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The many roles of histone deacetylases in development and physiology: implications for disease and therapy

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Abstract

Histone deacetylases (HDACs) are part of a vast family of enzymes that have crucial roles in numerous biological processes, largely through their repressive influence on transcription. The expression of many HDAC isoforms in eukaryotic cells raises questions about their possible specificity or redundancy, and whether they control global or specific programmes of gene expression. Recent analyses of HDAC knockout mice have revealed highly specific functions of individual HDACs in development and disease. Mutant mice lacking individual HDACs are a powerful tool for defining the functions of HDACs *in vivo* and the molecular targets of HDAC inhibitors in disease.

The distinctive patterns of gene expression that are associated with specialized embryonic and adult cell types, as well as the modulation of specific gene programmes in response to physiological and pathological signalling, require multiple levels of transcriptional control. This control occurs through transcriptional regulators that bind specific DNA sequences, leading to the modification of chromatin structure, which in turn controls the accessibility of DNA to regulatory factors. The main factor influencing chromatin structure is the state of amino-acid residues within histone tails, which serve as targets for a variety of reversible post-translational modifications that modulate nucleosome structure and gene transcription, both positively and negatively[1,2]. Acetylation, one of the most widespread modifications of histones, serves as a key modulator of chromatin structure and gene transcription, and provides a mechanism for coupling extracellular signals with the genome by regulated acetylation and deacetylation[3].

Histone acetylation modulates transcription in multiple ways. Acetylation of ε-amino groups of lysine residues within histone tails neutralizes their positive charge, thereby relaxing chromatin structure. This interferes with the generation of higher-order chromatin structures, and increasing the accessibility of transcription factors to their target genes[4]. Acetylated histones also serve as binding sites for bromodomain proteins, which often act as transcriptional activators. Conversely, histone deacetylation favours transcriptional

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Competing interests statement
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DATABASES
UniProtKB: http://www.uniprot.org
HDAC1 | HDAC2 | HDAC3 | HDAC4 | HDAC5 | HDAC6 | HDAC7 | HDAC8 | HDAC9 | HDAC10 | HDAC11 | MEF2

FURTHER INFORMATION
Olson laboratory homepage:
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ALL LINKS ARE ACTIVE IN THE ONLINE PDf

repression by allowing for chromatin compaction[5]. Direct acetylation and deacetylation of transcription factors has also been shown to have positive and negative consequences on gene transcription, respectively[6].

Histone acetylation is a dynamic process controlled by the antagonistic actions of two large families of enzymes — the histone acetyltransferases (HATs) and the histone deacetylases (HDACs). The balance between the actions of these enzymes serves as a key regulatory mechanism for gene expression and governs numerous developmental processes and disease states.

The vast majority of studies of HDAC functions have involved biochemical analyses *in vitro*, studies in cultured cells with HDAC inhibitors, HDAC knockdown by small interfering RNA (siRNA), or overexpression of HDACs. Although these experiments demonstrate the biochemical functions of HDACs as transcriptional repressors, they are non-predictive of their role *in vivo*. An important question regarding the functions and mechanisms of action of HDACs *in vivo* is whether HDACs act primarily to control global changes in the state of chromatin or whether they also have more specific functions in the regulation of key downstream genes and transcriptional programmes. The existence of many HDAC isoforms in eukaryotic cells also raises questions about possible specificity or redundancy of functions. Ongoing human clinical trials that are investigating the use of HDAC inhibitors as a treatment for a variety of disorders mean that it is vital these questions are answered. The recent creation of knockout mice lacking HDAC genes has revealed highly specific functions for individual HDAC isoforms during development and adulthood. These mutant mice are a powerful tool for defining the functions of HDACs *in vivo* and for identifying the molecular targets of HDAC inhibitors in disease. In this Review, we discuss the developmental and physiological functions of HDACs that are revealed by gene deletions in mice, and how these studies can inform future efforts to exploit HDACs in the settings of human disease.

Control of gene expression by HDACs

HDACs lack intrinsic DNA-binding activity and are recruited to target genes via their direct association with transcriptional activators and repressors, as well as their incorporation into large multiprotein transcriptional complexes[2,4]. Thus, the specificity of HDACs for regulation of distinct gene programmes depends on cell identity and the spectrum of available partner proteins in a cell, in addition to the signalling milieu of the cell. Although diminished histone acetylation at promoter regions generally correlates with gene silencing, consistent with the well-established functions of HDACs as transcriptional repressors, there is also evidence that HDACs can activate some genes. In yeast, for example, the HDAC Hos2 is required for gene activation, and deletion of the HDAC1 and 2 homologue, Rpd3, leads to repression of transcription at telomeric loci[7–9]. HDACs have also been linked to transcriptional activation of a subset of genes in higher eukaryotes[10] but, in settings in which HDAC inhibition leads to downregulation of specific genes, it is difficult to rule out possible secondary effects that result in transcriptional repression. It should be noted, however, that deletion or inhibition of HDACs often results in the upregulation or down-regulation of approximately equivalent percentages of genes[10–12].

It has also become clear in recent years that HDACs can act on numerous cellular substrates in addition to histones, and that acetylation might rival phosphorylation in its importance[6]. In this regard, class IIa deacetylases possess only minimal HDAC activity against acetylated histones, despite extensive evolutionary conservation of their deacetylase domain, pointing to the possible importance of other types of cellular substrates for their actions[13]. How these many facets of acetylation and deacetylation are controlled and integrated, and how they

influence the expression of specific genes as well as global changes in gene expression, are important issues for the field.

The HDAC superfamily

The HDAC superfamily is vast and ancient, dating back to prokaryotes. Here we focus on the mammalian HDACs and the lessons learned from genetic deletion models.

Mammalian genomes encode 11 proteins with a highly conserved deacetylase domain (FIG. 1). These proteins can be classified into four families (class I, IIa, IIb and IV), which differ in structure, enzymatic function, subcellular localization and expression patterns. In addition to these classical HDACs, mammalian genomes encode another group of deacetylases, the sirtuins, which are sometimes referred to as class III HDACs. There have been several recent reviews on the sirtuins[14–17], so they will not be covered here.

Class I HDACs

The class I HDAC family consists of HDAC1, 2, 3 and 8, which share homology with Rpd3 — a founding member from budding yeast[18–20]. These HDACs are expressed ubiquitously, localized predominantly to the nucleus and display high enzymatic activity toward histone substrates. They possess relatively simple structures, consisting of the conserved deacetylase domain with short amino- and carboxy-terminal extensions.

HDAC1 and HDAC2 are nearly identical and are generally found together in repressive complexes such as the sin3, NuRD, CoREST and PRC2 complexes[21]. HDAC3 is found in distinct complexes such as the N-CoR–SMRT complex, whereas no complex has been described for HDAC8 (REF. 19).

Class IIa HDACs

HDAC4, 5, 7 and 9 belong to the class IIa HDAC family. These HDACs have large N-terminal extensions with conserved binding sites for the transcription factor myocyte enhancer factor 2 (MEF2) and the chaperone protein 14-3-3, which render HDACs signal responsive. Following phosphorylation by kinases, such as calcium/calmodulin-dependent protein kinase (CaMK) and protein kinase D (PKD), these HDACs bind 14-3-3 and shuttle from the nucleus to the cytoplasm[22–25]. The dissociation of class II HDACs from MEF2 allows the HAT p300 to associate with MEF2 via the HDAC docking site, thereby converting MEF2 from a transcriptional repressor to a transcriptional activator[26–30]. The regulated phosphorylation of class IIa HDACs provides a mechanism for linking extracellular signals with transcription and has key roles in numerous tissues during development and disease.

In contrast to other HDACs, class IIa HDACs show relatively restricted expression patterns. HDAC5 and HDAC9 are highly enriched in muscles, the heart and brain[31,32]. HDAC4 is highly expressed in the brain and growth plates of the skeleton[33], and HDAC7 is enriched in endothelial cells and thymocytes[34] (T-cell precursors derived from the thymus).

The precise mechanism whereby class IIa HDACs repress transcription has not been fully elucidated. Highly purified recombinant class IIa HDACs possess only minimal catalytic activity, and the activity of class IIa HDACs purified from mammalian cells has been shown to be due to contaminating class I HDACs[13,35,36]. Moreover, MEF2-interacting transcription repressor (MITR), which is a splice variant of HDAC9 that lacks the HDAC domain, is as effective in repression of MEF2-target genes as the full-length HDAC9 protein, indicating that the intrinsic catalytic activity of class IIa HDACs is not required for repression[37–39].

The class IIa HDACs have been shown to recruit class I HDACs through their C-terminal HDAC domain, which probably accounts for a portion of their repressive activity[35]. In addition, the regulatory domains of class IIa HDACs interact with other transcriptional repressors, such as heterochromatin protein 1 (HP1) and C-terminal-binding protein (CTBP)[37,38,40]. Thus, they function as adaptors to nucleate multiple types of transcriptional regulators and to confer signal-responsiveness to downstream target genes.

Recently, the biochemical basis for the different activities of class I and class IIa HDACs has been elucidated. In the catalytic pocket of most HDACs, an ultra-conserved tyrosine acts as a transition-state stabilizer in the deacetylation reaction[41]. This tyrosine is changed to a histidine in vertebrate class IIa HDACs, and this conservative amino-acid change reduces the catalytic activity of the vertebrate enzymes more than 1,000-fold[36,42]. Although this activity is still measurable *in vitro*, it is unclear if it is of any biological relevance *in vivo*, especially given the observation that class IIa HDACs do not need their catalytic domain in order to be potent repressors.

Class IIb HDACs

HDAC6 and HDAC10 form the class IIb family. HDAC6 is the main cytoplasmic deacetylase in mammalian cells[43], whereas little is known about the functions of HDAC10 (REFS 44,45). Among the targets directly deacetylated by HDAC6 are cytoskeletal proteins such as α-tubulin and cortactin, transmembrane proteins such as the interferon receptor IFNαR, and chaperones[46–50]. HDAC6 is distinct from all other HDACs, as it harbours two deacetylase domains and a C-terminal zinc finger.

Class IV HDAC

HDAC11 is the sole class IV HDAC. expression of HDAC11 is enriched in the brain, heart, muscle, kidney and testis, but little is known about its function[51,52]. It is composed of a deacetylase domain that shows homology to class I and II HDAC domains, with small N- and C-terminal extensions.

Roles of class I HDACs in development

The ubiquitous expression, high deacetylase activity towards common substrates and high homology between class I HDACs suggests functional redundancy among these HDACs *in vivo*. However, deletion of each member of the class I HDAC family in mice leads to lethality in all cases, demonstrating the unique roles of each HDAC in the control of specific gene expression programmes.

HDAC1

HDAC1-null mice die before embryonic day 10.5 (E10.5) and display severe proliferation defects and general growth retardation[12,53]. Proliferation defects can also be observed in HDAC1-null embryonic stem (ES) cells and are associated with increased expression of the cyclin-dependent kinase inhibitors p21 and p27 (REF. 54). These cells show a significant reduction in total HDAC activity and modest hyperacetylation of histones H3 and H4, indicating that HDAC1 is a major deacetylase in ES cells. surprisingly, 3% of genes are downregulated and ~5% of genes are upregulated in HDAC1-null ES cells[54], suggesting that HDAC1 does not function simply as a global repressor of transcription, but instead regulates specific gene programmes by repressing or activating certain promoters.

Deletion of *hdac1* in zebrafish causes a variety of lethal defects in skeletal and neuronal elements. The specific target genes responsible for these phenotypes have not been defined, but they seem to be downstream of canonical and non-canonical Wnt signalling[55–59]. None

of the other HDACs has been genetically analysed in detail in zebrafish, although a recent report described disrupted liver development following morpholino- mediated knockdown of *hdac3* mRNA (REF. 60). surprisingly, conditional deletion of HDAC1 in tissues such as the heart, brain, skeletal muscle and smooth muscle is well tolerated in mice[12], although this is probably due to redundancy with HDAC2 in later development and postnatal life (see below)[12].

HDAC2

There is disagreement regarding the function of HDAC2 *in vivo*. One study found that HDAC2-null mice die within the first 24 hours after birth with severe cardiac malformations, including obliteration of the lumen of the right ventricle owing to excessive proliferation of cardiomyocytes, as well as bradycardia[12] (FIG. 2). By contrast, other studies have reported that mice harbouring a *lacZ* insertion in *Hdac2*, which is purported to create a null mutation, are viable[61]. The basis for these conflicting results is unclear. It is possible that different genetic backgrounds account for this difference. Alternatively, the *lacZ* insertion allele might be 'leaky' and allow adequate expression of HDAC2 for viability, a phenomenon that has previously been described for gene trap approaches[62].

The transcriptional targets of HDAC2 in the heart remain to be fully defined. However, the homeodomain-only protein (HOP), which functions as a positive and negative regulator of cardiomyocyte proliferation, has been shown to interact with HDAC2 (FIG. 2). Deletion of HOP also results in hyperproliferation of developing cardiomyocytes, suggesting that HDAC2 and HOP reside in a transcriptionally repressive complex to regulate cardiac proliferation and differentiation during development[63–65].

Redundant roles of HDAC1 and 2 in cardiac growth and development

Conditional null alleles for class I HDACs have permitted an analysis of their functions in specific tissues, bypassing the early lethality associated with global gene deletion. Given the lethal phenotypes resulting from global deletion of HDAC1 and HDAC2, it was surprising to find that deletion of either HDAC1 or HDAC2 in a variety of tissues, including the heart, brain, endothelial cells, smooth muscle, and neural crest cells did not yield obvious phenotypes. By contrast, deletion of both genes together results in severe phenotypes in all tissues examined, pointing to redundant functions of these HDACs during later development and adulthood[12].

Conditional deletion of HDAC1 and 2 together in the cardiac lineage has shown that a single wild-type allele of either gene is sufficient to support normal development, whereas deletion of all HDAC1 and 2 alleles results in neonatal lethality, accompanied by cardiac arrhythmias, dilated cardiomyopathy, and upregulation of genes encoding skeletal muscle-specific contractile proteins and calcium channels in the heart[12] (FIG. 2). The earlier that HDAC1 and HDAC2 are deleted, the more dramatic the phenotype. In the heart, deletion of HDAC1 and HDAC2 at E8.5 causes lethality 2 days later, whereas mice with a cardiac deletion at E10.5 survived for more than 3 weeks. Transcriptional analysis in these animals revealed that only 1.6% of transcripts were upregulated, and that deletion of HDAC1 and HDAC2 in the heart derepressed specific gene programmes involved in Ca^{2+} ion handling and in contractility. Cardiac expression of multiple fetal calcium channels is transcriptionally regulated by neuron-restrictive silencer factor (NRSF) through the recruitment of both class I and class IIa HDACs[66,67]. A dominant negative mutant of NRSF that is unable to bind repressors results in activation of the fetal gene programme, arrhythmogenesis and sudden death[68]. Thus, loss of HDAC1 and HDAC2 allows for the loss of repression by NRSF and other transcription factors, resulting in aberrant transcriptional

activity of genes involved in calcium flux and contractility, leading to cardiac arrhythmia and sudden death.

HDAC3

HDAC3 mutant mice die before E9.5 owing to defects in gastrulation[69–71]. The target genes responsible for this early phenotype are unknown, although loss of *Hdac3* seems to be associated with defective DNA double-stranded break repair[70]. Conditional deletions of HDAC3 have so far been described for the liver and heart. Loss of HDAC3 in the liver disrupts lipid and cholesterol homeostasis, leading to an accumulation of lipids and a decrease in glycogen storage[71]. These changes are caused by derepression of a gene programme that usually is under the control of nuclear hormone receptors such as the thyroid hormone receptor and peroxisome proliferator-activated receptor gamma (PPARγ), which control key steps in lipid and cholesterol biosynthesis early in the postnatal liver. Only minor increases could be observed in bulk histone acetylation and on the promoters of dysregulated genes, indicating that other class I HDACs are also likely to play a part in liver homeostasis.

Deletion of HDAC3 in cardiomyocytes also led to a dramatic upregulation of ligand-induced lipid storage in the heart[69]. These mice survive until 3–4 months of age, at which point they show massive cardiac hypertrophy and derepression of genes that control fatty-acid uptake and metabolism. In the heart, these gene programmes are under the control of the nuclear receptor PPARα, and derepression by loss of HDAC3 leads to abnormalities that mimic the metabolic derangements observed in diabetic cardiomyopathies. Furthermore, loss of HDAC3 in the heart results in robust interstitial fibrosis, which is phenotypically independent of rampant PPARα activity. However, it is currently unknown whether the transcription factors that regulate the fibrotic gene programme are directly repressed by HDAC3. Overexpresion of HDAC3 in the heart leads to increased thickness of the myocardium, which is due to increased cardiomyocyte hyperplasia without hypertrophy[72].

Class IIa HDACs in development and physiology

Each of the four class IIa HDACs have been deleted in mice and, although each gene seems to be dedicated to specific programmes of tissue-specific gene expression, commonalities between the different loss-of-function phenotypes point to similar mechanisms of action. Many of these modes of action reflect the repressive influence of these HDACs on the expression and function of the MEF2 transcription factor, as well as their signal responsiveness. Importantly, there is a high degree of redundancy between the class IIa HDACs. It is thus possible that each tissue has a hard-wired threshold for class IIa HDAC repression, and that the observed phenotypes reflect the cell types or gene programmes that are most sensitive to MEF2 and HDAC activity.

Regulation of skeletogenesis by HDAC4

HDAC4 has a central role in the formation of the skeleton[33]. Most of the bones in the vertebrate skeleton are formed from a cartilaginous template in which chondrocytes undergo hypertrophy, which is followed by apoptosis. Thereafter, osteoblasts, blood vessels and other cell types invade and produce the mature bone matrix[73]. HDAC4 is expressed in prehypertrophic chondrocytes *in vivo*, and mice with a global deletion of HDAC4 die during the first week of life owing to ectopic ossification of endochondral cartilage, which prevents expansion of the rib cage and leads to an inability to breathe (FIG. 3). This lethal phenotype is accompanied by precocious and ectopic hypertrophy of chondrocytes, resulting in the conversion of cartilaginous skeletal elements to ossified bone. Runt related transcription factor 2 (RUNX2) and the MEF2C transcription factor, which interact with HDAC4, have vital roles in the control of chondrocyte hypertrophy and bone formation[74]. In the absence of

HDAC4, transcriptional activation of these factors is unrestrained, leading to excessive bone formation[33]. Consistent with this mechanism, forced expression of RUNX2 or a constitutively active form of MEF2 in developing chondrocytes mimics the HDAC4 loss-of-function phenotype[75]. Conversely, forced expression of a signal-resistant mutant form of HDAC4 in chondrocytes *in vivo* inhibits chondrocyte hypertrophy and differentiation (FIG. 3).

Thus, by repressing the activity of MEF2C and RUNX2 in developing chondrocytes, HDAC4 is able to delay chondrocyte hypertrophy and thereby control the timing and extent of ossification of endochondral bones (FIG. 3). MEF2 directly regulates the expression of extracellular matrix protein genes, such as collagen type X alpha 1, and of vascular endothelial growth factor (VEGF), which is required for angiogenesis in the late stages of chondrocyte development[75]. In addition, RUNX2 activates the expression of the secreted growth factor Indian hedgehog (IHH), which has a number of functions in endochondral bone development. These functions are mediated by enhancing chondrocyte proliferation and stimulating the synthesis of parathyroid hormone-related peptide (PTHrP), which in turn inhibits differentiation of prehypertrophic to hypertrophic chondrocytes[76]. RUNX2 expression is also controlled by MEF2, and RUNX2 is a target for regulation by HDAC4 (REF. 75).

Control of cardiovascular growth and function by HDAC5 and 9

Mice lacking either HDAC5 or HDAC9 are viable, whereas compound mutant mice lacking both HDAC5 and 9 show a propensity for lethal ventricular septal defects and thin-walled myocardium, which typically arise from abnormalities in growth and maturation of cardiomyocytes[32]. Given the interaction between class IIa HDACs and MEF2, and the central role of MEF2 in the control of cardiomyocyte differentiation, the developmental cardiac defects in these double mutant mice probably result from super-activation of MEF2. This would be expected to lead to precocious differentiation and cell-cycle withdrawal of cardiomyocytes, causing hypocellularity of the myocardium. In addition, class IIa HDACs participate in multiprotein complexes and modulate the activities of numerous other transcription factors involved in myocardial growth, such as the serum response factor, myocardin and calmodulin binding transcription activator 2 (CAMTA2)[77]. Thus, the absence of HDAC5 and 9 probably perturbs the precisely coordinated gene expression programmes required for myocyte differentiation, proliferation and morphogenesis that underlie heart formation.

The adult heart typically responds to stress by a pathological growth response that ultimately leads to loss of cardiac function[78–80]. MEF2 is sufficient and necessary to drive the pathological cardiac hypertrophy and heart failure that takes place in response to injury[81]. HDAC5 and 9 have redundant roles in the suppression of cardiac growth in response to stress signalling. Mice lacking either HDAC5 or 9 are hypersensitive to cardiac stress resulting from excess workload or neurohumoral signalling (FIG. 4). These stimuli typically activate the calcineurin and CaMK–PKD pathways, which in turn lead to phosphorylation of class IIa HDACs, promoting their nuclear export[82]. Deletion of class IIa HDACs eliminates the counter-regulatory mechanism that restrains cardiac growth and sensitizes MEF2 and perhaps other transcription factors so that they become activated by stress-dependent intracellular signals.

Functions of class IIa HDACs in skeletal muscle

Numerous functions for class IIa HDACs have been described in skeletal muscle. Skeletal muscle fibres differ in their contractile and metabolic properties, which reflect different patterns of gene expression[83]. Slow-twitch, or type I, myofibres exhibit an oxidative

metabolism, are rich in mitochondria, are heavily vascularized and are resistant to fatigue. By contrast, fast-twitch, or type II, myofibres exhibit glycolytic metabolism, are involved in rapid bursts of contraction and fatigue rapidly. The calcium-dependent protein kinases CaMK and PKD have been implicated in the transduction of calcium signals that upregulate the expression of oxidative, slow fibre-specific genes in skeletal muscle[84]. MEF2 is a target for calcium signalling in skeletal muscle and is a key regulator of the slow myofibre phenotype. This function of MEF2 is mediated through its regulation by class IIa HDACs: in slow myofibres, class IIa HDACs are selectively degraded by the proteasome and MEF2 exerts a transcriptional activation function. Among the target genes of MEF2 in slow myofibres are type IIx myosin heavy chain, myosin light chain 2, slow troponin I and myoglobin. In support of this mode of function of class IIa HDACs in skeletal muscle, genetic deletion of class IIa HDACs in this tissue derepresses MEF2 and results in conversion of fast fibres to slow fibres[85] (FIG. 5).

HDAC9 has also been shown to modulate the response of skeletal muscle to motor innervation. Electrical activity from motor neurons represses the expression of many muscle genes, including those encoding acetylcholine receptor subunits. In response to denervation, these genes are derepressed, resulting in hypersensitivity of the muscle fibre to acetylcholine. Mice lacking HDAC9 are extremely sensitive to denervation-induced changes in gene expression, whereas mice that overexpress HDAC9 in skeletal muscle are rendered insensitive to the effects of denervation[86].

In addition to being a signal-responsive modulator of gene transcription, the expression of HDAC9 is tightly modulated. A highly conserved MEF2-binding site in the proximal promoter of the HDAC9 gene drives the expression of HDAC9, thereby establishing a negative feedback loop in which MEF2 drives the expression of its own repressor[87] (FIG. 5). This feedback loop is thought to provide robustness and fine-tuning to the gene programmes controlled by HDAC9 and MEF2, and to provide a myogenic ‘rheostat’ that modulates muscle differentiation in response to extracellular cues.

Control of endothelial function by HDAC7

During embryogenesis, HDAC7 is specifically expressed in the endothelial cells that form the inner lining of the cardiovascular system[34]. Genetic deletion of HDAC7 in mice results in embryonic lethality, owing to a loss of integrity of endothelial-cell interactions and consequent rupture of blood vessels and haemorrhaging (FIG. 6). Vascular disruption in HDAC7-null mice is accompanied by upregulation of matrix metalloproteinase 10 (MMP10), an endoprotease that is secreted by endothelial cells and that degrades the extracellular matrix, thereby perturbing endothelial-cell and smooth muscle-cell interactions. The inappropriate expression of MMP10 can be traced to its regulation by MEF2; in the absence of HDAC7, MEF2 activity is elevated, leading to pathological levels of MMP10 (FIG. 6). Concurrently, tissue inhibitor of metalloproteinase 1 (TIMP1) is downregulated in endothelial cells, presumably as a secondary consequence of vascular demise. The downregulation of TIMP1 in the face of enhanced expression of MMP10 would be expected to further exacerbate vascular destruction[34].

The involvement of HDAC7 in the control of MMP10 expression and vascular integrity has potentially important implications for a variety of human disorders. Vascular leakage causes circulatory collapse and contributes to the pathogenesis of numerous usually life-threatening diseases, such as atherosclerosis and aneurysm. Moreover, the imbalance between MMP and TIMP activity has been shown to profoundly influence vascular integrity following myocardial infarction and during tumour angiogenesis[88,89]. Hence, strategies to maintain the repressive influence of HDAC7 on MEF2 — for example, through inhibition of the kinase

cascades that lead to the phosphorylation of HDAC7 and its dissociation from MEF2 — would be expected to be beneficial with respect to maintaining vascular integrity.

Control of cytoskeletal dynamics by HDAC6

Mice with a deletion of HDAC6 are the only HDAC mutant animals published so far that do not have an obvious phenotype. However, they do display a dramatic increase in acetylated tubulin, in line with the notion that HDAC6 is the main tubulin deacetylase[43]. Given the large body of experimental *in vitro* evidence that clearly shows that HDAC6 has important functions in modulating the misfolded protein response and cytoskeletal dynamics, the lack of phenotype in the HDAC6 mutants is somewhat surprising. It is possible that redundancy with HDAC10 can explain the lack of *in vivo* phenotype.

Therapeutic actions of HDAC inhibitors

The involvement of histone acetylation and deacetylation in so many aspects of development and tissue homeostasis might suggest that systemic inhibition of HDACs with pharmacologic inhibitors would result in nonspecific and catastrophic effects as a consequence of global derepression of gene expression. Thus, it is striking that systemic HDAC inhibition with compounds that broadly inhibit most or all HDACs is well tolerated *in vivo* and blocks numerous disease-associated gene expression programmes in a seemingly specific manner.

Given the dramatic phenotypes that result from HDAC gene deletions, why are HDAC inhibitors so well tolerated *in vivo*? we propose three explanations, which are not mutually exclusive. First, a genetic deletion of an HDAC results in the complete absence of the enzyme, whereas inhibitors do not result in complete inhibition of activity. Second, a genetic deletion of an HDAC eliminates the gene product permanently, whereas the actions of an inhibitor are transient. Third, and perhaps most importantly, HDACs participate in multiprotein transcriptional complexes. Genetic deletion of an HDAC perturbs the complexes in which it would normally be associated, whereas inhibitors are believed to block enzymatic activity without necessarily disrupting the repressive complex.

Classical HDAC inhibitors such as trichostatin A (TSA) or suberoylanilide hydroxamic acid (SAHA) are mostly ‘pan–HDAC’ inhibitors; that is, they block, with similar affinities, the activity of all isoforms except class IIa HDACs. For example, the IC_{50} values for SAHA are: HDAC1 = 37.1 nM; HDAC3 = 44.6 nM; and HDAC6 = 40.9 nM[90]. Given that different HDAC isoforms govern dramatically different gene expression programmes in development and disease, it seems plausible that isoform-selective inhibitors should lead to improved efficacy and drug safety. The recent mechanistic insights into the biochemistry of class IIa HDACs should also be taken into account[36,41,91]. Many screening studies used class IIa HDACs purified from mammalian cells for the development of class IIa isoform-specific inhibitors and, surprisingly, compounds were identified that blocked class IIa but not class I activity. These compounds probably function as small-molecule inhibitors of protein–protein interactions and not as bona fide HDAC inhibitors[92]. As these molecules are entering clinical trials, it is important to realize that they might show biological properties distinct from classical HDAC inhibitors.

HDAC inhibitors from multiple chemical classes have entered clinical trials, and SAHA (marketed as Vorinostat, brand name Zolinza) has been approved for treatment of cutaneous manifestations of advanced, refractory T-cell lymphoma in a select group of patients[93]. The exact mechanism for the effect of HDAC inhibitors on tumour cells is currently unknown, and numerous explanations, such as changes in gene transcription, direct induction of apoptosis, production of reactive oxygen species and induction of cell-cycle arrest, have

been proposed[92,94–96]. The specific HDAC isoforms that mediate this antiproliferative effect also remain to be clearly identified. Genetic deletion of HDAC3 leads to cell-cycle dependent DNA damage coupled with defective double-stranded break repair[70]. HDAC3-null cells are thus sensitized to ionizing radiation, a phenomenon that has also been observed with HDAC inhibitors[97]. Therefore, some of the effects observed with HDAC inhibition might be mediated via HDAC3, although the involvement of other isoforms can not be ruled out[98]. The existence of conditional alleles for all class I HDACs might allow the creation of transformed cancer cell lines with conditional alleles for all the different class I HDAC isoforms (and their combinations), which would make a systematic analysis of HDAC requirement in cancer cells possible. These studies could then be extended by crossing conditional HDAC-null alleles into tumour-prone genetic backgrounds.

One of the most perplexing aspects of HDAC biology is that pharmacological inhibition of HDAC activity provides a therapeutic benefit in such a wide variety of disease states. TABLE 1 gives an overview of the disease states in which HDAC inhibition has been shown to be beneficial as well as the proposed mechanisms involved. These range from infectious and immunological diseases to traumatic shock, and from cardiac hypertrophy to neurodegenerative disease[99–105], and in certain circumstances HDAC inhibitors are even able to ‘cure’ genetic disease in humans[106]. Although a unifying theory explaining how reduced deacetylase activity is beneficial in such diverse pathophysiological states is currently unknown, it is tempting to speculate that most of these diseases have an epigenetic component (that is, aberrant histone acetylation), and that treatment with HDAC inhibitors resets the epigenetic memory of the cell to a pre-disease state.

Issues for the future

Although the interest in HDAC biology has intensified with the successful introduction of HDAC inhibitors in the clinical setting, we are still far from understanding the intricacies of protein acetylation. The number of identified acetylated non-histone proteins is rapidly increasing, raising questions regarding whether phenotypes resulting from HDAC gene deletion or from pharmacological inhibition reflect changes in chromatin structure and transcription, or if they reflect hyperacetylation of non-histone proteins. In the cases of non-histone substrates, it will be important to identify the proteins and understand how acetylation influences their actions. It also remains to be determined whether different HDAC isoforms have specific targets *in vivo*, as suggested by the specific phenotypes resulting from genetic deletion of individual HDACs. It is expected that unbiased profiling of hyperacetylated proteins in knockout or inhibitor-treated cells using mass spectrometry approaches will be a valuable tool in answering these questions. A recent study in human cancer cells showed the feasibility of this approach[107].

Given the variety of pre-clinical studies in which HDAC inhibitors have shown a therapeutic benefit, another major challenge will be to decipher the role of individual HDACs in specific disease processes and to develop isoform-specific HDAC inhibitors. Because HDACs can affect multiple targets, it will be important to develop inhibitors that selectively block the pathological actions of HDACs. Solving these challenges will most likely broaden the therapeutic window and possibly lead to the clinical application of HDAC inhibitors in a variety of non-oncological disease states.

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Glossary

Transition state	A particular state corresponding to the highest energy in a chemical reaction
Morpholino	A stable antisense oligonucleotide that is commonly used in zebrafish and *Xenopus laevis* to inhibit either the translation or splicing of mRNAs
Gene trap	A mutation strategy that uses insertion vectors to trap or isolate transcripts from flanking genes
Gastrulation	The process in animal embryos in which the endoderm and mesoderm move from the outer surface of the embryo to the inside, where they give rise to the internal organs
Interstitial fibrosis	Proliferation of fibroblasts resulting in increased collagen production and consequent organ dysfunction
Chondrocyte	The type of cell that produces and maintains the cartilaginous matrix
Osteoblast	The type of cell that is responsible for bone formation by secreting and mineralizing the bone matrix
Ossification	The process of bone formation in which soft connective tissue is converted into mineralized tissue
Endochondral bone	The parts of the skeleton which form by endochondral ossification, a process in which cartilage is replaced by bone.
Rheostat	A module for fine tuning a molecular circuit
IC_{50}	Inhibition concentration 50%. The concentration of inhibitor that is required to inhibit 50% of the activity of an enzyme compared with an unhibited control.

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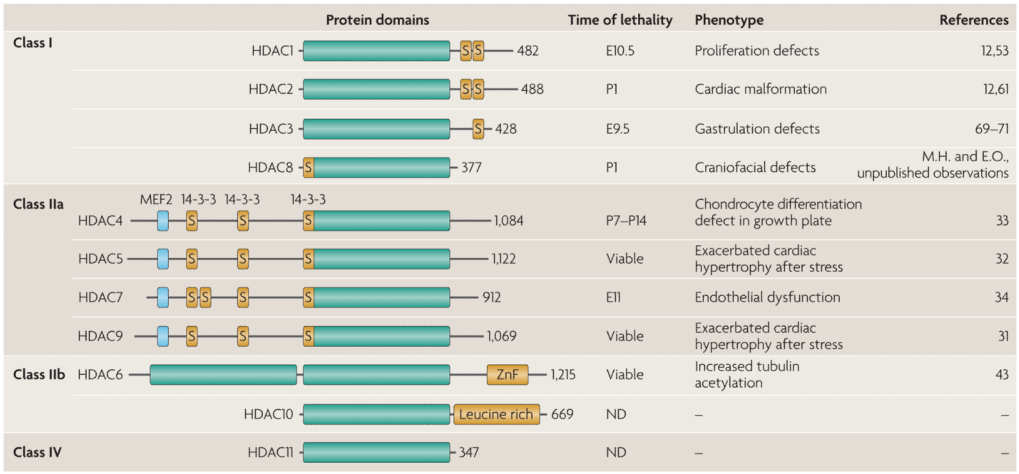

Figure 1. The histone deacetylase (HDAC) superfamily, showing protein domains, loss-of-function phenotypes in mice and time point of lethality of the knockouts

Green rectangles indicate the conserved HDAC domain; numbers following the HDAC domain indicate the number of amino acids. Myocyte enhancer factor 2 (MEF2)-binding sites are marked by a blue square, and binding sites for the 14-3-3 chaperone protein are also shown. E, embryonic day; ND, not determined; P, days postnatal; S, serine phosphorylation sites; ZnF, zinc finger.

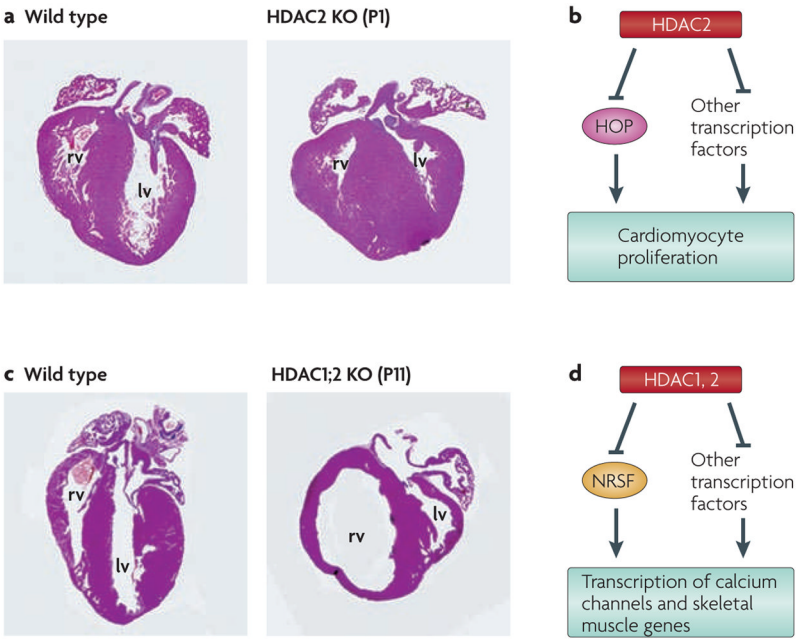

Figure 2. Control of heart development by histone deacetylase 1 (HDAC1) and HDAC2

a | Histological sections of hearts from wild type and HDAC2 knockout (KO) mice at postnatal day 1 (P1). Note the excessive number of cardiomyocytes in the mutant heart, which fill the chambers of the left ventricle (lv) and right ventricle (rv). **b** | Schematic of the role of HDAC2 in the repression of cardiomyocyte proliferation through inhibition of homeodomain-only protein (HOP). **c** | Histological sections of hearts from wild-type mice and mice with a cardiac deletion of HDAC1 and 2 at P11. Note the dilatation of the right ventricle in the mutant, which is indicative of heart failure. **d** | Schematic of the redundant roles of HDAC1 and 2 in regulation of calcium channel and skeletal muscle genes in cardiomyocytes via repression of neuron-restrictive silencer factor (NRSF) and other transcription factors. Parts **a** and **c** are reproduced, with permission, from REF. 12 © (2007) Cold Spring Harbor Laboratory Press.

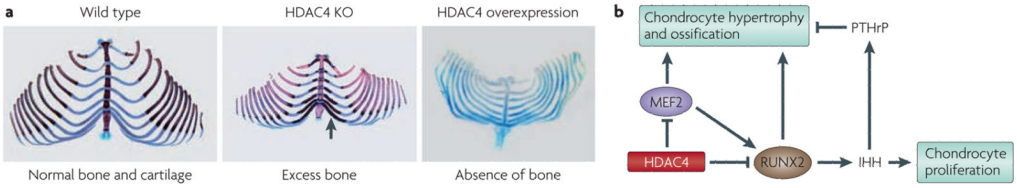

Figure 3. Control of chondrocyte hypertrophy by histone deacetylase 4 (HDAC4)

a | Ribs from neonatal mice stained for bone (red) and cartilage (blue). Deletion of HDAC4 results in ossification of cartilage by the arrowhead), whereas overexpression of HDAC4 in the cartilage of transgenic mice prevents ossification. **b** | Schematic of the repressive influence of HDAC4 on myocyte enhancer factor 2 (MEF2) and runt related transcription factor 2 (RUNX2) in the pathway for chondrocyte proliferation and hypertrophy. IHH, Indian hedgehog; KO, knockout; PTHrP, parathyroid hormone-related peptide. Part **a** is reproduced, with permission, from REF. 33 © (2007) Cell Press.

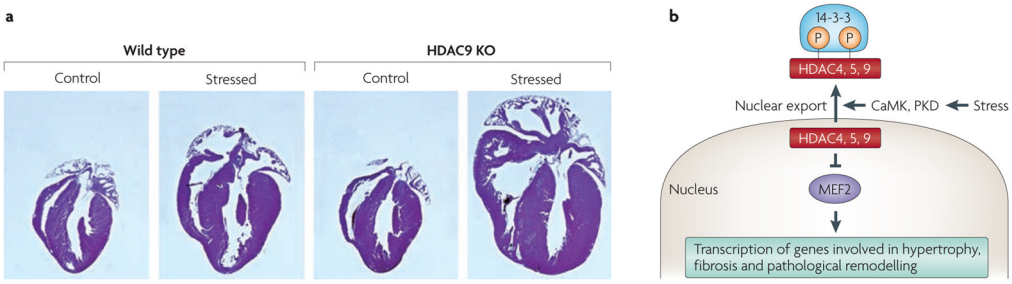

Figure 4. Control of pathological cardiac hypertrophy by class IIa histone deacetylases (HDACs)

a | Histological sections of hearts from wild-type and HDAC9 knockout (KO) adult mice. Mice were subjected to cardiac stress by expression of a cardiac-specific transgene encoding activated calcineurin, which drives pathological hypertrophy. Note that HDAC9 knockout mice have normal hearts in the absence of stress, but display cardiomegaly in response to stress, owing to loss of the growth-inhibitory function of HDAC9. **b** | Schematic of the repressive influence of class IIa HDACs on myocyte enhancer factor 2 (MEF2) and pathological cardiac remodelling. Stress-inducible kinases, such as calcium/calmodulin-dependent protein kinase (CaMK) and protein kinase D (PKD), induce the phosphorylation of class IIa HDACs, which creates docking sites for the 14-3-3 chaperone protein, resulting in nuclear export with consequent activation of MEF2 and its downstream target genes, which are involved in cardiac remodelling. Part **a** is reproduced, with permission, from REF. 31 (2007) © Cell Press.

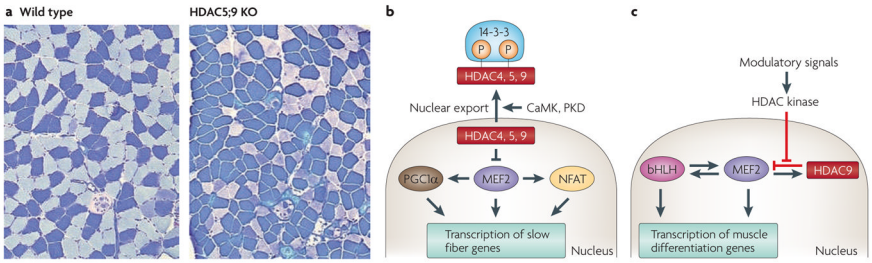

Figure 5. Control of slow myofibre gene expression by class IIa histone deacetylases (HDACs)

a | Histological sections of soleus muscle from wild-type and HDAC5;9 double mutant knockout (KO) mice stained for type I myosin heavy chain, a marker of type I slow myofibres. Note the increase in slow myofibres after deletion of class IIa HDACs. **b** | Schematic of the repressive influence of class IIa HDACs on myocyte enhancer factor 2 (MEF2), which acts together with PGC-1α (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha) and NFAT (nuclear factor of activated T-cells) to promote the formation of slow myofibres. Signalling by calcium/calmodulin-dependent protein kinase (CaMK) and protein kinase D (PKD) induces the phosphorylation of class IIa HDACs, which creates docking sites for the 14-3-3 chaperone protein, resulting in nuclear export with consequent activation of slow myofibre genes. **c** | A MEF2-dependent negative feedback loop for the control of HDAC9 expression during muscle differentiation. Myogenic basic helix-loop-helix (bHLH) transcription factors activate the expression of MEF2, which then amplifies and sustains the expression of myogenic bHLH genes. Myogenic bHLH factors and MEF2 also cooperate to activate skeletal muscle differentiation genes. In addition, MEF2 activates the expression of HDAC9, which in turn represses MEF2 activity. Signals that influence myogenesis activate HDAC kinases and thereby repress HDAC9 activity, providing a ‘rheostat’ mechanism for the control of myogenesis. Part **a** is reproduced from REF. 85.

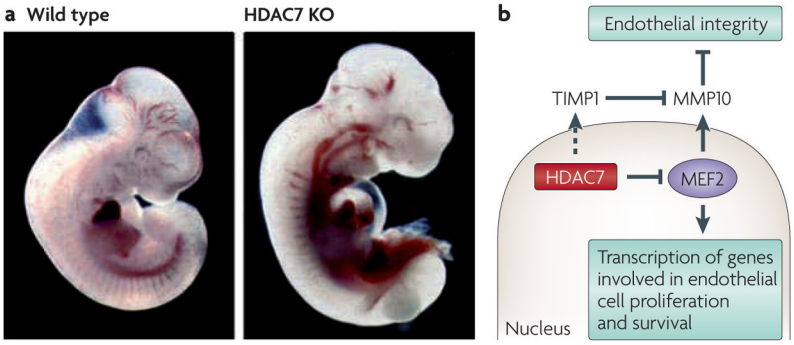

Figure 6. Control of endothelial integrity by histone deacetylase 7 (HDAC7)

a | Wild-type and HDAC7 knockout (KO) embryos at embryonic day 10.5 (E10.5). The absence of HDAC7 results in vascular rupture, pericardial oedema and haemorrhaging throughout the mutant embryos. **b** | Schematic of the role of HDAC7 in maintenance of vascular integrity. HDAC7 is expressed specifically in endothelial cells, where it represses the activity of myocyte enhancer factor 2 (MEF2). In the absence of HDAC7, MEF2 activity is elevated, resulting in upregulation of matrix metalloproteinase 10 (MMP10) and degradation of cell–cell interactions required for vascular integrity. Deletion of HDAC7 also leads to downregulation of tissue inhibitor of metalloproteinase 1 (TIMP1), presumably through indirect mechanisms, which further enhances MMP10 activity.

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Table 1

Clinical and experimental use of histone deacetlyase (HDAC) inhibitors in diverse disease states

Disease	Proposed mechanism	Refs
Humans		
HIV infection	De-silencing of latent virus	99
Cutaneous T-cell lymphoma	Upregulation of tumor-suppressor genes, induction of apoptosis	108, 109
GPI deficiency	Increased PIGM expression by hyper-acetylation of histones on promoter	106
Ulcerative colitis	Inhibition of NF-κB activation	110
Sickle cell disease	Increase in fetal haemoglobin expression	111
Mice		
Arthritis	Inhibition of TNFα expression and of inflammation	112
Asthma	Inhibition of cytokine expression and T-cell infiltration	113
Autoimmune encephalitis	Upregulation of antioxidant, antiexcitotoxicity and proneuronal factors	114
Colitis	Suppression of pro-inflammatory cytokines	115
Cardiac hypertrophy	Unknown	102, 116
Dementia	Dendrite sprouting, increased synapse number	100
Graft versus host disease	Reduction of pro-inflammatory cytokines	101, 117
Hepatitis	Inhibition of TNFα and INFγ	118
Muscular dystrophy	Induction of follistatin	119
Systemic lupus erythematosus	Downregulation of pro-inflammatory cytokines	120
Spinal muscular atrophy	Activation of survival motor neuron 2 gene	121
Rats		
Haemorrhagic shock	Reduction of TNFα expression	122
Brain trauma	Inhibiting neuroinflammation	104

GPI, glycosylphosphatidylinositol; INFγ, interferon gamma, NF-κB, nuclear factor kappa B; PIGM, phosphatidylinositol glycan anchor biosynthesis, class M; TNFα, tumour-necrosis factor alpha.