDAC, histone deacetylase; HF, heart failure; MF, myofibroblast; MI, myocardial infarction; and TSA, trichostatin-A.

abnormalities in skull development¹²⁵; however, a specific role for HDAC8 in the heart has not been described.

Class II HDACs

Studies in genetically modified mouse models have revealed that class IIa HDACs generally act as negative regulators of cardiomyocyte hypertrophy. A major mechanism by which class IIa HDACs function is via repression of MEF2 function, an effect that is independent of deacetylase activity and mediated by physical interaction between the HDAC N-terminal domain and the MEF2.^{113,114} Overexpression of HDAC4, 5, or 9 suppresses MEF2-dependent transcription and agonist-induced hypertrophy in cultured cardiomyocytes.^{114,126,127} In response to hypertrophic stimuli, class IIa HDACs are phosphorylated by stress responsive kinases (eg, CaMK and PKD) on conserved N-terminal serine residues.^{114,126,128} HDAC phosphorylation promotes dissociation of the HDAC-MEF2 inhibitory complex and triggers HDAC nuclear export. These

events release tonic repression of MEF2, allowing it to recruit coactivator proteins (eg, HATs), interact with other TFs (eg, nuclear factor of activated T cells and GATA4), and trans-activate a prohypertrophic gene program.¹²⁹ In this regard, class IIa HDACs function as signal-responsive repressors of MEF2 function and pathological hypertrophic growth.¹¹⁴ In support of this general mechanism, mice deficient in either *Hdac5* or *Hdac9* develop exaggerated pathological hypertrophy in response to pressure overload.^{114,127} The relationships between particular stress-activated kinases and downstream HDAC targets can be specific as demonstrated by the selective interaction CaMKII with HDAC4.^{129,130} In addition to regulation by upstream kinases, phosphorylation-independent HDAC4 nuclear export is triggered by stress-activated oxidation of conserved cysteine thiols (Cys-667 and Cys-669) in the C-terminal deacetylase domain, an event that is inhibited by thioredoxin 1.¹³¹

Little is known about class IIb HDACs in the heart. HDAC6 activity has been shown to be increased in rodent myocardium after pathological stress.^{132,133} A recent study found that the partially selective HDAC6 inhibitor tubastatin-A was protective effects in a canine model of tachypacing-induced atrial dysfunction.¹³⁴ The role of HDAC10 in the heart is unknown. Additional studies of class IIb HDAC function using more selective compounds and genetic models are required.

Bromodomain and Extra Terminal Bromodomains (Kac Readers)

The body of work highlighted above demonstrates that the interplay between epigenetic writers (eg, HATs) and erasers (eg, HDACs), which dynamically position acetyl-lysine (Kac) on target proteins, is an important mechanism of gene control during pathological cardiac growth. In contrast, the role of epigenetic reader proteins of any type in the heart was not known until recently. Studies from our group⁴⁶ and the laboratory of Spiltoir et al¹¹¹ have established the importance of bromodomain and extra terminal (BET) family acetyl-lysine reader proteins as critical effectors of pathological cardiac hypertrophy and HF pathogenesis. Although pathological hypertrophy has been associated with increased histone acetylation at regulatory genomic regions^{135,136} and increased activity of P-TEFb,⁴⁷ the signaling events linking local chromatin hyperacetylation to Pol II dynamics in the heart remained poorly understood. BETs are a conserved family of proteins consisting of the ubiquitously expressed Brd2, Brd3, Brd4, and the testis-specific BrdT, all of which have 2 tandem N-terminal Kac recognition domains (bromodomains) that bind acetylated histone. Bromodomain 4 has been shown to facilitate transcriptional activation in other cellular systems via interactions with Mediator¹³⁷ and P-TEFb complexes.^{138,139} Furthermore, the CDK9 component of P-TEFb kinase is required for cardiomyocyte hypertrophy in vitro, whereas excessive activation of P-TEFb (via transgenic overexpression of Cyclin-T1 in cardiomyocytes) is sufficient to cause cardiac hypertrophy in vivo.⁴⁷ Therefore, we hypothesized that BETs might be critical in signaling between hyperacetylated enhancers and Pol II at transcriptional start sites in the stressed heart. Our ability to probe the role of BETs in the heart was enabled by the development of the small-molecule JQ1, a first-in-class, potent, and selective BET bromodomain inhibitor.⁶⁸ JQ1 competitively inhibits BET bromodomain binding to Kac,^{38,46} thereby displacing these reader proteins from acetylated chromatin and suppressing downstream signaling events to Pol II (Figure 2).^{68,69} These studies demonstrated that BET inhibition with nanomolar doses of JQ1 or *Brd4*-specific siRNAs blocked agonist-stimulated hypertrophy in cultured cardiomyocytes. In adult mice, JQ1 potently inhibited cardinal features of pathological hypertrophy and HF progression in vivo in response to pressure overload or chronic phenylephrine infusion. Transcriptomic analyses revealed that BETs coactivated several canonical prohypertrophic TFs, such as nuclear factor of activated T cells, GATA4, and nuclear factor κ -light-chain-enhancer of activated B cells, to regulate a broad, but specific gene expression program. Epigenomic studies revealed that a major mechanism by which BETs coactivated gene transcription was via recruiting P-TEFb activity to transcriptional

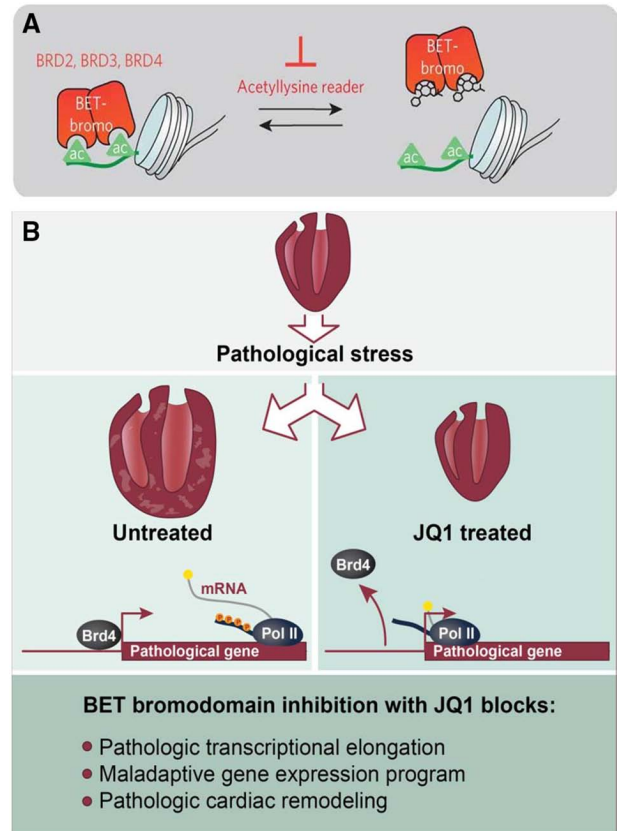


Figure 2. Bromodomain (BRD) inhibitors in heart failure. **A**, Schematic of bromodomain and extra terminal (BET) bromodomain acetyl-lysine readers in the absence (left) and presence (right) of small-molecule BET inhibitor. Reprinted from Fierz and Muir³⁸. **B**, Mice subject to cardiac pressure overload (transaortic constriction vs sham, 4 weeks) exhibit Pol II pause release, pathological gene induction, cardiomegaly, fibrosis, and heart failure. BET bromodomain inhibition with JQ1 suppresses transcriptional elongation in the heart and potentially blocks progression of pathological remodeling in vivo. Reprinted from Anand et al with permission of the publisher.⁴⁶ Copyright © 2013, Elsevier.

start sites and promoting pause release of Pol II. These studies suggested a model in which BET coactivator proteins function as essential signal transducers between activated enhancers (which are bound by prohypertrophic TFs and undergo dynamic histone hyperacetylation) and poised Pol II near-transcriptional start sites. In this regard, BETs act as rheostats on stress-activated gene induction in the heart via regulation of P-TEFb and transcriptional elongation (Figure 2).^{46,111} These studies provide an impetus for developing BET inhibitors as investigational therapeutic agents in heart disease. Conditional genetic models will be required to annotate gene and cell-type-specific roles for BETs in vivo further.

Histone PTMs: Lysine Methylation

Methylation of histones on specific lysine residues is also a major regulator of chromatin state. Several specific histone methylations are each associated with characteristic activating or repressive transcriptional functions (eg, H3K4me3 marks active promoters, H3K9me3 marks heterochromatin, and H3K4me1 marks poised enhancers).¹⁰ Locus-specific histone methylation is dynamically regulated by a families of histone

methyltransferase and demethylase enzymes exhibiting varying specificity for both histone targets (eg, H3K4 and H3K27) and the types of methyl marks catalyzed (me1, me2, and me3).¹⁰ Proteins harboring methyl-lysine recognition motifs (eg, chromodomains) function in downstream chromatin-dependent signal transduction.⁴¹ Although numerous studies have implicated histone methylation in several developmental and disease contexts,^{140–142} our understanding of this mode of chromatin remodeling in cardiac hypertrophy and HF is nascent. ChIP-chip studies from Dahl salt-sensitive rat left ventricular hypertrophy models and failing human hearts revealed dynamic, genome-wide changes in H3K4me3 and H3K9me3 marks.¹⁴³ More recently, ChIP-Seq and RNA-Seq studies in adult cardiomyocytes isolated from pressure-overloaded mouse hearts demonstrated dynamic and locus-specific alterations in several histone methyl marks that were correlated with changes in transcript abundance.¹³⁵ The histone demethylase JMJD2A/KDM4A, which catalyzes demethylation of H3K9me3 and H3K36me3,¹⁴⁴ has been implicated as a positive regulator of pathological cardiac hypertrophy.¹⁴⁵ JMJD2A protein abundance is increased ≈7- to 8-fold in LV tissue from patients with hypertrophic cardiomyopathy, and mice with cardiomyocyte-specific deletion of *Jmjd2a* are partially protected from trans-aortic constriction-induced hypertrophy. Conversely, mice overexpressing JMJD2A in cardiomyocytes (at 8-fold higher than control) have preserved baseline LV structure/function but develop exaggerated pathological hypertrophy after transaortic constriction. JMJD2A was shown to induce a specific subset of cardiac genes (such as the prohypertrophic target *Fhl1*) and was associated with altered H3K9me3 and SRF/myocardin enrichment at this locus. The extent to which JMJD2A effects are FHL1 dependent is unknown. The authors of this study note that JMJD2A manipulation produces a relatively limited profile of differentially expressed transcripts in the mouse heart when assessed by cDNA microarrays.¹⁴⁵ Further cardiac studies using ChIP-Seq for JMJD2A and key histone marks coupled with RNA-Seq in these mouse models will help elucidate such target specificity. This study suggests that inhibition of JMJD2A function in the heart might be a therapeutic strategy in HF.

Although H3K4me3 (enriched near promoters) and H3K36me2/3 (enriched within gene bodies and 3' ends) are associated with actively transcribed genes, H3K27me3 marks are associated with gene and chromatin silencing.¹⁴⁶ The EZH2 subunit of Polycomb repressive complex 2 (PRC2), which catalyzes the H3K27 mono-, di-, and trimethylations, has been subject of much interest.^{147,148} Human PRC2 consists of a minimum of 4 core subunits: Main methyltransferase subunit of Polycomb repressive complex 2 (EZH2), EED, SU(Z)12 and RbAp48.¹⁴⁹ The C-terminal SET domain of EZH2 is essential for PRC2 transmethylation function and the EED and Su(Z)12 subunits are required for full catalytic activity. EZH2 activity is regulated by several mechanisms, including phosphorylation of PRC proteins, variation in subunit isoforms, and interaction with specific histone modifications.¹⁴⁶ The precise mechanisms by which PRC2 mediates chromatin silencing are just beginning to be understood.¹⁴⁹ PRC2-dependent gene silencing is critical to normal cardiac development and post-natal maturation. EZH2 deletion in mouse cardiac progenitors results in hypertrophied adult hearts characterized by fibrotic,

noncompacted right ventricles, excessive activation of the homeodomain TF Six1, and derepression of a Six1-dependent skeletal muscle gene program.¹⁵⁰ Importantly, this study supports the contention that epigenetic perturbations during organogenesis can affect disease phenotype during adulthood. In a separate study, EZH2 inactivation in differentiated cardiomyocytes using *Nkx2-5^{Cre}* resulted in lethal congenital heart malformations, in part, because of inappropriate spatiotemporal gene control, including derepression of the cell cycle inhibitor INK4A/B and several potent TFs that drive noncardiomyocyte gene expression programs.¹⁵¹ Beyond H3K27, EZH2 methylation substrates may also include key cardiac TFs, such as GATA4. In cultured cardiomyocytes and fetal mouse hearts, EZH2 binds and methylates GATA4 at lysine 299 resulting in decreased GATA4 function.¹⁵² EZH2 can also bind lncRNAs (eg, *Xist* and *HOTAIR*), which facilitate recruitment of PRC2 to specific chromatin sites.^{153–155} Finally, interaction of EZH2 with primary micro-RNA-208b was shown to regulate aspects of myosin heavy chain isoform switching in the adult mouse heart during pressure overload.¹⁵⁶

DNA Methylation of Cytosine

Methylation of cytosine carbon 5 (5mC) at symmetrical DNA CG dinucleotide residues (CpG) is an important mechanism of gene regulation, particularly of transcriptional silencing.^{18,157–159} 5mC has been shown to play an important repressive role in imprinting, X chromosome inactivation, and retrotransposon silencing.^{10,159} Reversible DNA methylation occurs at CpG sites by 3 highly conserved DNA methyltransferase enzymes (DNMTs), DNMT3A, and DNMT3B (which catalyzes de novo methylation) and DNMT1 (which maintains 5mC through cell division).^{159,160} The TET family of dioxygenase enzymes catalyzes the oxidation of methylcytosine to hydroxymethylcytosine, an intermediary for demethylation, and along with redox-dependent DNA 5-dehydroxymethylases, plays key roles in cytosine carbon 5 demethylation.¹⁶¹ Overall, between 60% and 80% of the ≈28 million CpG sites in the human genome are methylated.¹⁵⁹ The elucidation of complete human methylomes has established that the 5mC is not simply a stable repressive mark, but rather an epigenetic modification that is dynamically deposited and removed, can exist in non-CpG sequence contexts, and is enriched at bodies of actively transcribed genes.^{10,159} CpG methylation at promoters and TSSs is the best-studied methylation pattern to date and is thought to repress gene activity through multiple mechanisms, including disruption of TF–DNA interactions, recruitment of repressive methyl-CpG binding proteins, and modulation of other chromatin-binding proteins and histone modifications.^{160,162} Evidence for several additional noncanonical roles for DNA methylation is evolving.¹⁶⁰ For example, hypermethylation within gene bodies may repress gene expression via regulation of transcriptional elongation and cotranscriptional mRNA processing (eg, alternative splicing).¹⁶⁰ The importance of DNA methylation during vertebrate development is underscored by the finding that deletion of DNMT1, DNMT3a, or DNMT3b is embryonically lethal in mice.^{163,164}

DNA methylation has not been widely studied to date in HF¹⁶⁵ and available studies are summarized in Table 3.^{143,166–169}

Table 3. Histone Protein and DNA Methylation in HF: Human Studies

Subjects	Method	Findings	Reference
HF=4 Control=4	ChIP-Seq	Histone H3K4me3 and H3K9me3 marks markedly differed between HF vs controls and mapped to HF-specific pathways	143
HF=8 Control=6	MeDIP	Differential promoter region DNA methylation for three angiogenesis-related genes (<i>AMOTL2</i> , <i>ARHGAP24</i> , <i>PECAM1</i>) observed and related to differential expression in HF vs controls	168
HF=4 Control=4	MeDIP ChIP-Seq	Distinct genome-wide promoter, intragenic and gene body CpG island methylation, promoter region DNA methylation and gene body H3K36me3 patterns observed in HF vs controls	169
HF=39 Control=36	Infinium HumanMethylation 27 platform	CpG island DNA methylation patterns for 20 genes including <i>LY75</i> , <i>ERBB3</i> , <i>HOXB13</i> and <i>ADORA2A</i> differed in DCM vs controls	166
HF=10 Control=10	MeDIP	57 promoters exhibited differential DNA methylation with greatest differences in <i>AURKB</i> , <i>BTNL9</i> , <i>CLDN5</i> and <i>TK1</i> in HF vs controls	167

H3K4me3 indicates H3K4 trimethylation; and HF, heart failure.

Taken together, these studies suggest that (1) differential DNA methylation occurs between failing and control hearts near selected genes, particularly in promoter regions, in a manner correlating with altered gene expression in HF, (2) these different methylation patterns may provide an opportunity for diagnostic discrimination between failing and control hearts, and (3) differential methylation patterns may identify novel genomic loci involved in HF pathogenesis. Genome-wide profiling of the cardiac methylome in experimental systems and human hearts will be particularly informative.

Long Noncoding RNAs

Although the role of lncRNAs in epigenetic regulation during development, differentiation, and cancer is established,¹⁷⁰ our understanding of lncRNAs in cardiac development and disease is just beginning.¹⁷¹ A recent study that used RNA-seq in human heart tissue samples from patients with left ventricular assist devices reported that the dominant transcriptomic feature of mechanically unloaded left ventricles was a predominance of differentially expressed lncRNA species, as opposed to mRNAs or miRNAs.¹⁷² The lncRNA Braveheart (*Bvht*) is the best characterized lncRNA in the cardiomyocyte.¹⁷³ *Bvht* was shown to be required for cardiomyocyte lineage specification from mesoderm and maintenance of cardiac fate in neonatal mouse cardiomyocytes.¹⁷³ *Bvht* interacts with the SU(Z)12 component of Polycomb 2 repressive complex, and *Bvht* deficiency in cardiac progenitor cells leads to increased enrichment of SU(Z)12 at promoters of genes involved in cardiac differentiation.¹⁷³ Thus, *Bvht* seems to function during cardiac differentiation via an epigenetic mechanism involving locus-specific inhibition of PRC2 activity. In addition to the seminal discovery of *Bvht*, other lncRNA species have been implicated in cardiac biology. lncRNA natural antisense transcripts¹⁷⁴ have been shown to modulate expression of troponin I,¹⁷⁵ myosin heavy and light chains,¹⁷⁶ atrial natriuretic peptide,¹⁷⁷ and atrial myosin light chain 1.¹⁷⁸ Inappropriate amplification of a noncoding RNA derived from the DMPK 3' untranslated region CTG triplet has been implicated in the pathogenesis of myotonic dystrophy (DM1), possibly via inappropriate activation of the TF Nkx2.5.¹⁷⁹ Finally, in a genome-wide association study in humans, polymorphisms in the locus encoding the lncRNA ANRIL conferred strong genetic susceptibility for coronary artery disease.¹⁸⁰ ANRIL, a natural antisense

transcript of the protein-coding INK4b/ARF/INK4a complex, has been shown to interact with PRC1 to modulate H3K27me and transcriptional repression in cancer cells, raising the possibility that related epigenetic mechanisms might be at play in cardiovascular disease.¹⁸¹

Future Directions and Conclusions

An era increasingly devoted to deciphering the fundamental epigenetic mechanisms in HF pathogenesis has dawned. Leveraging next-generation sequencing technologies and contemporary modes of probing the epigenome (ChIP-Seq, Hi-C, and TAB-Seq), detailed chromatin state maps and TF cisomes in both animal HF models and human HF myocardial tissue are now becoming possible. When coupled with gene expression profiles (eg, RNA-Seq), these analyses will be essential to understand how alterations in chromatin structure conspire with TFs and noncoding RNAs to drive HF pathogenesis. Such analyses may reveal genomic loci critical for chromatin-dependent signaling (eg, important enhancer regions) whose misregulation confer susceptibility to human heart HF. The use of gene editing technologies, patient-derived cells, and chemical biological approaches will be essential to delineate specific roles for chromatin regulators and critical *cis*-regulatory genomic elements (eg, enhancers) in cardiac biology.

Evolving research consistently underscores the rich heterogeneity and functional inter-relationship of the genome, epigenome, and transcriptome in human HF.¹⁸² Acquired forms of human HF exhibit substantial phenotypic heterogeneity, reflecting complex interactions between environmental stressors, the genome, and the epigenome.¹⁸³ Even familial cardiomyopathies that are known to be caused by single-gene mutations exhibit substantial phenotypic diversity, as evidenced by variable intra- and interfamily expressivity and incomplete penetrance.¹⁸⁴ This clinical heterogeneity is undoubtedly influenced by epigenetic mechanisms that transduce environmental signals and gene-gene interactions that drive differential transcriptional responses in the myocardium. Regulation via ATP-dependent CRCs, histone modifications, DNA methylation, and noncoding RNAs is a dynamic process that varies throughout development, physiology, and the various stages of HF pathogenesis. A deeper understanding of these dynamic epigenetic mechanisms may unmask novel

HF risk factors and identify subclinical states along the disease spectrum, affording earlier diagnostic and therapeutic opportunities.

Deciphering fundamental epigenetic HF mechanisms may also usher in a new era of therapies for established HF. Although targeting cardiac transcription has been an area of great therapeutic interest, direct pharmacological modulation of TFs has proven extremely difficult: many of these DNA-binding proteins reside within the nucleus in low abundance and lack structural features readily accessible to small molecules. In addition, the therapeutic window of putative TF modulating drugs is also narrow because many of the TFs hyperactivated in HF (eg, GATA4 and MEF2) are also key determinants of cardiomyocyte identity. The therapeutic manipulation of chromatin regulators, already a burgeoning area in cancer drug discovery and exemplified by the use of small-molecule BET bromodomain inhibitors in experimental HF,^{46,111} may afford novel opportunities to target pathological gene control in human HF.

Sources of Funding

S.M. Haldar was supported by National Institutes of Health (DK093821) and an Individual Biomedical Research Award from The Hartwell Foundation.

Disclosures

None.

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KEY WORDS: chromatin ■ etiology ■ heart failure ■ histones ■ hypertrophy ■ transcription factors

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Circ Heart Fail. 2014;7:850-863

doi: 10.1161/CIRCHEARTFAILURE.114.001193

Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 1941-3289. Online ISSN: 1941-3297

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