SYMPOSIUM REVIEW

Roles and post-translational regulation of cardiac class IIa histone deacetylase isoforms

Kate L. Weeks and Metin Avkiran

The Journal of Physiology

Abstract   Cardiomyocyte hypertrophy is an integral component of pathological cardiac remodelling in response to mechanical and chemical stresses in settings such as chronic hypertension or myocardial infarction. For hypertrophy to ensue, the pertinent mechanical and chemical signals need to be transmitted from membrane sensors (such as receptors for neurohormonal mediators) to the cardiomyocyte nucleus, leading to altered transcription of the genes that regulate cell growth. In recent years, nuclear histone deacetylases (HDACs) have attracted considerable attention as signal-responsive, distal regulators of the transcriptional reprogramming that in turn precipitates cardiomyocyte hypertrophy, with particular focus on the role of members of the class IIa family, such as HDAC4 and HDAC5. These histone deacetylase isoforms appear to repress cardiomyocyte hypertrophy through mechanisms that involve protein interactions in the cardiomyocyte nucleus, particularly with pro-hypertrophic transcription factors, rather than via histone deacetylation. In contrast, evidence indicates that class I HDACs promote cardiomyocyte hypertrophy through mechanisms that are dependent on their enzymatic activity and thus sensitive to pharmacological HDAC inhibitors. Although considerable progress has been made in understanding the roles of post-translational modifications (PTMs) such as phosphorylation, oxidation and proteolytic cleavage in regulating class IIa HDAC localisation and function, more work is required to explore the contributions of other PTMs, such as ubiquitination and sumoylation, as well as potential cross-regulatory interactions between distinct PTMs and between class IIa and class I HDAC isoforms.

Kate L. Weeks, PhD, studied biochemistry and molecular biology at the University of Melbourne before undertaking a PhD on the protective effects of exercise and PI3K signaling in cardiovascular disease at the Baker IDI Heart and Diabetes Institute, under the supervision of Associate Professor Julie McMullen. She was awarded an Overseas Research Fellowship from the National Heart Foundation of Australia in 2012 to undertake postdoctoral training in Professor Avkiran's laboratory, where she is investigating the roles of histone deacetylases and protein phosphatases in pathological cardiac remodelling. **Metin Avkiran**, PhD DSc, is Professor of Molecular Cardiology and Deputy Head of Cardiovascular Division at King's College London British Heart Foundation Centre of Research Excellence, and currently serves as President of the International Society for Heart Research. His laboratory's research focuses on studying the molecular signaling mechanisms that regulate cardiac function in health and disease.

Introduction

Histone deacetylases (HDACs) are an ancient family of enzymes that catalyse the removal of acetyl groups from the ε-amino group of specific acetyl lysine residues within their protein substrates. Deacetylation of histones in nucleosomes induces chromatin condensation, which represses transcription by preventing binding of transcription factors and other components of the transcriptional machinery to gene promoter and enhancer regions. Conversely, acetylation of histones by histone acetyltransferases (HATs) induces chromatin relaxation, resulting in increased gene transcription. Thus, HDACs and HATs serve as important and opposing epigenetic regulators of gene expression.

Of the four classes of non-sirtuin HDACs (I, IIa, IIb and IV; see Fig. 1), class I and IIa are the best studied with regard to cardiac biology and pathology. Genetically modified mouse models and the use of pharmacological HDAC inhibitors in experimental models of cardiovascular disease have revealed important roles for both class I and IIa HDACs in the regulation of cardiac structure and function (see Tables 1 and 2). Administration of small molecule HDAC inhibitors, such as trichostatin A (TSA), suberanilohydroxamic acid (SAHA) and valproic acid, blocks pathological cardiac changes in a range of experimental settings (see Table 1). For example, administration of TSA 2 weeks after the induction of pressure overload reversed cardiac hypertrophy in mice (Kee *et al.* 2006), and administration of SAHA at reperfusion reduced infarct size and improved cardiac function in a rabbit model of ischaemia–reperfusion injury (Xie *et al.* 2014). The pharmacophore of most HDAC inhibitors developed to date contains a zinc-binding group that chelates the zinc ion required for catalytic activity (Bertrand, 2010; McKinsey, 2011). As the principal mechanism by which class IIa HDACs regulate cardiomyocyte hypertrophy is not dependent on their catalytic activity (Zhang *et al.* 2002*a*), and class IIa HDACs are relatively insensitive to HDAC inhibitors (Lahm *et al.* 2007; Bradner *et al.* 2010), the efficacy of HDAC inhibitors in attenuating pathological cardiac remodelling in animal models is likely to be a consequence of their inhibition of class I HDAC isoforms. Although we are not aware of conclusive clinical evidence regarding the therapeutic potential of HDAC inhibitors in the context of heart failure, in patients with epilepsy treatment with valproic acid appears to be associated with a reduced risk of myocardial infarction (Olesen *et al.* 2011).

Class IIa HDACs are endogenous inhibitors of cardiomyocyte hypertrophy and are therefore referred to as 'anti-hypertrophic'. Class IIa HDACs suppress cardiomyocyte hypertrophy when localised in the nucleus by repressing the activity of pro-hypertrophic transcription factors, such as members of the myocyte enhancer factor-2 (MEF2) family (Lu *et al.* 2000; Zhang *et al.* 2002*a*), and by recruiting epigenetic regulators such as class I HDACs and histone methyltransferases to DNA promoter regions (Fischle *et al.* 2002; Zhang *et al.* 2002*b*; Hohl *et al.* 2013). Nuclear export of class IIa HDACs permits the induction of hypertrophic genes by alleviating their repressive interactions with transcription factors and allowing the recruitment of HATs and histone demethylases. The relative importance of and interplay
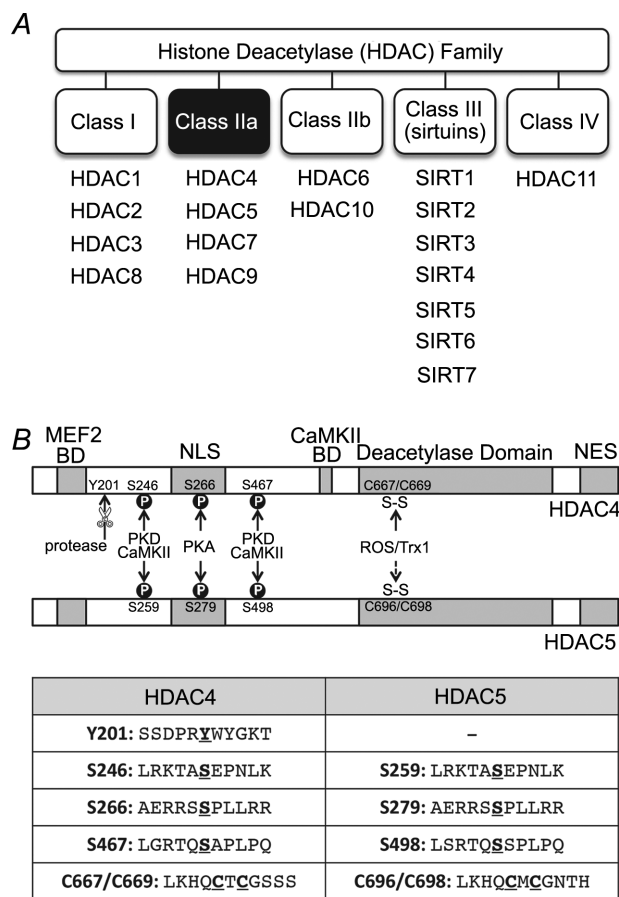

Figure 1. Histone deacetylase classification
A, the histone deacetylase (HDAC) family is comprised of four classes. Class II HDACs are further categorised into subclasses. Class I and class IIa HDAC isoforms are the best characterised with regards to cardiomyocyte hypertrophy and cardiac remodelling. This review focuses primarily on the class IIa subclass. *B*, HDAC4 and HDAC5 are highly homologous, sharing a conserved MEF2 binding domain (BD), a nuclear localisation signal (NLS), a deacetylase domain and a nuclear export sequence (NES). Phosphorylation by protein kinases at conserved serine residues within the N-terminal region is a key determinant of subcellular localisation. Oxidation of conserved cysteine residues within the deacetylase domain is a phosphorylation-independent post-translational mechanism that can also affect HDAC4/5 localisation. Proteolytic cleavage of HDAC4 at a unique site results in the generation of an N-terminal fragment that represses MEF2 activity. HDAC4 also contains a CaMKII binding domain, which is not present in HDAC5.

Table 1. HDAC inhibitors attenuate pathological remodelling in experimental models of cardiac injury

Inhibitor	Isoform selectivity	Effect of inhibitor on cardiac phenotype
Trichostatin A (TSA)	Pan HDACi	Blunted cardiac hypertrophy induced by chronic isoprenaline infusion in mice (Kook et al. 2003)
		Blunted cardiac hypertrophy induced by ascending aortic banding in mice and rats (Kee et al. 2006)
		Prevented cardiac hypertrophy induced by chronic angiotensin II infusion in mice (Kee et al. 2006)
		Reversed established cardiac hypertrophy induced by ascending aortic banding in mice (Kee et al. 2006)
		Blunted left ventricular hypertrophy and attenuated cardiac fibrosis induced by transverse aortic banding in mice (Kong et al. 2006)
		Reduced infarct size in mouse models of ischaemia–reperfusion injury (Granger et al. 2008; Xie et al. 2014)
		Reduced infarct size and improved functional parameters in Langendorff isolated perfused mouse hearts (Zhang et al. 2010)
		Attenuated pathological remodelling and improved survival in a mouse model of myocardial infarction (Zhang et al. 2012)
Valproic acid (VPA)	Weak class I HDACi	Blunted cardiac hypertrophy induced by ascending aortic banding in mice (Kee et al. 2006)
		Prevented/reversed cardiac hypertrophy and fibrosis in DOCA-salt hypertensive rats (Kee et al. 2013)
		Attenuated left ventricular remodelling and improved systolic function in a rat model of myocardial infarction (Lee et al. 2007)
		Reduced blood pressure and prevented the development of left ventricular hypertrophy and fibrosis in a genetic rat model of hypertension (Cardinale et al. 2010)
		Attenuated right ventricular hypertrophy and fibrosis induced by pulmonary artery banding in rats (Cho et al. 2010)
		Attenuated left and right ventricular hypertrophy in a rat model of pulmonary hypertension induced by monocrotaline injection (Cho et al. 2010)
		Prevented cardiac hypertrophy induced by chronic angiotensin II infusion in mice (Kee et al. 2006)
Scriptaid	Pan HDACi	Blunted left ventricular hypertrophy induced by transverse aortic banding in mice (Kong et al. 2006)
		Reduced infarct size in a mouse model of ischaemia–reperfusion injury (Granger et al. 2008)
SAHA	Pan HDACi	Reduced blood pressure and attenuated left ventricular hypertrophy and fibrosis in DOCA-salt hypertensive rats (Iyer et al. 2010)
		Reduced infarct size and improved systolic function in a mouse model of ischaemia–reperfusion injury (Xie et al. 2014)
		Reduced infarct size and improved systolic function in rabbits when administered prior to ischaemia–reperfusion injury or at reperfusion (Xie et al. 2014)
Apicidin	Class I HDACi	Blunted left ventricular hypertrophy, attenuated cardiac fibrosis and improved systolic function in mice subjected to transverse aortic banding (Gallo et al. 2008)
SK-7041	Class I/pan HDACi	Prevented cardiac hypertrophy induced by ascending aortic banding in mice (Kee et al. 2006)

between class I and class IIa HDAC isoforms in regulating cardiomyocyte hypertrophy and other components of pathological cardiac remodelling such as fibrosis are the subject of intense research. The recent discovery that cardiac class IIa HDACs may regulate the acetylation status and activity of class I HDACs (Eom et al. 2014) reveals a new layer of complexity that warrants further investigation as part of this effort.

HDACs are subject to various post-translational modifications, including phosphorylation, proteolytic cleavage, oxidation, ubiquitination and sumoylation. This review will focus on the roles and regulation of class IIa HDACs in the context of cardiomyocyte hypertrophy and cardiac remodelling, with emphasis on post-translational modifications that alter the function of individual members of this class. For broader reviews

Table 2. Loss- and gain-of-function HDAC mouse models

Isoform	Mouse model	Reported cardiac phenotype	References
HDAC1	Global knockout	Embryonic lethality between E9.5 and E10.5 due to proliferation defects.	Lagger *et al.* 2002
	Global knockout	Embryonic lethality by E9.5.	Montgomery *et al.* 2007
	Cardiomyocyte-specific knockout	No gross cardiac abnormalities basally. Comparable hypertrophic response to chronic isoprenaline administration and transverse aortic constriction (TAC) as littermate controls.	Montgomery *et al.* 2007
	Cardiomyocyte-specific transgenic	No evidence of cardiac hypertrophy at 2–3 months of age.	Trivedi *et al.* 2007
HDAC2	Global knockout	Increased cardiomyocyte hyperplasia during perinatal period. Resistant to isoprenaline- and TAC-induced cardiac remodelling. Resistant to cardiac hypertrophy induced by transgenic expression of the homeobox gene, *Hop.*	Trivedi *et al.* 2007
	Global knockout	Neonatal lethality due to ventricular defects. Increased cardiomyocyte hyperplasia and apoptosis in P1 hearts. Bradycardia.	Montgomery *et al.* 2007
	Cardiomyocyte-specific knockout	No gross cardiac abnormalities. Comparable hypertrophic response to chronic isoprenaline administration as littermate controls.	Montgomery *et al.* 2007
	Cardiomyocyte-specific transgenic	Developed pathological cardiac hypertrophy by 8 weeks of age. Further increase in heart mass when crossed with transgenic mice overexpressing the homeobox gene, *Hop.*	Trivedi *et al.* 2007
HDAC1/2	Double cardiomyocyte-specific knockout	Postnatal lethality due to the development of dilated cardiomyopathy and cardiac arrhythmias. Phenotype attributed to dysregulation of genes encoding ion channels and sarcomeric proteins.	Montgomery *et al.* 2007
HDAC3	Global knockout	Embryonic lethality by E9.5.	Montgomery *et al.* 2008
	Cardiomyocyte-specific knockout	Developed significant left ventricular hypertrophy, atrial enlargement, interstitial fibrosis and systolic dysfunction by 12 weeks of age. Phenotype attributed to dysregulation of metabolic genes.	Montgomery *et al.* 2008
	Cardiomyocyte-specific transgenic	Significant thickening of the ventricular walls and diminished lumen volume at birth due to increased cardiomyocyte proliferation. Normalisation of heart morphology by 2–3 months of age. Similar hypertrophic response to chronic isoprenaline treatment as wild-type littermates.	Trivedi *et al.* 2007; Trivedi *et al.* 2008
HDAC4	Global knockout	Lethality prior to weaning due to severe growth retardation resulting from premature ossification of developing bones. No obvious cardiac phenotype.	Vega *et al.* 2004
	Cardiomyocyte-specific knockout	Cardiac phenotype has not been extensively described. Normal dP/dt_{max}, dP/dt_{min} and cardiac output in isolated working hearts. Elevated *Nppa* expression (marker of pathological cardiac hypertrophy) compared with wild-type littermates.	Hohl *et al.* 2013
HDAC5	Global knockout	Developed cardiac hypertrophy by 8 months of age. Displayed exaggerated hypertrophic response to TAC and transgenic expression of activated calcineurin. Similar hypertrophic response to wild-type littermates in response to chronic isoprenaline administration.	Chang *et al.* 2004
HDAC6	Global knockout	Protected from developing systolic dysfunction in response to chronic angiotensin II infusion or TAC, despite the same degree of left ventricular hypertrophy and fibrosis as wild-type littermates.	Demos-Davies *et al.* 2014

Continued

Table 2. Continued

Isoform	Mouse model	Reported cardiac phenotype	References
HDAC7	Global knockout	Embryonic lethality due to cardiovascular defects.	Chang *et al.* 2006
HDAC8	Global knockout	Perinatal lethality due to skull defects. No obvious cardiovascular abnormalities.	Haberland *et al.* 2009
HDAC9	Global knockout	Developed cardiac hypertrophy by 8 months of age. Displayed exaggerated hypertrophic response to TAC and transgenic expression of activated calcineurin. Similar hypertrophic response to wild-type littermates in response to chronic isoprenaline infusion.	Zhang *et al.* 2002*a*; Chang *et al.* 2004
HDAC5/9	Double global knockout	High incidence of embryonic and perinatal lethality due to haemorrhages and ventricular defects. Cardiac hypertrophy was observed in the small percentage of double knockout mice that survived until adulthood.	Chang *et al.* 2004

covering other HDAC classes and their potential as targets for cardiac pharmacotherapy, the reader is referred to recent review articles from the laboratories of two leading investigators in the field, Timothy McKinsey (McKinsey, 2012) and Joseph Hill (Xie & Hill, 2013).

Class IIa HDACs

The HDAC superfamily can be divided into four classes, based on sequence similarity and functional domains (see Fig. 1A). Class I and II HDACs are conserved amongst prokaryotes and eukaryotes, while class IV HDACs are present in all organisms except fungi (Gregoretti *et al.* 2004). Class III HDACs are more commonly known as sirtuins and constitute an unrelated class of NAD-dependent deacetylases (Landry *et al.* 2000).

The class II HDACs can be further divided into two subclasses. Class IIa includes HDAC4, HDAC5, HDAC7, HDAC9 and a truncated splice variant of HDAC9 known as MEF2-interacting transcriptional repressor (MITR) or HDAC-related protein (HDRP). Class IIb includes HDAC6 and HDAC10. Unlike class I HDACs, which are widely expressed (Yang *et al.* 1997; Hu *et al.* 2000), class IIa HDAC expression is restricted to a subset of tissues and cell types. In humans and mice, expression of HDAC4, HDAC5 and HDAC9 is highest in heart, brain and skeletal muscle (Grozinger *et al.* 1999; Zhou *et al.* 2001), whereas HDAC7 expression is restricted to endothelial cells within the heart, lung and skeletal muscle (Kao *et al.* 2000; Chang *et al.* 2006). Expression of MITR/HDRP is highest in heart and brain (Zhou *et al.* 2000; Zhang *et al.* 2001). Serial analysis of gene expression (SAGE) has identified HDAC5 as the most abundant HDAC transcript in the adult human heart (de Ruijter *et al.* 2003).

Structure and function of class IIa HDACs

With the exception of MITR/HDRP, which lacks a deacetylase domain, class IIa HDACs consist of a C-terminal deacetylase domain and an extensive N-terminal regulatory domain. Class IIa HDACs have very low deacetylase activity towards native acetyl lysine residues, despite being capable of deacetylating the synthetic substrate trifluoro acetyl lysine *in vitro* (Bradner *et al.* 2010). This is due to the replacement of a conserved tyrosine residue with a histidine residue within the deacetylase domain of class IIa HDACs, which markedly impairs catalytic activity (Lahm *et al.* 2007). It has been proposed that the deacetylase domain of class IIa HDACs functions as a binding domain, as class IIa HDACs bind acetyl lysine substrates with comparable affinity to class I and IIb HDACs, despite being unable to deacetylate these substrates (Bradner *et al.* 2010). It has also been suggested that deacetylase activity associated with class IIa HDACs purified from cells or tissue most likely arises from the presence of HDAC3 in class IIa HDAC repressor complexes (Fischle *et al.* 2002).

As illustrated in Fig. 1B, the regulatory domain of class IIa HDACs contains a MEF2 binding motif, a nuclear localisation signal (NLS), and several conserved serine residues that act as docking sites for 14-3-3 proteins when phosphorylated. The best characterised HDAC kinases in the context of cardiac biology and pathology are Ca^{2+}–calmodulin-dependent protein kinase II (CaMKII) and protein kinase D (PKD). However, in various cell types and *in vitro* settings, class IIa HDACs appear to be substrates also for protein kinase A (PKA), G protein-coupled receptor kinase-5, microtubule affinity-regulating kinases, salt-inducible kinases and AMP-dependent protein kinases (Chang *et al.* 2005; Dequiedt *et al.* 2006; Berdeaux *et al.* 2007; McGee *et al.* 2008; Ha *et al.* 2010; Zhang *et al.* 2011; Walkinshaw *et al.* 2013*a*). Association with 14-3-3 proteins masks the NLS and sequesters HDACs in the cytoplasm, following unveiling of a C-terminal nuclear export sequence and transport out of the nucleus by the nuclear export receptor CRM1 (Grozinger & Schreiber, 2000; McKinsey *et al.* 2001; Harrison *et al.*

2004). Nucleo-cytoplasmic shuttling appears to be the primary mechanism regulating class IIa HDAC function, as exclusion from the nucleus prevents the repressive interaction of HDACs with transcription factors, resulting in increased transcription of target genes (Miska *et al.* 1999; Lu *et al.* 2000; Harrison *et al.* 2004). In the case of MEF2, nuclear export of class IIa HDACs permits interaction with the HAT, E1 binding protein p300 (p300), which promotes pro-hypertrophic gene transcription via its effects on histone acetylation and the formation of enhanceosomes (Wei *et al.* 2008; He *et al.* 2011). p300 is also capable of directly acetylating MEF2, which enhances DNA binding and transcriptional activity (Ma *et al.* 2005).

Regulation of cardiac remodelling by class IIa HDACs

The majority of the evidence implicating class IIa HDACs in the regulation of cardiomyocyte hypertrophy and cardiac remodelling comes from genetically modified mouse models (see Table 2), most of which have been generated in the laboratory of Eric Olson, and *in vitro* studies in primary and immortalised cell lines. Heterologously expressed HDAC4 and HDAC5 are predominantly nuclear, but accumulate in the cytoplasm upon exposure to pro-hypertrophic stimuli, such as the α_1-adrenergic receptor agonist phenylephrine (PE) and endothelin-1 (ET-1) (Harrison *et al.* 2004; Vega *et al.* 2004*a*; Ago *et al.* 2008; Peng *et al.* 2009; Backs *et al.* 2011; Haworth *et al.* 2012; Chang *et al.* 2013). Blocking class IIa HDAC nuclear export with CRM1 inhibitors, such as leptomycin B or its derivatives, or by mutation of the HDAC 14-3-3 docking sites to non-phosphorylatable alanine residues, prevents cardiomyocyte hypertrophy (Harrison *et al.* 2004; Vega *et al.* 2004*a*; Monovich *et al.* 2009), providing strong evidence that such nuclear export is required for the induction of the hypertrophic response.

Mice globally deficient in either HDAC5 or HDAC9 were viable, but developed cardiac hypertrophy even in the absence of an imposed stress by approximately 8 months of age (Zhang *et al.* 2002*a*; Chang *et al.* 2004). In contrast, compound deletion of both *Hdac5* and *Hdac9* resulted in embryonic lethality due to haemorrhage and ventricular defects (Chang *et al.* 2004). The small percentage of double-knockout mice that survived to adulthood were severely growth retarded and displayed significant cardiac hypertrophy, demonstrating that HDAC5 and HDAC9 have important functions in embryonic and postnatal cardiac development.

Mice with individual deletion of *Hdac5* or *Hdac9* displayed an exaggerated hypertrophic response to pressure overload, induced by constriction of the thoracic aorta, suggesting that these HDAC isoforms function to limit cardiac enlargement following haemodynamic overload (Zhang *et al.* 2002*a*; Chang *et al.* 2004).

Both genotypes also displayed profound hypertrophic growth compared with wild-type littermates when crossed with transgenic mice expressing activated calcineurin, an important transducer of pro-hypertrophic signalling (Molkentin *et al.* 1998). The findings that HDAC5 and HDAC9 knockout mice respond similarly to hypertrophic stimuli and that HDAC5/9 double-knockout mice are embryonically lethal suggest that these isoforms are activated by similar signalling pathways and have overlapping functions. Functional redundancy of class IIa HDACs has been observed also in the context of fibre type switching in skeletal muscle; deletion of individual class IIa HDAC isoforms had no effect on the proportion of type I and type II fibres in mouse soleus muscle, whereas heterozygous or homozygous deletion of multiple isoforms (*Hdac4/5*, *Hdac5/9* or *Hdac4/5/9*) increased the percentage of type I fibres (Potthoff *et al.* 2007).

Mice with global deletion of *Hdac4* die prior to weaning due to severe growth retardation resulting from the premature ossification of developing bones (Vega *et al.* 2004*b*). Although no obvious cardiac abnormalities were observed in these mice, it was impossible to examine the effects of *Hdac4* deletion on stress-induced cardiac hypertrophy as the mice died prior to adulthood. Mice with cardiomyocyte-specific deletion of *Hdac4* have since been generated (Hohl *et al.* 2013), but the effects of hypertrophic stress on their cardiac phenotype have not been described in detail to date. In their recent collaborative study, the laboratories of Johannes Backs and Christoph Maack have used these mice, as well as tissue samples from failing and non-failing human myocardium, to investigate the epigenetic regulation of *Nppa* and *Nppb*, fetal genes encoding the atrial and B-type natriuretic peptides ANP and BNP, which are re-expressed during cardiac hypertrophy and heart failure (Hohl *et al.* 2013). Their findings support the hypothesis that class IIa HDACs repress MEF2-dependent gene transcription by recruiting the methyltransferase SUV39H1 and the adaptor protein heterochromatin protein 1 (HP1) to promoter regions, as part of a corepressor complex (Zhang *et al.* 2002*b*). Nuclear export of HDAC4 in response to elevated cardiac load may increase expression of ANP and BNP, not via effects on histone acetylation but via histone demethylation by JMJC domain-containing demethylases, following dissociation of HP1 and SUV39H1 (Hohl *et al.* 2013). Whether similar mechanisms operate in HDAC4-mediated regulation of other hypertrophic genes, or in the regulation of ANP and BNP expression by other class IIa HDACs, has not been reported.

Mice with global deletion of *Hdac7* die during embryogenesis due to cardiovascular defects (Chang *et al.* 2006). Conditional deletion of *Hdac7* in endothelial cells phenocopied global deletion, whereas mice with conditional deletion of *Hdac7* in cardiomyocytes were viable (Chang *et al.* 2006). Thus, any effect of HDAC7

on cardiac remodelling is likely to arise predominantly from its function in endothelial cells. In this context, PKD-mediated phosphorylation of HDAC7 following vascular endothelial growth factor (VEGF) stimulation of endothelial cells led to nuclear export of HDAC7, increased MEF2 activity and enhanced angiogenesis in an *ex vivo* assay (Ha *et al.* 2008*a*). Nuclear export of HDAC5 was shown to have a similar pro-angiogenic effect (Ha *et al.* 2008*b*). As insufficient angiogenesis is a critical determinant of the transition from compensatory cardiac hypertrophy to decompensated heart failure (Shiojima *et al.* 2005), it is likely that class IIa HDACs regulate pathological cardiac remodelling via their functions in multiple cell types, including endothelial cells, and not just in cardiomyocytes. It follows from this that any new therapies targeted at class IIa HDAC nucleocytoplasmic shuttling may need to exhibit cell specificity, in order to limit cardiomyocyte hypertrophy but not compromise angiogenesis during cardiac remodelling.

Post-translational modifications regulating class IIa HDAC function

Phosphorylation. HDAC5 contains at least 17 phospho-acceptor residues that are conserved across species, which suggests that phosphorylation is a fundamental post-translational modification regulating HDAC5 folding and function (Greco *et al.* 2011). The best characterised HDAC5 phosphorylation sites are Ser259 and Ser498, which flank the NLS, and Ser279, which lies within the NLS. Homologous sites are present in HDAC4 (see Fig 1B) and HDAC9. As mentioned previously, phosphorylation of Ser259 and Ser498 in HDAC5 by CaMKII or PKD induces nuclear export (McKinsey *et al.* 2000*a*,*b*; Vega *et al.* 2004*a*; Backs *et al.* 2006; Backs *et al.* 2008). In contrast, phosphorylation of Ser279 by protein kinase A (PKA) has been proposed to promote nuclear retention (Ha *et al.* 2010; Chang *et al.* 2013; Walkinshaw *et al.* 2013*b*), possibly by inducing a conformational change that prevents binding of 14-3-3 proteins at phosphorylated Ser259 and Ser498 (Ha *et al.* 2010).

Expression and activity of CaMKIIδ and PKD are elevated in failing human myocardium (Bossuyt *et al.* 2008), and both kinases contribute to pathological remodelling in rodents (Fielitz *et al.* 2008; Backs *et al.* 2009). HDAC4 contains a unique CaMKII docking site that promotes interaction with activated CaMKII and subsequent phosphorylation of Ser467 and Ser632 (Backs *et al.* 2006). HDAC5 lacks a CaMKII docking site, but can be phosphorylated by CaMKII when bound to HDAC4 via its coiled-coil domain (Backs *et al.* 2008). Nuclear CaMKIIδ phosphorylates HDAC5 following InsP$_3$-induced Ca^{2+} release from the nuclear envelope (Wu *et al.* 2006),

while cytosolic CaMKIIδ could maintain HDACs in a phosphorylated state once exported to the cytoplasm (Backs *et al.* 2006). PKD1, which is predominantly cytosolic under basal conditions, associates with the sarcolemma upon PE stimulation, then rapidly translocates to the nucleus where it phosphorylates HDAC5 (Bossuyt *et al.* 2011).

Intriguingly, recent evidence suggests that nuclear export of HDAC5 may occur independently of increases in Ser259 and Ser498 phosphorylation. Stimulation with the β-adrenergic receptor (β-AR) agonist isoprenaline (isoproterenol) induced nuclear export of HDAC5 in adult rat ventricular myocytes, but this was accompanied by reduced phosphorylation of the relevant 14-3-3 docking sites (Haworth *et al.* 2012). The mechanism(s) responsible for reduced HDAC5 phosphorylation in this experimental setting is not yet known. Of interest, all three subunits of the heterotrimeric protein phosphatase 2A (PP2A) holoenzyme co-immunoprecipitate with HDAC5 when stably expressed in HEK293 cells (Greco *et al.* 2011) and PP2A has been found to dephosphorylate HDAC4 *in vitro* (Paroni *et al.* 2008). Whether PP2A-mediated dephosphorylation contributes to reduced HDAC5 phosphorylation at Ser259 and Ser498 in response to β-adrenergic stimulation in cardiac myocytes, and the role of any such dephosphorylation in regulating the nuclear localisation of HDAC5, require further investigation.

The phosphorylation status of Ser279 appears to be a key determinant of HDAC5 localisation, as mutation to a phosphomimetic aspartic acid residue promoted nuclear accumulation and rendered HDAC5 resistant to nuclear export induced by PE or ET1 stimulation in neonatal rat and adult rabbit cardiac myocytes (Ha *et al.* 2010; Chang *et al.* 2013). Furthermore, mutation of Ser279 to alanine promoted cytoplasmic accumulation in U2OS cells, consistent with the hypothesis that phosphorylation of Ser279 promotes nuclear retention (Greco *et al.* 2011). However, no change in basal distribution was observed with a Ser279Ala HDAC5 mutant in adult rabbit cardiac myocytes (Chang *et al.* 2013). In the same study, it was proposed that phosphorylation of Ser279 by β-AR-mediated activation of PKA promotes nuclear import/retention of HDAC5, as isoprenaline and forskolin, an activator of adenylyl cyclase, induced nuclear import of wild-type HDAC5, but had no effect on the localisation of the Ser279Ala mutant (Chang *et al.* 2013). As noted above, the β-AR agonist isoprenaline has been reported to induce HDAC5 nuclear export and increased MEF2 activity in adult rat ventricular myocytes (Haworth *et al.* 2012). Thus the regulation of HDAC5 localisation and function by β-AR stimulation and the roles of phosphorylation/dephosphorylation at regulatory serine residues appear to warrant additional investigation.

With regards to adrenergic regulation of class IIa HDACs, very recent evidence suggests that increased

HDAC4/5 phosphorylation and consequent MEF2 activation following ET1 stimulation may occur primarily through stimulation of α_1- and β_1-ARs on cardio-myocytes, following activation of presynaptic ET_A receptors and subsequent inhibition of noradrenaline reuptake into sympathetic nerve terminals (Lehmann *et al.* 2014). This observation suggests that there is significant crosstalk between different neurohormonal stimuli in the regulation of class IIa HDACs, not only downstream of pertinent G protein-coupled receptors within cardio-myocytes but also through neuronal presynaptic receptors.

Proteolytic cleavage. An additional mechanism by which PKA may regulate the function of HDAC4 has been described in a study by the laboratories of Backs and Olson (Backs *et al.* 2011). HDAC4 contains a cleavage site (Tyr201), which is not present in other class IIa HDACs (see Fig. 1B), and activation of PKA leads to HDAC4 cleavage at this site by an as-yet-unidentified serine protease, resulting in the production of an N-terminal fragment (HDAC4-NT) that accumulates in the nucleus where it represses MEF2 activity. HDAC4-NT was detected in hearts from wild-type mice following isoprenaline administration over a four-hour period, but not in hearts from mice lacking the catalytic α-subunit of PKA, confirming that PKA-dependent generation of HDAC4-NT can occur *in vivo* downstream of β-AR stimulation. It has been suggested that this mechanism may allow cardiomyocytes to exhibit differential hyper-trophic responses to acute adrenergic activation in physio-logical stress situations and to sustained neurohormonal stimulation during prolonged periods of cardiac stress in disease (Backs *et al.* 2011). Accordingly, during acute β-AR stimulation, PKA-mediated generation of HDAC4-NT would rein in MEF2 activity, attenuating hypertrophic gene transcription. In settings of sustained neuro-hormonal stimulation, also involving other mediators such as ET-1, angiotensin II and reactive oxygen species (see Oxidation section below), CaMKII- and PKD-mediated, phosphorylation-dependent as well as phosphorylation-independent mechanisms of class IIa HDAC nuclear export would predominate, leading to MEF2 activation and pathological cardiac remodelling. In this context, selective stimulation of β-ARs is sufficient to induce cardiomyocyte hypertrophy and cardiac remodelling (Osadchii, 2007) and β-AR antagonists are clinically proven therapies for chronic heart failure (Bristow, 2011). It seems likely, therefore, that any HDAC4-NT-mediated anti-hypertrophic consequences of β-AR stimulation are indeed short lasting, and/or that mechanisms that are independent of class IIa HDACs make a predominant contribution to cardiomyocyte hyper-trophy and cardiac remodelling during chronic β-AR stimulation.

There is evidence that HDAC4 can be also cleaved by caspase-2 and caspase-3 at Asp289 (Paroni *et al.* 2004). The resulting N-terminal fragment augments apoptosis via the repression of serum response factor (SRF) and Runx2 (Liu *et al.* 2004; Paroni *et al.* 2004, 2007; Backs *et al.* 2011). Whether this proteolytic cleavage event occurs in the heart and has a functional role during cardiac stress responses has not been explored.

Oxidation. Junichi Sadoshima and colleagues have ident-ified oxidation as a novel phosphorylation-independent post-translational modification regulating subcellular localisation of HDAC4 in cardiomyocytes (Ago *et al.* 2008) and suggested that nicotinamide adenine dinucleotide phosphate oxidase 4 (Nox4) plays an important role in generating the relevant oxidative signal (Matsushima *et al.* 2013). Although the precise mechanism of oxidation-induced nuclear export is unknown, it has been proposed that formation of an intra-molecular disulphide bond between two conserved cysteine residues (Cys667 and Cys669 in HDAC4; see Fig. 1B) disrupts zinc coordination, leading to a conformational change that exposes the nuclear export signal to CRM1 (Ago *et al.* 2008). HDAC5 appears to be regulated in a similar manner, as isoprenaline-induced nuclear export of HDAC5 was blocked by overexpression of the disulphide oxidoreductase protein thioredoxin-1 or in the presence of the antioxidant N-acetylcysteine (Haworth *et al.* 2012).

Ubiquitination. Ubiquitination plays a critical role in cell homeostasis, regulating protein turnover by targeting proteins for degradation by the proteasome. Reversible ubiquitination of protein substrates also serves an important role in signal transduction and has been shown to modulate gene expression in several cardiac disease settings (Portbury *et al.* 2012). Although HDAC ubiquitination has not been studied in the heart, a study in skeletal muscle suggests that ubiquitination is an important post-translational modification that regulates class IIa HDAC function (Potthoff *et al.* 2007). In this study, ubiquitination and subsequent degradation of class IIa HDACs promoted the expression of slow twitch fibre-specific genes by alleviating their repressive interaction with MEF2 (Potthoff *et al.* 2007). Proteasomal inhibition in mice resulted in increased HDAC4 and HDAC5 expression and reduced MEF2 activity in various skeletal muscles, while Cre-mediated deletion of multiple class IIa HDACs resulted in a switch from fast- to slow-twitch fibres, demonstrating that ubiquitination is an important post-translational mechanism that maintains appropriate levels of HDAC expression. Whether ubiquitination of class IIa HDACs is important in cardiac biology is yet to be explored, but this seems likely.

Sumoylation. Sumoylation, the covalent attachment of small ubiquitin-like modifier (SUMO) proteins to target proteins, is emerging as an important post-translational mechanism regulating cardiovascular homeostasis (Kho *et al.* 2011; Wang *et al.* 2011). HDAC4 is sumoylated in HeLa cells, possibly as it enters the nucleus through the nuclear pore complex (Kirsh *et al.* 2002). Interestingly, interaction with HDAC4 or HDAC5 potentiates the sumoylation of MEF2, which renders MEF2 transcriptionally inactive, and ablation of the SUMO conjugation site in HDAC4 dampens the repressive effect of HDAC4 on MEF2-dependent gene transcription (Gregoire & Yang, 2005; Zhao *et al.* 2005). Whether altered sumoylation of class IIa HDACs plays a role in the pathogenesis of cardiovascular disease has not been investigated.

Summary

Figure 2 summarises the current state of knowledge regarding the opposing roles of class IIa and class I HDAC isoforms in regulating pathological cardiac remodelling and outlines the multiple post-translational modifications that have already been shown to regulate class IIa HDAC function, as well as those that warrant further
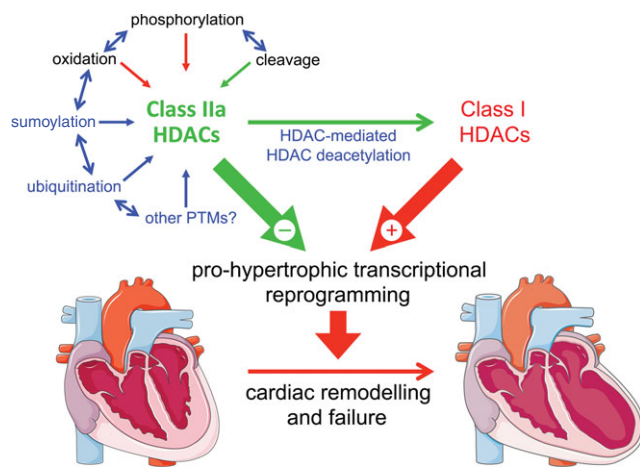
investigation, in this context. Over the past decade or more, substantial evidence has accumulated that class IIa HDAC isoforms such as HDAC4 and HDAC5 are important regulators of cardiomyocyte hypertrophy and pathological cardiac remodelling, principally through their repressive effects on MEF2 transcription factor activity when enriched in the nucleus. Importantly, the subcellular localisation, integrity and MEF2 interaction of class IIa HDAC isoforms appear to be regulated by multiple post-translational mechanisms such as phosphorylation, oxidation and proteolytic cleavage, and potentially sumoylation, ubiquitination and proteasomal degradation. Greater understanding of such mechanisms, and potential cross-regulatory interactions between them, may allow the development of new therapeutic approaches towards harnessing the 'anti-hypertrophic' actions of class IIa HDAC isoforms under conditions of chronic mechanical and neurohormonal stress and thereby attenuating pathological cardiac remodelling. This is particularly relevant given recent reports that class IIa HDACs can additionally regulate the expression and activity of 'pro-hypertrophic' class I HDACs (Spallotta *et al.* 2013; Eom *et al.* 2014). A greater understanding of the interplay between different HDAC classes at different stages of disease, as well as the interplay between HDACs and other epigenetic regulators, such as bromodomain and extra-terminal acetyl-lysine reader proteins (Anand *et al.* 2013; Spiltoir *et al.* 2013), is likely to aid the development of new targeted therapies for the prevention or reversal of pathological cardiac remodelling and heart failure.

Figure 2. A schematic diagram of the current state of play and avenues for future investigation
Class I and class IIa HDACs are now recognised as important and opposing regulators of pathological cardiac remodelling, through mechanisms that are either dependent on (class I) or independent of (class IIa) histone acetyl lysine deacetylase activity. The figure also illustrates various post-translational modifications (PTMs) of class IIa HDACs whose role in regulating pathological cardiac remodelling is either relatively well established (black) or requires further investigation (blue). Potential interactions and cross-regulation between different PTMs remain unknown, and recent evidence (Eom *et al.* 2014) for deacetylation of HDAC2 (a class I HDAC) by HDAC5 (a class IIa HDAC) should trigger further investigation of regulatory crosstalk between distinct HDAC family members.

References

Ago T, Liu T, Zhai P, Chen W, Li H, Molkentin JD, Vatner SF & Sadoshima J (2008). A redox-dependent pathway for regulating class II HDACs and cardiac hypertrophy. *Cell* **133**, 978–993.

Anand P, Brown JD, Lin CY, Qi J, Zhang R, Artero PC, Alaiti MA, Bullard J, Alazem K, Margulies KB, Cappola TP *et al.* (2013). BET bromodomains mediate transcriptional pause release in heart failure. *Cell* **154**, 569–582.

Backs J, Backs T, Bezprozvannaya S, McKinsey TA & Olson EN (2008). Histone deacetylase 5 acquires calcium/calmodulin-dependent kinase II responsiveness by oligomerization with histone deacetylase 4. *Mol Cell Biol* **28**, 3437–3445.

Backs J, Backs T, Neef S, Kreusser MM, Lehmann LH, Patrick DM, Grueter CE, Qi X, Richardson JA, Hill JA *et al.* (2009). The delta isoform of CaM kinase II is required for pathological cardiac hypertrophy and remodeling after pressure overload. *Proc Natl Acad Sci USA* **106**, 2342–2347.

Backs J, Song K, Bezprozvannaya S, Chang S & Olson EN (2006). CaM kinase II selectively signals to histone deacetylase 4 during cardiomyocyte hypertrophy. *J Clin Invest* **116**, 1853–1864.

Backs J, Worst BC, Lehmann LH, Patrick DM, Jebessa Z, Kreusser MM, Sun Q, Chen L, Heft C, Katus HA & Olson EN (2011). Selective repression of MEF2 activity by PKA-dependent proteolysis of HDAC4. *J Cell Biol* **195**, 403–415.

Berdeaux R, Goebel N, Banaszynski L, Takemori H, Wandless T, Shelton GD & Montminy M (2007). SIK1 is a class II HDAC kinase that promotes survival of skeletal myocytes. *Nat Med* **13**, 597–603.

Bertrand P (2010). Inside HDAC with HDAC inhibitors. *Eur J Med Chem* **45**, 2095–2116.

Bossuyt J, Chang CW, Helmstadter K, Kunkel MT, Newton AC, Campbell KS, Martin JL, Bossuyt S, Robia SL & Bers DM (2011). Spatiotemporally distinct protein kinase D activation in adult cardiomyocytes in response to phenylephrine and endothelin. *J Biol Chem* **286**, 33390–33400.

Bossuyt J, Helmstadter K, Wu X, Clements-Jewery H, Haworth RS, Avkiran M, Martin JL, Pogwizd SM & Bers DM (2008). Ca^{2+}/calmodulin-dependent protein kinase IIdelta and protein kinase D overexpression reinforce the histone deacetylase 5 redistribution in heart failure. *Circ Res* **102**, 695–702.

Bradner JE, West N, Grachan ML, Greenberg EF, Haggarty SJ, Warnow T & Mazitschek R (2010). Chemical phylogenetics of histone deacetylases. *Nat Chem Biol* **6**, 238–243.

Bristow MR (2011). Treatment of chronic heart failure with beta-adrenergic receptor antagonists: a convergence of receptor pharmacology and clinical cardiology. *Circ Res* **109**, 1176–1194.

Cardinale JP, Sriramula S, Pariaut R, Guggilam A, Mariappan N, Elks CM & Francis J (2010). HDAC inhibition attenuates inflammatory, hypertrophic, and hypertensive responses in spontaneously hypertensive rats. *Hypertension* **56**, 437–444.

Chang CW, Lee L, Yu D, Dao K, Bossuyt J & Bers DM (2013). Acute β-adrenergic activation triggers nuclear import of histone deacetylase 5 and delays G$_q$-induced transcriptional activation. *J Biol Chem* **288**, 192–204.

Chang S, Bezprozvannaya S, Li S & Olson EN (2005). An expression screen reveals modulators of class II histone deacetylase phosphorylation. *Proc Natl Acad Sci USA* **102**, 8120–8125.

Chang S, McKinsey TA, Zhang CL, Richardson JA, Hill JA & Olson EN (2004). Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development. *Mol Cell Biol* **24**, 8467–8476.

Chang S, Young BD, Li S, Qi X, Richardson JA & Olson EN (2006). Histone deacetylase 7 maintains vascular integrity by repressing matrix metalloproteinase 10. *Cell* **126**, 321–334.

Cho YK, Eom GH, Kee HJ, Kim HS, Choi WY, Nam KI, Ma JS & Kook H (2010). Sodium valproate, a histone deacetylase inhibitor, but not captopril, prevents right ventricular hypertrophy in rats. *Circ J* **74**, 760–770.

Demos-Davies KM, Ferguson BS, Cavasin MA, Mahaffey JH, Williams SM, Spiltoir JI, Schuetze KB, Horn TR, Chen B, Ferrara C *et al.* (2014). HDAC6 contributes to pathological responses of heart and skeletal muscle to chronic angiotensin II signaling. *Am J Physiol Heart Circ Physiol* **307**, H252–H258.

Dequiedt F, Martin M, Von Blume J, Vertommen D, Lecomte E, Mari N, Heinen MF, Bachmann M, Twizere JC, Huang MC *et al.* (2006). New role for hPar-1 kinases EMK and C-TAK1 in regulating localization and activity of class IIa histone deacetylases. *Mol Cell Biol* **26**, 7086–7102.

de Ruijter AJ, van Gennip AH, Caron HN, Kemp S & van Kuilenburg AB (2003). Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J* **370**, 737–749.

Eom GH, Nam YS, Oh JG, Choe N, Min HK, Yoo EK, Kang G, Nguyen VH, Min JJ, Kim JK *et al.* (2014). Regulation of acetylation of histone deacetylase 2 by p300/CBP-associated factor/histone deacetylase 5 in the development of cardiac hypertrophy. *Circ Res* **114**, 1133–1143.

Fielitz J, Kim MS, Shelton JM, Qi X, Hill JA, Richardson JA, Bassel-Duby R & Olson EN (2008). Requirement of protein kinase D1 for pathological cardiac remodeling. *Proc Natl Acad Sci USA* **105**, 3059–3063.

Fischle W, Dequiedt F, Hendzel MJ, Guenther MG, Lazar MA, Voelter W & Verdin E (2002). Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. *Mol Cell* **9**, 45–57.

Gallo P, Latronico MV, Gallo P, Grimaldi S, Borgia F, Todaro M, Jones P, Gallinari P, De Francesco R, Ciliberto G *et al.* (2008). Inhibition of class I histone deacetylase with an apicidin derivative prevents cardiac hypertrophy and failure. *Cardiovasc Res* **80**, 416–424.

Granger A, Abdullah I, Huebner F, Stout A, Wang T, Huebner T, Epstein JA & Gruber PJ (2008). Histone deacetylase inhibition reduces myocardial ischemia-reperfusion injury in mice. *FASEB J* **22**, 3549–3560.

Greco TM, Yu F, Guise AJ & Cristea IM (2011). Nuclear import of histone deacetylase 5 by requisite nuclear localization signal phosphorylation. *Mol Cell Proteomics* **10**, M110 004317.

Gregoire S & Yang XJ (2005). Association with class IIa histone deacetylases upregulates the sumoylation of MEF2 transcription factors. *Mol Cell Biol* **25**, 2273–2287.

Gregoretti IV, Lee YM & Goodson HV (2004). Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *J Mol Biol* **338**, 17–31.

Grozinger CM, Hassig CA & Schreiber SL (1999). Three proteins define a class of human histone deacetylases related to yeast Hda1p. *Proc Natl Acad Sci USA* **96**, 4868–4873.

Grozinger CM & Schreiber SL (2000). Regulation of histone deacetylase 4 and 5 and transcriptional activity by 14-3-3-dependent cellular localization. *Proc Natl Acad Sci USA* **97**, 7835–7840.

Ha CH, Jhun BS, Kao HY & Jin ZG (2008a). VEGF stimulates HDAC7 phosphorylation and cytoplasmic accumulation modulating matrix metalloproteinase expression and angiogenesis. *Arterioscler Thromb Vasc Biol* **28**, 1782–1788.

Ha CH, Kim JY, Zhao J, Wang W, Jhun BS, Wong C & Jin ZG (2010). PKA phosphorylates histone deacetylase 5 and prevents its nuclear export, leading to the inhibition of gene transcription and cardiomyocyte hypertrophy. *Proc Natl Acad Sci USA* **107**, 15467–15472.

Ha CH, Wang W, Jhun BS, Wong C, Hausser A, Pfizenmaier K, McKinsey TA, Olson EN & Jin ZG (2008*b*). Protein kinase D-dependent phosphorylation and nuclear export of histone deacetylase 5 mediates vascular endothelial growth factor-induced gene expression and angiogenesis. *J Biol Chem* **283**, 14590–14599.

Haberland M, Mokalled MH, Montgomery RL & Olson EN (2009). Epigenetic control of skull morphogenesis by histone deacetylase 8. *Genes Dev* **23**, 1625–1630.

Harrison BC, Roberts CR, Hood DB, Sweeney M, Gould JM, Bush EW & McKinsey TA (2004). The CRM1 nuclear export receptor controls pathological cardiac gene expression. *Mol Cell Biol* **24**, 10636–10649.

Haworth RS, Stathopoulou K, Candasamy AJ & Avkiran M (2012). Neurohormonal regulation of cardiac histone deacetylase 5 nuclear localization by phosphorylation-dependent and phosphorylation-independent mechanisms. *Circ Res* **110**, 1585–1595.

He J, Ye J, Cai Y, Riquelme C, Liu JO, Liu X, Han A & Chen L (2011). Structure of p300 bound to MEF2 on DNA reveals a mechanism of enhanceosome assembly. *Nucleic Acids Res* **39**, 4464–4474.

Hohl M, Wagner M, Reil JC, Muller SA, Tauchnitz M, Zimmer AM, Lehmann LH, Thiel G, Bohm M, Backs J & Maack C (2013). HDAC4 controls histone methylation in response to elevated cardiac load. *J Clin Invest* **123**, 1359–1370.

Hu E, Chen Z, Fredrickson T, Zhu Y, Kirkpatrick R, Zhang GF, Johanson K, Sung CM, Liu R & Winkler J (2000). Cloning and characterization of a novel human class I histone deacetylase that functions as a transcription repressor. *J Biol Chem* **275**, 15254–15264.

Iyer A, Fenning A, Lim J, Le GT, Reid RC, Halili MA, Fairlie DP & Brown L (2010). Antifibrotic activity of an inhibitor of histone deacetylases in DOCA-salt hypertensive rats. *Br J Pharmacol* **159**, 1408–1417.

Kao HY, Downes M, Ordentlich P & Evans RM (2000). Isolation of a novel histone deacetylase reveals that class I and class II deacetylases promote SMRT-mediated repression. *Genes Dev* **14**, 55–66.

Kee HJ, Bae EH, Park S, Lee KE, Suh SH, Kim SW & Jeong MH (2013). HDAC inhibition suppresses cardiac hypertrophy and fibrosis in DOCA-salt hypertensive rats via regulation of HDAC6/HDAC8 enzyme activity. *Kidney Blood Press Res* **37**, 229–239.

Kee HJ, Sohn IS, Nam KI, Park JE, Qian YR, Yin Z, Ahn Y, Jeong MH, Bang YJ, Kim N, Kim JK, Kim KK, Epstein JA & Kook H (2006). Inhibition of histone deacetylation blocks cardiac hypertrophy induced by angiotensin II infusion and aortic banding. *Circulation* **113**, 51–59.

Kho C, Lee A, Jeong D, Oh JG, Chaanine AH, Kizana E, Park WJ & Hajjar RJ (2011). SUMO1-dependent modulation of SERCA2a in heart failure. *Nature* **477**, 601–605.

Kirsh O, Seeler JS, Pichler A, Gast A, Muller S, Miska E, Mathieu M, Harel-Bellan A, Kouzarides T, Melchior F & Dejean A (2002). The SUMO E3 ligase RanBP2 promotes modification of the HDAC4 deacetylase. *EMBO J* **21**, 2682–2691.

Kong Y, Tannous P, Lu G, Berenji K, Rothermel BA, Olson EN & Hill JA (2006). Suppression of class I and II histone deacetylases blunts pressure-overload cardiac hypertrophy. *Circulation* **113**, 2579–2588.

Kook H, Lepore JJ, Gitler AD, Lu MM, Wing-Man Yung W, Mackay J, Zhou R, Ferrari V, Gruber P & Epstein JA (2003). Cardiac hypertrophy and histone deacetylase-dependent transcriptional repression mediated by the atypical homeodomain protein Hop. *J Clin Invest* **112**, 863–871.

Lagger G, O'Carroll D, Rembold M, Khier H, Tischler J, Weitzer G, Schuettengruber B, Hauser C, Brunmeir R, Jenuwein T & Seiser C (2002). Essential function of histone deacetylase 1 in proliferation control and CDK inhibitor repression. *EMBO J* **21**, 2672–2681.

Lahm A, Paolini C, Pallaoro M, Nardi MC, Jones P, Neddermann P, Sambucini S, Bottomley MJ, Lo Surdo P, Carfi A *et al.* (2007). Unraveling the hidden catalytic activity of vertebrate class IIa histone deacetylases. *Proc Natl Acad Sci USA* **104**, 17335–17340.

Landry J, Sutton A, Tafrov ST, Heller RC, Stebbins J, Pillus L & Sternglanz R (2000). The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc Natl Acad Sci USA* **97**, 5807–5811.

Lee TM, Lin MS & Chang NC (2007). Inhibition of histone deacetylase on ventricular remodeling in infarcted rats. *Am J Physiol Heart Circ Physiol* **293**, H968–H977.

Lehmann LH, Rostosky JS, Buss SJ, Kreusser MM, Krebs J, Mier W, Enseleit F, Spiger K, Hardt SE, Wieland T *et al.* (2014). Essential role of sympathetic endothelin A receptors for adverse cardiac remodeling. *Proc Natl Acad Sci USA* **111**, 13499–13504.

Liu F, Dowling M, Yang XJ & Kao GD (2004). Caspase-mediated specific cleavage of human histone deacetylase 4. *J Biol Chem* **279**, 34537–34546.

Lu J, McKinsey TA, Nicol RL & Olson EN (2000). Signal-dependent activation of the MEF2 transcription factor by dissociation from histone deacetylases. *Proc Natl Acad Sci USA* **97**, 4070–4075.

Ma K, Chan JK, Zhu G & Wu Z (2005). Myocyte enhancer factor 2 acetylation by p300 enhances its DNA binding activity, transcriptional activity, and myogenic differentiation. *Mol Cell Biol* **25**, 3575–3582.

McGee SL, van Denderen BJ, Howlett KF, Mollica J, Schertzer JD, Kemp BE & Hargreaves M (2008). AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes* **57**, 860–867.

McKinsey TA (2011). Isoform-selective HDAC inhibitors: closing in on translational medicine for the heart. *J Mol Cell Cardiol* **51**, 491–496.

McKinsey TA (2012). Therapeutic potential for HDAC inhibitors in the heart. *Annu Rev Pharmacol Toxicol* **52**, 303–319.

McKinsey TA, Zhang CL, Lu J & Olson EN (2000*a*). Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* **408**, 106–111.

McKinsey TA, Zhang CL & Olson EN (2000*b*). Activation of the myocyte enhancer factor-2 transcription factor by calcium/calmodulin-dependent protein kinase-stimulated binding of 14-3-3 to histone deacetylase 5. *Proc Natl Acad Sci USA* **97**, 14400–14405.

McKinsey TA, Zhang CL & Olson EN (2001). Identification of a signal-responsive nuclear export sequence in class II histone deacetylases. *Mol Cell Biol* **21**, 6312–6321.

Matsushima S, Kuroda J, Ago T, Zhai P, Park JY, Xie LH, Tian B & Sadoshima J (2013). Increased oxidative stress in the nucleus caused by Nox4 mediates oxidation of HDAC4 and cardiac hypertrophy. *Circ Res* **112**, 651–663.

Miska EA, Karlsson C, Langley E, Nielsen SJ, Pines J & Kouzarides T (1999). HDAC4 deacetylase associates with and represses the MEF2 transcription factor. *EMBO J* **18**, 5099–5107.

Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, Grant SR & Olson EN (1998). A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* **93**, 215–228.

Monovich L, Koch KA, Burgis R, Osimboni E, Mann T, Wall D, Gao J, Feng Y, Vega RB, Turner BA *et al.* (2009). Suppression of HDAC nuclear export and cardiomyocyte hypertrophy by novel irreversible inhibitors of CRM1. *Biochim Biophys Acta* **1789**, 422–431.

Montgomery RL, Davis CA, Potthoff MJ, Haberland M, Fielitz J, Qi X, Hill JA, Richardson JA & Olson EN (2007). Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and contractility. *Genes Dev* **21**, 1790–1802.

Montgomery RL, Potthoff MJ, Haberland M, Qi X, Matsuzaki S, Humphries KM, Richardson JA, Bassel-Duby R & Olson EN (2008). Maintenance of cardiac energy metabolism by histone deacetylase 3 in mice. *J Clin Invest* **118**, 3588–3597.

Olesen JB, Hansen PR, Abildstrom SZ, Andersson C, Weeke P, Schmiegelow M, Erdal J, Torp-Pedersen C & Gislason GH (2011). Valproate attenuates the risk of myocardial infarction in patients with epilepsy: a nationwide cohort study. *Pharmacoepidemiol Drug Saf* **20**, 146–153.

Osadchii OE (2007). Cardiac hypertrophy induced by sustained beta-adrenoreceptor activation: pathophysiological aspects. *Heart Fail Rev* **12**, 66–86.

Paroni G, Cernotta N, Dello Russo C, Gallinari P, Pallaoro M, Foti C, Talamo F, Orsatti L, Steinkuhler C & Brancolini C (2008). PP2A regulates HDAC4 nuclear import. *Mol Biol Cell* **19**, 655–667.

Paroni G, Fontanini A, Cernotta N, Foti C, Gupta MP, Yang XJ, Fasino D & Brancolini C (2007). Dephosphorylation and caspase processing generate distinct nuclear pools of histone deacetylase 4. *Mol Cell Biol* **27**, 6718–6732.

Paroni G, Mizzau M, Henderson C, Del Sal G, Schneider C & Brancolini C (2004). Caspase-dependent regulation of histone deacetylase 4 nuclear–cytoplasmic shuttling promotes apoptosis. *Mol Biol Cell* **15**, 2804–2818.

Peng Y, Lambert AA, Papst P & Pitts KR (2009). Agonist-induced nuclear export of GFP–HDAC5 in isolated adult rat ventricular myocytes. *J Pharmacol Toxicol Methods* **59**, 135–140.

Portbury AL, Ronnebaum SM, Zungu M, Patterson C & Willis MS (2012). Back to your heart: ubiquitin proteasome system-regulated signal transduction. *J Mol Cell Cardiol* **52**, 526–537.

Potthoff MJ, Wu H, Arnold MA, Shelton JM, Backs J, McAnally J, Richardson JA, Bassel-Duby R & Olson EN (2007). Histone deacetylase degradation and MEF2 activation promote the formation of slow-twitch myofibers. *J Clin Invest* **117**, 2459–2467.

Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R, Colucci WS & Walsh K (2005). Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. *J Clin Invest* **115**, 2108–2118.

Spallotta F, Tardivo S, Nanni S, Rosati JD, Straino S, Mai A, Vecellio M, Valente S, Capogrossi MC, Farsetti A *et al.* (2013). Detrimental effect of class-selective histone deacetylase inhibitors during tissue regeneration following hindlimb ischemia. *J Biol Chem* **288**, 22915–22929.

Spiltoir JI, Stratton MS, Cavasin MA, Demos-Davies K, Reid BG, Qi J, Bradner JE & McKinsey TA (2013). BET acetyl-lysine binding proteins control pathological cardiac hypertrophy. *J Mol Cell Cardiol* **63**, 175–179.

Trivedi CM, Lu MM, Wang Q & Epstein JA (2008). Transgenic overexpression of Hdac3 in the heart produces increased postnatal cardiac myocyte proliferation but does not induce hypertrophy. *J Biol Chem* **283**, 26484–26489.

Trivedi CM, Luo Y, Yin Z, Zhang M, Zhu W, Wang T, Floss T, Goettlicher M, Noppinger PR, Wurst W *et al.* (2007). Hdac2 regulates the cardiac hypertrophic response by modulating Gsk3 beta activity. *Nat Med* **13**, 324–331.

Vega RB, Harrison BC, Meadows E, Roberts CR, Papst PJ, Olson EN & McKinsey TA (2004*a*). Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylase 5. *Mol Cell Biol* **24**, 8374–8385.

Vega RB, Matsuda K, Oh J, Barbosa AC, Yang X, Meadows E, McAnally J, Pomajzl C, Shelton JM, Richardson JA *et al.* (2004*b*). Histone deacetylase 4 controls chondrocyte hypertrophy during skeletogenesis. *Cell* **119**, 555–566.

Walkinshaw DR, Weist R, Kim GW, You L, Xiao L, Nie J, Li CS, Zhao S, Xu M & Yang XJ (2013*a*). The tumor suppressor kinase LKB1 activates the downstream kinases SIK2 and SIK3 to stimulate nuclear export of class IIa histone deacetylases. *J Biol Chem* **288**, 9345–9362.

Walkinshaw DR, Weist R, Xiao L, Yan K, Kim GW & Yang XJ (2013*b*). Dephosphorylation at a conserved SP motif governs cAMP sensitivity and nuclear localization of class IIa histone deacetylases. *J Biol Chem* **288**, 5591–5605.

Wang J, Chen L, Wen S, Zhu H, Yu W, Moskowitz IP, Shaw GM, Finnell RH & Schwartz RJ (2011). Defective sumoylation pathway directs congenital heart disease. *Birth Defects Res A Clin Mol Teratol* **91**, 468–476.

Wei JQ, Shehadeh LA, Mitrani JM, Pessanha M, Slepak TI, Webster KA & Bishopric NH (2008). Quantitative control of adaptive cardiac hypertrophy by acetyltransferase p300. *Circulation* **118**, 934–946.

Wu X, Zhang T, Bossuyt J, Li X, McKinsey TA, Dedman JR, Olson EN, Chen J, Brown JH & Bers DM (2006). Local InsP3-dependent perinuclear Ca2+ signaling in cardiac myocyte excitation-transcription coupling. *J Clin Invest* **116**, 675–682.

Xie M & Hill JA (2013). HDAC-dependent ventricular remodeling. *Trends Cardiovasc Med* **23**, 229–235.

Xie M, Kong Y, Tan W, May H, Battiprolu PK, Pedrozo Z, Wang ZV, Morales C, Luo X, Cho G *et al.* (2014). Histone deacetylase inhibition blunts ischemia/reperfusion injury by inducing cardiomyocyte autophagy. *Circulation* **129**, 1139–1151.

Yang WM, Yao YL, Sun JM, Davie JR & Seto E (1997). Isolation and characterization of cDNAs corresponding to an additional member of the human histone deacetylase gene family. *J Biol Chem* **272**, 28001–28007.

Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA & Olson EN (2002*a*). Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell* **110**, 479–488.

Zhang CL, McKinsey TA & Olson EN (2001). The transcriptional corepressor MITR is a signal-responsive inhibitor of myogenesis. *Proc Natl Acad Sci USA* **98**, 7354–7359.

Zhang CL, McKinsey TA & Olson EN (2002*b*). Association of class II histone deacetylases with heterochromatin protein 1: potential role for histone methylation in control of muscle differentiation. *Mol Cell Biol* **22**, 7302–7312.

Zhang L, Qin X, Zhao Y, Fast L, Zhuang S, Liu P, Cheng G & Zhao TC (2012). Inhibition of histone deacetylases preserves myocardial performance and prevents cardiac remodeling through stimulation of endogenous angiomyogenesis. *J Pharmacol Exp Ther* **341**, 285–293.

Zhang LX, Zhao Y, Cheng G, Guo TL, Chin YE, Liu PY & Zhao TC (2010). Targeted deletion of NF-kappaB p50 diminishes the cardioprotection of histone deacetylase inhibition. *Am J Physiol Heart Circ Physiol* **298**, H2154–H2163.

Zhang Y, Matkovich SJ, Duan X, Gold JI, Koch WJ & Dorn GW 2nd (2011). Nuclear effects of G-protein receptor kinase 5 on histone deacetylase 5-regulated gene transcription in heart failure. *Circ Heart Fail* **4**, 659–668.

Zhao X, Sternsdorf T, Bolger TA, Evans RM & Yao TP (2005). Regulation of MEF2 by histone deacetylase 4- and SIRT1 deacetylase-mediated lysine modifications. *Mol Cell Biol* **25**, 8456–8464.

Zhou X, Marks PA, Rifkind RA & Richon VM (2001). Cloning and characterization of a histone deacetylase, HDAC9. *Proc Natl Acad Sci USA* **98**, 10572–10577.

Zhou X, Richon VM, Rifkind RA & Marks PA (2000). Identification of a transcriptional repressor related to the noncatalytic domain of histone deacetylases 4 and 5. *Proc Natl Acad Sci USA* **97**, 1056–1061.

Additional information

Competing interests

None declared.

Funding