Control of Cardiac Growth by Histone Acetylation/Deacetylation

Johannes Backs, Eric N. Olson

Abstract—Histones control gene expression by modulating the structure of chromatin and the accessibility of regulatory DNA sequences to transcriptional activators and repressors. Posttranslational modifications of histones have been proposed to establish a “code” that determines patterns of cellular gene expression. Acetylation of histones by histone acetyltransferases stimulates gene expression by relaxing chromatin structure, allowing access of transcription factors to DNA, whereas deacetylation of histones by histone deacetylases promotes chromatin condensation and transcriptional repression. Recent studies demonstrate histone acetylation/deacetylation to be a nodal point for the control of cardiac growth and gene expression in response to acute and chronic stress stimuli. These findings suggest novel strategies for “transcriptional therapies” to control cardiac gene expression and function. Manipulation of histone modifying enzymes and the signaling pathways that impinge on them in the settings of pathological cardiac growth, remodeling, and heart failure represents an auspicious therapeutic approach. (Circ Res. 2006;98:15-24.)

Key Words: cardiac hypertrophy ■ histone acetyltransferase ■ histone deacetylase ■ gene expression ■ epigenetics

Genomic DNA within the nuclei of eukaryotic cells is highly compacted with histone and nonhistone proteins in a dynamic polymer called chromatin. The basic unit of chromatin is the nucleosome, which comprises 146 base pairs of DNA wrapped around a histone octamer that consists of 2 copies each of histones H2A, H2B, H3, and H4. Nucleosomes interact to create a condensed structure that limits access of genomic DNA to transcription factors, thereby repressing gene expression. Residues within histone tails are subject to diverse posttranslational modifications, including phosphorylation, acetylation, and methylation, which together establish a “histone code” that governs the higher-order structure of chromatin and gene expression. The sum of these biochemical modifications is referred to as “epigenetic information.”

Consistent with transient versus long-term epigenetic memory, some histone modifications (eg, phosphorylation and acetylation) are highly dynamic, whereas others (eg, methylation) are more stable. Most studies, to date, have focused on the role of histone acetylation/deacetylation in the control of gene transcription. Acetylation of conserved lysine residues in histone tails by histone acetyltransferases (HATs) stimulates gene expression by neutralizing positive charge, resulting in destabilization of histone–histone and histone–DNA interactions that limit access of transcription factors to DNA. The stimulatory effect of HATs on gene expression is countered by histone deacetylases (HDACs), which promote chromatin condensation and thereby repress transcription (Figure 1). A common feature of many HATs and HDACs is their ability to interact with nonhistone nuclear proteins, such as DNA-binding transcription factors, coactivators, and corepressors, thereby activating or repressing specific genes. The association of HATs and HDACs with sequence-specific DNA-binding factors confers target gene-specificity to their actions.
In addition to their roles as transcriptional activators and repressors, HATs and HDACs serve as intranuclear targets for signaling cascades initiated at the cell surface and thereby couple extracellular signals to the genome. Recent studies of genetically altered mice have begun to reveal functions of these chromatin-remodeling enzymes in the control of gene programs that govern growth and development of the heart and other tissues. Here, we describe the roles of HATs and HDACs as key regulators of cardiac growth and gene expression and potential therapeutic targets in the settings of pathological cardiac hypertrophy and heart failure.

**Stress-Dependent Cardiac Growth and Remodeling**

A variety of cardiovascular disorders, including myocardial infarction, arterial hypertension, and altered contractility resulting from mutations of sarcomeric proteins provoke the adult heart to become enlarged because of hypertrophic growth of cardiomyocytes.\(^5,6\) Stress-induced hypertrophy may initially normalize ventricular wall stress, but prolonged hypertrophic growth of the heart frequently leads to heart failure, cardiac arrhythmias, and sudden death.\(^5\) The latter abnormalities are often associated with myocyte dropout attributable to apoptosis or necrosis, fibrosis of the ventricular wall, and abnormalities in sarcomere assembly that result in ventricular dilatation.

At the cellular level, hypertrophy is accompanied by an increase in cardiomyocyte size, enhanced protein synthesis, heightened organization of sarcomeres, and reinduction of a fetal cardiac gene program that ultimately weakens cardiac performance.\(^5\) Numerous extracellular agonists and, in particular, those that act through G-protein–coupled receptors, such as α- and β-adrenergic agonists, endothelin, angiotensin, and 5-hydroxytryptamine, promote cardiomyocyte hypertrophy.\(^5,10\) Increased cardiac workload, cell stretch, and abnormalities in structure and function of the sarcomere and cytoskeleton also result in activation of the hypertrophic response.\(^12\)

A complex web of signaling pathways has been implicated in the transmission of stress signals leading to cardiac hypertrophy.\(^3\) These pathways, many of which rely on calcium, have been extensively reviewed elsewhere and will not be discussed in detail here. The extent to which the signaling pathways activated by different pathological stimuli overlap or diverge remains an important issue. Other key questions in the field are whether there are nodal points of convergence among hypertrophic pathways and how pathological signals are transmitted to downstream targets in the nucleus that reprogram cardiac gene expression. Whether the signaling pathways that drive pathological growth of the heart are distinct from or related to those involved in normal postnatal growth of the heart or physiological growth in response to exercise is also an issue of fundamental importance, particularly with respect to the development of therapies to prevent or reverse pathological cardiac growth without disrupting salutary processes in the heart.

**Transcriptional Reprogramming of the Stressed Myocardium**

The stressed myocardium displays characteristic changes in its pattern of gene expression that alter cardiac function. A hallmark of the cardiac stress response is a myosin heavy chain (MHC) isoform switch in which the embryonic β-MHC isoform is upregulated with concomitant downregulation of the adult α-MHC isoform.\(^13,14\) This isoform transition, which has been proposed to contribute to diminished myofibrillar ATPase activity and impaired contractility, has been well documented in rodents, in which α-MHC is the predominant adult isoform.\(^15\) However, its significance in humans, where the β-isoform predominates, has been less certain. Nevertheless, there is evidence that improvement of cardiac function in heart failure patients receiving β-adrenoceptor antagonists correlates with an increase in α- and decrease in β-MHC expression.\(^16\) Moreover, partial replacement of β-MHC with α-MHC in the ventricles of transgenic rabbits confers cardioprotection in the setting of tachycardia-induced cardiomyopathy.\(^17\)

Other well-characterized changes in gene expression in the hypertrophic and failing heart include upregulation of genes encoding natriuretic peptides and downregulation of the genes encoding sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) and phospholamban, with consequent abnormalities in calcium handling by the cardiomyocyte.\(^18,19\) As discussed below, most studies to date have focused on the mechanisms involved in fetal gene induction in the stressed myocardium. Equally important, but less understood, are the mechanisms that repress expression of α-MHC and other genes in the diseased heart.

Numerous transcription factors have been implicated in stress-dependent gene expression in the heart including the zinc-finger protein GATA4, the Rel transcription factor NFAT, the MADS box proteins SRF and MEF2, and the cAMP-responsive transcription factor CREB.\(^20-28\) Most of
these transcription factors do not change in abundance in the stressed myocardium. Thus, their enhanced activity must depend on posttranslational mechanisms. HATs and HDACs play a central role in modulating the activities of these transcription factors during pathological cardiac growth.

**Histone Acetyltransferases**

HATs fall into at least 5 families.29 The most extensively studied HATs in muscle are p300 and the closely related coactivator, CREB-binding protein (CBP), which play critical roles in physiological and pathological growth of cardiac myocytes. p300 possesses intrinsic HAT activity that modifies chromatin and associated transcription factors, thereby relaxing chromatin structure and promoting gene activation. The importance of p300 in normal cardiac transcription is illustrated by the phenotype of p300 knockout mice, which die between days 9 and 11.5 of gestation and show reduced expression of muscle structural proteins such as β-MHC and α-actinin, as well as cardiac structural defects and reduced trabeculation.30 A gene knock-in approach demonstrated the HAT domain of p300 to be essential for heart formation.31

During agonist-induced hypertrophy of cardiomyocytes, p300 transcriptional activity is enhanced.32 Moreover, ectopic overexpression of p300 and CBP stimulates, and dominant-negative mutants of p300 block agonist-mediated cardiac growth.33,34 Transgene-mediated expression of p300 in the adult mouse heart also results in hypertrophy and heart failure.34 Besides acetylation of histone tails, p300 also serves as an adaptor for hypertrophy-responsive transcription factors, such as GATA4, SRF, and MEF2, and these interactions are required for the full transcriptional activities of these factors.35–38 The activity of p300/CBP is enhanced by signaling pathways that promote cardiac hypertrophy, providing a mechanism for stimulation of the fetal gene program by hypertrophy-inducing factors.

**Histone Deacetylases**

The action of HATs is counterpoised by HDACs. There are currently 18 known human HDACs, which fall into 3 classes based on their homology with 3 distinct yeast HDACs.39 Among them, class II HDACs have been shown to repress growth of myocytes.40,41 However, there is also increasing evidence that class I HDACs might exert opposite effects and promote cellular growth.42,43 The potential functions of class III HDACs in the heart, which act as NAD-dependent deacetylases, remain to be fully deciphered.

**Control of Cardiac Growth by Class II HDACs**

The prototypical class II HDACs—HDAC4, HDAC5, HDAC7, and HDAC9—are unique in several aspects of function and regulation.39,40 First, class II HDACs are highly expressed in muscle, brain, and T cells, whereas class I and class III HDACs are expressed ubiquitously. Second, class II HDACs contain in addition to the catalytic domain a large N-terminal extension that mediates interactions with transcriptional repressors and activators.8,39,44–46 The N-terminal regulatory region of these class II HDACs also serves as a target for phosphorylation, which governs the nuclear export of these proteins (Figure 2).

Gene-targeting studies in the mouse have provided the most striking evidence of the importance of class II HDACs as signal-responsive suppressors of postnatal cardiac growth. Mice lacking either HDAC5 or HDAC9 are viable and show no evidence of cardiac abnormalities at early age. However, by about 6 months of age, mutant animals develop spontaneous cardiac hypertrophy that appears to reflect sensitization to age-related cardiac insults.41 HDAC547 and HDAC941 mutant mice also develop profoundly enlarged hearts in response to pressure overload resulting from aortic constriction or constitutive cardiac activation of calcineurin, a transducer of cardiac stress signals.40 In contrast, these mice display a normal hypertrophic response to chronic β-adrenergic stimulation with isoproterenol, which acts through the cAMP-signaling pathway. These findings indicate that HDAC5 and -9 function as antagonists of a specific subset of hypertrophic signaling pathways that are dependent on intracellular calcium signaling. Mice lacking HDAC5 or -9 also display a
normal cardiac growth response to exercise, indicating that these chromatin-remodeling enzymes are dedicated to the repression of signaling pathways involved in pathological but not physiological hypertrophy. Thus, these HDACs are potentially attractive drug targets.

The adult heart is exquisitely sensitive to the level of expression of HDAC5 and -9, such that deletion of even a single copy of either gene results in a heightened response to cardiac stress. These findings highlight the functions of HDAC5 and -9 as modifiers of cardiac disease phenotypes or susceptibility genes to cardiovascular disease. Hence, one might imagine individuals with polymorphisms in these HDACs that would display an especially severe response to a cardiac insult such as hypertension or myocardial infarction, but would otherwise display normal cardiac function in the absence of cardiac injury. It would be of interest to screen for such individuals in the population.

Mice homozygous for null alleles of either HDAC5 or -9 and heterozygous for the other allele are viable, whereas only a small fraction of HDAC5/9 double-null animals survives, and they display growth retardation. The majority of double-mutant mice die during late embryogenesis with thin-walled myocardium and ventricular-septal defects, suggesting that these HDACs are also required for heart development, presumably by regulating the activities of cardiac transcription factors.

Mice lacking HDAC4 and HDAC7 (Chang S., Young B., Li S., Qi X., Richardson J.A., Olson E.N., unpublished data, 2005) are not viable, which has precluded an analysis of the potential functions of these HDACs in cardiac hypertrophy. Moreover, MEF2 interaction with HDACs is mutually exclusive. Thus, mice lacking HDAC4 and HDAC7 in the adult heart and other tissues.

MEF2 Is a Critical Target of Class II HDACs
Abnormal cardiac growth in HDAC-knockout animals correlates with superactivation of the MEF2 transcription factor, which suggests a causal relationship between MEF2 activity and the hypertrophic response. Indeed, prior studies established that MEF2 proteins associate directly with class II HDACs via an 18-amino-acid motif present only in these HDACs, Class I HDACs lack this domain and fail to directly regulate the activity of MEF2. Class II HDACs form a complex with MEF2 on gene regulatory elements, resulting in repression of genes harboring MEF2-binding sites. p300 has also been shown to associate with the same domain of MEF2 that is occupied by class II HDACs, but interaction of MEF2 with HATs and HDACs is mutually exclusive. Thus, MEF2 acts as platform to respond to positive or negative transcriptional signals by exchanging HATs and class II HDACs (Figure 2).

There is strong evidence pointing to MEF2 as a critical target for class II HDACs, but it is unlikely to serve as the sole transcriptional effector of class II HDACs. Indeed, class II HDACs associate, directly or indirectly, with and repress the transcriptional activities of SRF, GATA factors, NFAT, and myocardin, each of which have been shown to be involved in cardiac hypertrophy. Moreover, MEF2 interacts with GATA and NFAT transcription factors, which also associate with each other. Thus, class II HDACs have the potential to repress hypertrophy-responsive genes recruited via MEF2 to gene regulatory regions that do not contain direct binding sites for MEF2. An important issue is whether there are specific gene targets responsible for hypertrophic growth or whether hypertrophy results secondarily to changes in cardiac function. How transcriptional changes are coupled to the translational events associated with hypertrophy also remains to be fully resolved.

Class II HDACs Act As Signal-Responsive Repressors
How do class II HDACs determine the response of the heart to stress? Class II HDACs levels do not appear to change in stressed myocardium. Instead, these HDACs are shuttled from the nucleus to the cytoplasm in response to stress signals, providing a posttranslational mechanism to override HDAC-mediated repression of cardiac growth. This redistribution of HDACs enables MEF2 and other transcriptional activators and coactivators to associate with HATs, resulting in increased local histone acetylation and activation of downstream genes that promote cellular growth.

Nucleocytoplasmic shuttling of class II HDACs is dependent on phosphorylation of 2 serine-containing motifs found exclusively in the amino-terminal extensions of HDAC4, HDAC5, HDAC7, and HDAC9 (Figures 2 and 3). When phosphorylated, these motifs associate with a chaperone protein, termed 14-3-3, which results in masking of the nuclear localization sequence located between the phosphorylation sites and unmasking of a nuclear export sequence at the C termini of the HDACs. The nuclear export sequence is subsequently bound by the CRM1 nuclear export receptor, which escorts class II HDACs from the nucleus to the cytoplasm. Nuclear export of class II HDACs serves as an important step to reinforce the mechanism for activation of transcription factors that are repressed by interaction with these HDACs. However, nuclear export is not absolutely essential for signal-dependent activation of HDAC target genes because a naturally occurring splice variant of HDAC9, referred to as MITR, dissociates from MEF2 on phosphorylation, allowing for MEF2 activation, but it remains nuclear because it lacks the C-terminal nuclear export sequence.

Recently, inhibitors of CRM1-dependent nuclear export were shown to prevent cardiomyocyte hypertrophy and pathological cardiac gene expression in vitro, at least in part through their ability to prevent nuclear export of HDAC5, although there are undoubtedly other targets. Importantly, these inhibitors did not perturb the actions of thyroid hormone or insulin-like growth factor 1, which stimulate physiological hypertrophy. These findings underscore the distinctions between the signaling pathways involved in physiological and pathological hypertrophy and raise the possibility that specific antagonists of the latter pathways might be developed for treatment of pathological cardiac hypertrophy. Although CRM1 inhibitors possess significant toxicity, they have been advanced into clinical trials for cancer and human immunodeficiency virus infection.

Adenoviral overexpression of constitutive active mutants of HDAC4, -5, and -9 prevent agonist-induced cardiomyo-
cyte hypertrophy. Thus, the phosphorylation of class II HDACs appears to be an essential step in the process of cardiac hypertrophy in vitro. Forced overexpression of a signal-resistant HDAC5 mutant in the hearts of transgenic mice in vivo results in heart failure, presumably because MEF2 (or other HDAC targets) requires a basal level of activity for cardiac homeostasis.

Not only do HATs and class II HDACs compete for association with MEF2, there is also competition between MEF2 and calmodulin (CaM) for association with class II HDACs (Figure 2). Structural analysis of HDAC4 and -5 revealed a CaM-binding motif within the MEF2-binding regions of these proteins. On binding of Ca$^{2+}$/CaM, the Ca$^{2+}$/CaM complex associates with class II HDACs, thereby displacing MEF2 and providing a signal in addition to HDAC phosphorylation to derepress MEF2 target genes. In addition, CaM also interacts with Cabin1, another transcriptional repressor of MEF2. As a consequence of an increase in intracellular calcium concentration, CaM competes with MEF2 for binding to Cabin1, resulting in the dissociation of MEF2 from its repressor Cabin1 and, consequently, in MEF2 activation. Cabin1 docks on the same region of MEF2 as class II HDACs and represses MEF2 activity by (1) recruiting the mSin3 corepressor and class I HDACs to MEF2 target genes and (2) by displacing p300 and NFAT from MEF2.

The crystal structure of the MEF2 DNA-binding domain associated with Cabin1 and class II HDACs has been solved, providing detailed structural information that may allow the eventual development of specific small molecular modulators of these interactions.

**Class II HDAC Kinases**

The kinase(s) that phosphorylate class II HDACs have become the focus of intense interest because they serve to connect extracellular stimuli with the genome by governing the nuclear localization and functions of class II HDACs. Class II HDACs share high amino acid homology in the regions surrounding the phosphorylation sites in their N-terminal domains and all contain docking sites for 14-3-3 proteins. CaM kinases, which have been implicated in hypertrophy and heart failure in rodents and humans, phosphorylate the signal-responsive serines in class II HDACs, resulting in their dissociation from MEF2 and nuclear export in a 14-3-3 protein- and CRM1-dependent manner (Figure 3). There is also increasing evidence that specific CaMK isoforms, such as CaMKII, which appears to play a critical role in pathological cardiac hypertrophy, selectively target specific class II HDACs. CaMKII binds and phosphorylates HDAC4 but not other class II HDACs because HDAC4 contains a unique CaMKII docking site that is not present in other class II HDACs (Backs J., Backs T., Song K., Olson E.N., unpublished data, 2005). Interestingly, there is also evidence that HDAC4 can confer CaMKII responsiveness to other class II HDACs through heterodimerization (Backs J., Backs T., Song K., Olson E.N., unpublished data, 2005).
Recently, we and others have shown that protein kinase D (PKD) also transmits hypertrophic signals from G-protein-coupled receptors to the regulatory phosphorylation sites in class II HDACs with consequent induction of cardiac hypertrophy. PKD, which is phosphorylated and activated by protein kinase C (PKC), physically associates with HDAC5 and promotes phosphorylation of the 14-3-3 binding sites. Importantly, small molecule inhibitors that target PKC and PKD, but not CaMK, abolish agonist-mediated nuclear export of HDAC5 in cardiac myocytes, which suggests a predominant role for this pathway in the control of HDAC5 in the heart.

Expression Screen for Regulators of HDAC Phosphorylation

In an effort to identify regulators of HDAC phosphorylation, we designed a eukaryotic expression screen in which a GAL4-dependent luciferase reporter was expressed in COS cells with the N-terminal regulatory domain of HDAC5 fused to the DNA-binding domain of yeast GAL4 and 14-3-3 fused to the transcription activation domain of the viral coactivator VP16. In transfected COS cells, HDAC5 is not phosphorylated, so it cannot associate with 14-3-3–VP16. Transfection of COS cells with a cDNA library results in activation of luciferase expression by cDNAs encoding HDAC5 kinases or modulators of such kinases that create 14-3-3 docking sites for HDAC5 in cardiac myocytes, which suggests a predominant role for this pathway in the control of HDAC5 in the heart.

This screen has yielded a plethora of regulators of HDAC5 phosphorylation, including G-protein–coupled receptors, kinases, regulators of Rho signaling, and transcriptional regulators that we believe to control the expression of HDAC5 kinases. Among the strong inducers of HDAC5 phosphorylation identified in this screen were G-protein–coupled receptors for lysophosphatidic acid and sphingosine-1-phosphate, which have been implicated in cardiac hypertrophy as well as cardiovascular development. Activators of Rho signaling, such as RhoA, RhoC, and Rho guanine nucleotide exchange factors were also discovered as powerful inducers of HDAC5 phosphorylation in this assay. The mechanism whereby Rho signaling leads to HDAC phosphorylation remains to be defined. The possible involvement of Rho kinase in this process warrants further consideration.

In addition, we identified microtubule-associating regulatory kinase 2 (Mark2) as a potent HDAC kinase. Mark2, which is highly expressed in the heart, phosphorylates microtubule-associates proteins triggering microtubule disruption. Given the influence of the cytoskeleton on cardiac function, it will be of interest to further explore the potential functions of Mark2 in the heart and its possible role in connecting signals from the cytoskeleton with the nucleus via HDAC phosphorylation.

Other Posttranslational Modifications of Class II HDACs

Besides phosphorylation-dependent nuclear export of class II HDACs, there is increasing evidence that sumoylation and caspase-dependent proteolysis modify class II HDAC function. Self-aggregated HDAC4 and MTR are covalently modified by SUMO1, which likely takes place at the nuclear pore complex. Recently, class II HDACs have been shown to induce sumoylation of MEF2 resulting in a enhanced repression of MEF2 activity. Evidence was provided that sumoylation of HDAC4 itself inhibits HDAC4-induced sumoylation of the transcriptional activation domain of MEF2. Thus, SUMO modification of HDAC4 relieves MEF2 from HDAC4 repression. Importantly, HDAC4 promotes sumoylation on a lysine residue that is also subject to acetylation by CBP, a close homologue of p300. Interestingly, this lysine is deacetylated specifically by the class III HDAC, SIRT1. These findings provide further insights into the mechanisms whereby class II HDACs compete with HATs to regulate MEF2 activity and how class III HDACs may control these processes. The observation that sumoylation of a class II HDAC itself negatively regulates these MEF2 modifications raises interesting questions about how sumoylation of class II HDACs is regulated.

Limited proteolysis of proteins is known to affect their function. Recently, caspase was shown to cleave HDAC4 at aspartic acid 289, disjoining the C-terminal half, which shuttles to the cytosol, from the N-terminal half, which accumulates into the nucleus. Binding of class II HDACs to MEF2 is mediated by the N-terminal extension of HDACs. Likewise, the N-terminal cleavage product was shown to act as a strong repressor of MEF2. Of note, the N-terminal cleavage product contains only 1 of the 2 conserved serines that serve as 14-3-3 protein docking sites, suggesting that this cleavage product is less responsive to kinase signaling. Thus, these findings provide a mechanism by which the MEF2-specific repressive function of class II HDACs is potentiated independently of HDAC phosphorylation. More work is warranted to investigate if cleavage or the inhibition of cleavage of class II HDACs also controls cardiac growth.

Implications for Other Cell Types

The ability of class II HDACs to control cardiac growth and remodeling appears to reflect a general mechanism that operates in diverse cell types during embryogenesis and postnatal development. For example, HDAC4 null mice display premature ossification of developing bones attributable to ectopic and early onset chondrocyte hypertrophy, mimicking the phenotype that results from constitutive chondrocyte expression of Runx2, a transcription factor necessary for chondrocyte hypertrophy. HDAC4, which is expressed in prehypertrophic chondrocytes, regulates chondrocyte hypertrophy and endochondral bone formation by interacting with and inhibiting the activity of Runx2. Conversely, overexpression of HDAC4 in proliferating chondrocytes in vivo inhibits chondrocyte hypertrophy and differentiation, mimicking a Runx2 loss-of-function phenotype. These results establish HDAC4 as a central regulator of chondrocyte hypertrophy and skeletogenesis and support the idea that class II HDACs govern cellular growth in general. Recent studies have also revealed functions for class II HDACs in patterning of the aortic arch and angiogenesis (unpublished results).
Paradoxical Effects of HDAC Inhibitors on Cardiac Growth

Several chemical inhibitors of HDACs have been identified, including trichostatin A (TSA), sodium butyrate, and HC-toxin. Based on the repressive effects of class II HDACs on cardiac growth and fetal gene activation, we anticipated that such inhibitors would promote cardiac hypertrophy by mimicking the effect of HDAC deletion. Paradoxically, however, HDAC inhibitors impose a dose-dependent blockade to hypertrophy and fetal gene activation in a manner that correlates with increased histone acetylation.97 In a recent study, it was demonstrated that inhibition of HDACs by TSA upregulates α-MHC expression in cultured cardiac myocytes, as well as in an in vivo model of hypothyroid rats, whereas it downregulates the expression of α- and β-tubulins and prevents the induction of tubulins in response to the hypertrophic agonist angiotensin II.94 HDAC inhibitors therefore have the potential not only to antagonize deleterious cardiac growth but also to improve contractility in the failing heart.

How might the antigrrowth effect of HDAC inhibitors then be explained? MITR, the splicing variant of HDAC9 that contains only the N-terminal extension of class II HDACs but lacks the catalytic domain, can repress MEF2 as efficiently as the full-length HDAC9 protein,41 indicating that the deacetylase domain is dispensable for the antigrrowth effect of class II HDACs. However, it has also been reported that class II HDACs regulate transcription by bridging the enzymatically active SMRT/N-CoR–HDAC3 complex95 and that MITR recruits HDAC150 (Figure 2). Therefore, it cannot be ruled out that class II HDACs recruit the deacetylase activity of class I HDACs to exert their fully repressive transcriptional activity.

We propose that distinct HDACs play positive or negative roles in the control of cardiac growth by regulating opposing sets of target genes via their interactions with different sets of transcription factors.98 Given the antigrrowth function of class II HDACs, class I HDACs might induce cardiac growth. Of note, the HDAC inhibitors used in the studies mentioned above do not antagonize NAD-dependent class III HDACs. Therefore, we speculate that class I HDACs are required for repression of genes whose products repress hypertrophy. We can imagine at least three possibilities. (1) Class I HDACs may be required to block expression of genes that encode repressors of cardiac growth. Accordingly, inhibition of these HDACs could result in derepression of such antihypertrophic genes and a consequent block to hypertrophy. (2) Class I HDACs could result in derepression of such antihypertrophic repressors of cardiac growth. Accordingly, inhibition of these could result in derepression of such antihypertrophic genes and a consequent block to hypertrophy. (3) Class I HDACs to exert their fully repressive transcriptional activity.

Potential Roles of Class I HDACs in Cardiac Growth

Based on the phenotype of HDAC1 knockout mice, a role for this class I HDAC in the control of growth has been demonstrated.100 HDAC1 null animals die before embryonic day 10.5 apparently because of severe proliferation defects, indicated by elevated levels of the cyclin-dependent kinase inhibitors p21(WAF1/CIP1) and p27(KIP1). Intriguingly, p21, which is upregulated in cancer cells treated with HDAC inhibitors,101 has been implicated as a repressor of cardiac hypertropy.102,103 Whereas HDAC1 is not expressed in the heart before embryonic day 10.5, it is detectable in the adult heart.104 Therefore, inhibition of HDAC1 in postnatal cardiac myocytes might upregulate p21 and thereby block agonist-induced hypertrophic responses in these cells. Other components of antigrrowth networks may also be controlled by class I HDACs, including the myocyte-enriched calcineurin interacting protein (MCIP),105 glycogen-synthase kinase-3β,106,107 A20,108 phospholipase A2,109 PKG,110 c-Jun N-terminal kinase (JNK),111,112 or, as an interesting possibility, class II HDACs.

A recent study implicates another class I HDAC, HDAC2, in cardiac hypertropy.43 Cardiac overexpression of a heart-enriched nuclear protein, homeodomain-only protein (HOP), induces hypertrophic growth and subsequent heart failure. HOP associates with and represses SRF113,114 apparently by recruiting HDAC2.43 In the same study, it was reported that HDAC inhibitors could block HOP-induced cardiac growth, suggesting HOP as a potential target of HDAC inhibitor-mediated alleviation of cardiac hypertropy.

Regardless of the precise mechanism, the fortuitous discovery that HDAC inhibitors prevent cardiac hypertropy and normalize cardiac gene expression in the face of stress points to intriguing possibilities for the use of such inhibitors in the treatment of hypertrophy and heart failure in humans.

Potential Roles of Class III HDACs in Cardiac Growth

Class III HDACs function in a wide array of cellular processes, including gene silencing, longevity, and DNA damage repair. One member of this family, Sir2α, is expressed in nuclei of cardiac myocytes. In a recent study,115 the effects of Sir2 inhibition were compared with the effects of TSA on cardiomyocytes. In contrast to TSA, the Sir2 inhibitors nicotinamide and sirtinol as well as expression of dominant-negative Sir2α induced myocyte apoptosis, which was inhibited in the presence of dominant-negative p53, suggesting that Sir2α inhibition causes apoptosis through p53. Overexpression of Sir2α protected cardiac myocytes

including factors implicated in cardiac remodeling, such as tubulin and GATA transcription factors.34,98

In an effort to identify target genes of HDACs in cardiomyocytes, Kaneda et al performed genome-wide screening by differential chromatin scanning in the absence and presence of TSA.99 Interestingly, the acetylation status of a subset of HDAC target genes was also affected by the hypertrophic agonist cardiotoxin-1, supporting the notion that hypertrophic genes are indeed regulated by histone deacetylation.

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from apoptosis in response to serum starvation and significantly increased the size of cardiac myocytes. Furthermore, Sir2 expression was increased significantly in hearts from dogs with heart failure induced by rapid pacing superimposed on stable, severe hypertrophy. Thus, an increase in Sir2 expression during heart failure may play a cardioprotective role in vivo.

**Concluding Remarks**

Based on in vivo and in vitro models of cardiac hypertrophy and remodeling, it is apparent that histone-modifying enzymes act as key regulators of pathological cardiac growth by serving as nodal points in the transmission of cardiac stress signals to a progrowth gene program. Given the multiplicity of distinct HDACs, there is a need to identify specific functions of each and to develop small molecule inhibitors that can selectively modulate the activities of individual HDAC isoforms. The further generation of mice lacking individual HDAC genes will facilitate the identification of such inhibitors and promises to continue to reveal unexpected functions of HDACs in vivo. Nevertheless, HDAC inhibitors are already in clinical development for treatment of cancer.\(^{116}\)

Thus, the discovery that these HDAC inhibitors repress cardiac hypertrophy in the face of stress may ultimately impact the treatment of heart failure in humans.

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