Journal of Cellular Biochemistry 93:57–67 (2004)

Regulation of Histone Deacetylase Activities

Nilanjan Sengupta and Edward Seto*

H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida 33612

Abstract Histone deacetylases (HDACs) are enzymes that catalyze the removal of acetyl groups from lysine residues in both histone and non-histone proteins. They play a key role in the regulation of gene transcription and many other biological processes involving chromatin. Significantly, recent studies suggest that HDACs are critically involved in cell-cycle regulation, cell proliferation, differentiation, and in the development of human cancer. HDAC inhibitors currently are being exploited as potential anti-cancer agents. As expected for vital regulators of many cellular processes, the activities of HDACs are tightly controlled and precisely regulated by multiple mechanisms. The activities of most if not all HDACs are regulated by protein–protein interactions. In addition, many HDACs are regulated by post-translational modifications as well as by subcellular localization. Less studied, but perhaps equally important, is the regulation of some HDACs by control of expression, availability of cofactors, and by proteolytic processing. A complete understanding of how HDACs are regulated will contribute not only to our overall knowledge of chromatin structure and gene control, but will offer tremendous insight into approaches for developing therapeutic HDAC inhibitors with improved specificity.
J. Cell. Biochem. 93: 57–67, 2004.

Key words: HDAC; histone deacetylation; chromatin; gene regulation; protein–protein interaction; post-translational modification; subcellular localization

The packaging of eukaryotic DNA into chromatin poses a fundamental accessibility problem. All reactions within the chromatin substrate including transcription, replication, recombination, and repair, must be initiated and regulated by DNA-binding factors. The interaction of these factors with their target DNA requires chromatin to be partially unwound. Many studies have established that such chromatin flexibility is achieved by two principal molecular mechanisms. First, ATP-dependent chromatin remodeling factors alter histone–DNA interactions such that nucleosomal DNA becomes more accessible to interacting proteins [Becker and Horz, 2002; Lusser and Kadonaga, 2003]. Second, the amino-terminal tails of the core histone proteins are subjected to a variety of covalent post-translational modifications including acetylation, phosphorylation, methylation, ubiquitination, and ADP-ribosylation [Strahl and Allis, 2000; Zhang and Reinberg, 2001; Berger, 2002]. These modifications play essential roles in generating the dynamic state of chromatin.

Acetylation, which is linked predominantly to transcriptional activation, is the most extensively studied post-translational histone modification to date. This process involves the transfer of an acetyl group from the acetyl coenzyme A metabolic intermediary to the ε-amino group of lysine residues in histone tails, catalyzed by a group of enzymes known as histone acetyltransferases (HAT) [Roth et al., 2001]. It is generally accepted that the primary effect of acetylation is to partially neutralize the positive charge of histones, thus decreasing their affinity for DNA and thereby generating a permissive structure for the binding of proteins to DNA. Additionally, acetylated histone tails can recruit other chromatin-associated proteins.

The functional importance of acetylation lies in its highly reversible nature that depends on the accuracy and efficiency of the reverse reaction, histone deacetylation, which is catalyzed by a group of enzymes known as histone deacetylases (HDACs). Broadly speaking, HDACs promote transcriptional repression

Grant sponsor: National Institutes of Health; Grant number: R01 GM58486.

*Correspondence to: Edward Seto, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612. E-mail: setoe@moffitt.usf.edu

Received 24 April 2004; Accepted 27 April 2004

DOI 10.1002/jcb.20179

Published online 20 July 2004 in Wiley InterScience (www.interscience.wiley.com).

and silencing [Cress and Seto, 2000; Ng and Bird, 2000; Grozinger and Schreiber, 2002; Thiagalingam et al., 2003; Verdin et al., 2003; Yang and Seto, 2003]. Mammalian HDACs have been divided into three different classes (I, II, and III) based on sequence homology to yeast HDACs, reduced potassium dependency 3 (Rpd3), histone deacetylase 1 (Hda1), and silent information regulator 2 (Sir2), respectively. Class I and II HDACs are sensitive to the inhibitor trichostatin A (TSA), whereas class III is insensitive to TSA and requires the coenzyme NAD^+ as a cofactor. Most HDACs do not contain intrinsic DNA-binding activities; other cellular factors are required for their proper recruitment to specific locations in the genome. In addition to histones, many HDACs can deacetylate non-histone proteins in vitro and in vivo.

HDAC proteins are critical regulators of fundamental cellular events, including cell-cycle control, differentiation, and apoptosis, and their misregulation is involved in tumorigenesis [Marks et al., 2001c]. HDACs have been implicated in mediating the function of oncogenic translocation products in specific forms of leukemia and lymphoma [Melnick and Licht, 2002]. Importantly, HDAC inhibitors can induce growth arrest, differentiation, and/or apoptosis of transformed cells and therefore, are being explored as therapeutic agents for the treatment of certain forms of cancer [McLaughlin et al., 2003; Secrist et al., 2003; Yoshida et al., 2003].

As expected with proteins that occupy an essential physiological role, the activities of the HDAC proteins are highly regulated by multiple distinct mechanisms. However, much of the work so far on HDACs has gone into understanding their functions and mechanisms of action, and how they relate to cancer. Less effort has been made in elucidating how these proteins are regulated. The focus of this article is to provide a much-needed overview and discussion of our current knowledge regarding the regulation of HDACs. A thorough evaluation of HDAC regulation will eventually improve our overall understanding of the biology of HDACs.

REGULATION BY PROTEIN COMPLEX FORMATION

The activity of HDACs can be separated into two areas, enzymatic activity (the ability to deacetylate histones or other non-histone protein substrates), and functional activity (the ability to regulate transcription, for example). Several HDACs exist as a component in stable large multi-subunit complexes, and most if not all HDACs interact with other cellular proteins. Results from many studies in different laboratories suggest that with the exceptions of yeast HOS3 and mammalian HDAC8, most purified recombinant HDACs are enzymatically inactive [Carmen et al., 1999; Hu et al., 2000; Lee et al., 2004]. Any protein that associates with HDACs, therefore, has the potential to activate or inhibit the enzymatic activity of HDACs. Likewise, HDACs, in general, have no DNA binding activity, therefore, any DNA-binding protein that targets HDACs to DNA or to histones potentially can affect HDAC function.

Early studies of human HDAC1 and HDAC2 revealed that they exist together in at least three distinct multi-protein complexes called the Sin3, the NuRD/NRD/Mi2, and the CoREST complexes [Hassig et al., 1997; Laherty et al., 1997; Zhang et al., 1997, 1998b; Tong et al., 1998; Ayer, 1999; Ng and Bird, 2000; Humphrey et al., 2001; You et al., 2001]. Sin3 and NuRD complexes share a core comprised of four proteins: HDAC1, HDAC2, RbAp46, and RbAp48. In addition, each complex contains unique polypeptides (Sin3, SAP18, and SAP30 in the Sin3 complex; Mi2, MTA-2, and MBD3 in the NuRD complex). The first clue underscoring the importance of associated proteins in modulating HDAC enzymatic activity came from purification studies of the NuRD complex. In an elegant reconstitution approach using purified subunits, Zhang et al. [1999] showed that the HDAC activity of the core complex was severely compromised compared to the native holocomplex. The addition of MTA2 to the core complex was sufficient to direct the formation of an enzymatically active complex. MBD3, another component of the NuRD complex, was found to mediate the association of MTA2 with the core HDAC complex. Shortly afterwards, another study in yeast identified Sds3p as an integral component of the Sin3/Rpd3 HDAC complex [Lechner et al., 2000]. Using an sds3Δ strain, the authors showed that in the absence of Sds3p, the Rpd3p complex lacks HDAC activity. Additionally, Sds3p could promote the integrity of the Sin3 complex, and the association of Sin3p with Rpd3p was severely compromised in the absence of Sds3p. Subsequently, another study extended these findings and

identified mammalian SDS3 as a key component of the Sin3 corepressor complex that augments enzymatic activity of HDAC1 in vivo [Alland et al., 2002]. Like MTA2 and SDS3, in the CoREST complex, the association of HDAC1/2 with CoREST is essential for HDAC enzymatic activity [You et al., 2001].

Perhaps the best example of HDAC regulation by protein–protein interaction emanated from studies of HDAC3. Data from early studies suggested that nuclear receptor corepressors, silencing mediator of retinoid and thyroid receptor (SMRT) and nuclear receptor corepressor (N-CoR), function as platforms for recruitment of HDACs [Alland et al., 1997; Heinzel et al., 1997]. Surprisingly however, the interaction between HDAC3 and SMRT/N-CoR resulted in the stimulation of HDAC3 enzymatic activity [Wen et al., 2000; Guenther et al., 2001; Zhang et al., 2002]. No enhancement of HDAC3 activity was seen with an N-CoR mutant that did not bind HDAC3. Thus, it appears that the enzymatic activity of HDAC3 is specifically regulated by the availability of interacting SMRT/N-CoR. The activation of HDAC3 is mediated by a deacetylase-activating domain (DAD) present in SMRT and N-CoR. This domain was found to be necessary and sufficient for HDAC3 enzymatic activation in reconstitution experiments using purified components.

A more recent study by Guenther et al. [2002] identified an additional level of HDAC3 regulation. They showed that the enzymatic activation of HDAC3 by SMRT requires an energy-dependent prior priming of HDAC3 by the protein folding machinery, TCP-1 ring complex (TriC). Upon SMRT binding, TriC dissociates from HDAC3, yielding an enzymatically active HDAC complex. Incidentally, another ATP-dependent chaperone protein, HSP70, has been implicated in enhancing the catalytic activity of HDAC1, 2, and 3 [Johnson et al., 2002]. Unlike HDAC3, the class II HDACs cannot be activated by SMRT/N-CoR. Instead, Fischle et al. [2001, 2002] showed that the enzymatic activity of HDAC4, 5, and 7 is dependent on the association with the HDAC3/SMRT/N-CoR complex. These studies point toward a model in which HDAC4, 5, and 7 are not active deacetylases but rather, exert their functional effects by recruiting preexisting enzymatically active SMRT/N-CoR complexes containing HDAC3.

The three proteins that activate class I HDAC activities, MTA2, CoREST, and SMRT/N-CoR all possess SANT domains. The SANT domain is a putative DNA binding domain present in a number of transcriptional regulators, including SWI3, ADA3, N-CoR, and TFIIIB. Both SMRT/N-CoR and CoREST contain two SANT domains, of which only one is necessary for activating HDAC enzymatic activity. In the case of SMRT, the second SANT motif regulates HDAC3 by targeting the enzyme to histones [Yu et al., 2003]. Deletion of the HDAC-interacting SANT domain results in loss of HDAC enzymatic activity thereby reinforcing the idea that SANT domain proteins clearly are important regulators of HDAC activity. However, not all HDAC complexes contain SANT domain proteins. For example, SANT domain-containing proteins have not been identified in the well-characterized Sin3/HDAC complex. It is conceivable that the HDAC activator SDS3 may function by recruitment of a SANT domain protein. Alternatively, other components in the Sin3 complex can substitute for the function of a SANT domain protein to activate HDAC activity.

REGULATION BY POST-TRANSLATIONAL MODIFICATIONS

Phosphorylation

Phosphorylation is a leading post-translational mechanism for controlling many enzyme activities. In germinating *Zea mays* embryos, HD1 can be separated into multiple forms by HPLC. Phosphorylation of one of these enzyme forms, HD1-A, causes a change in substrate specificity of the enzyme [Brosch et al., 1992]. Another maize deacetylase, HD2, consists of three polypeptides, one of which, p39, is phosphorylated by protein kinase CK2 [Lusser et al., 1997].

All mammalian HDACs possess potential phosphorylation sites and many of them have been found to be phosphorylated in vitro and in vivo. In one study, human HDAC1 was analyzed by ion trap mass spectrometry, and two phosphorylated residues, Ser^{421} and Ser^{423}, located in the protein's C-terminal, were identified [Pflum et al., 2001]. The protein kinase CK2 was shown to phosphorylate HDAC1 in vitro. Site directed mutations of Ser^{421} and Ser^{423} to alanine in HDAC1 reduced enzymatic as well as transcriptional repression activity, and similar results were obtained with a C-terminal deletion mutant of HDAC1. Importantly, complex

formation, including association with RbAp48, MTA-2, mSin3A, and CoREST, is severely impaired in these mutants. There are two non-mutually exclusive possibilities to explain how phosphorylation can affect HDAC activity. Conceivably, phosphorylation of HDAC1 can alter its conformation into a more favorable enzymatically active form. Alternatively, phosphorylation might increase the ability of HDAC1 to interact with proteins, such as MTA2 and SDS3, which can activate its activity and consequently enhance its enzymatic activity.

In a separate study, Cai et al. [2001] also reported phosphorylation of HDAC1. However, this study differs from the previous one in three important observations: (i) a phosphorylation site was found to exist between residues 387 and 409, (ii) a single alanine mutation in any of the CK2 sites failed to reflect any change in the level of phosphorylation compared to wildtype HDAC1, and (iii) phosphorylation of HDAC1 did not affect the deacetylase activity of the protein.

A third study took a different approach to explore the regulation of HDACs by phosphorylation [Galasinski et al., 2002]. Treatment of cells with the phosphatase inhibitor okadaic acid (OA) resulted in altered chromatographic elution as well as an altered deacetylase activity profile of fractionated HDAC1 and HDAC2. Furthermore, the authors showed that both HDAC1 and HDAC2 exist in three different phosphorylation forms: unphosphorylated, basally phosphorylated, and hyperphosphorylated. Mitotic arrest resulted in hyperphosphorylation of HDAC2 but not HDAC1. Consistent with the earlier observation by Pflum et al. [2001] that phosphorylation promotes enzymatic activity, dephosphorylation of hyperphosphorylated HDACs led to a small but significant decrease in deacetylase activities in OA-treated cells. Unexpectedly, HDAC hyperphosphorylation induced by OA treatment was found to disrupt the association of HDAC1 and HDAC2 with each other as well as with both mSin3A and YY1 corepressors. This observation argues that not only phosphorylation per se but also the extent of HDAC phosphorylation in vivo are equally important in the final physiological outcome.

Like HDAC1, HDAC2 is a phosphoprotein and phosphorylation occurs at multiple serine residues located in the C-terminal end of the protein [Tsai and Seto, 2002]. In HDAC2, besides Ser^{422} and Ser^{424} (which correspond to Ser^{421} and Ser^{423} of HDAC1), Ser^{394} also can be phosphorylated by CK2. Unlike HDAC1, which can be phosphorylated in vitro by CK2, protein kinase A (PKA), and protein kinase G (PKG), only CK2 can phosphorylate HDAC2 in vitro. Phosphorylation of HDAC2 is necessary for both enzymatic activity as well as for association with the corepressors mSin3 and Mi2. However, unlike HDAC1, phosphorylation of HDAC2 does not affect transcriptional repression, at least in transient transfection coupled with luciferase reporter assays. In a study of breast cancer cells by Sun et al. [2002], the transcriptional repressors Sp1 and Sp3 were shown to associate with HDAC1 and CK2-phosphorylated HDAC2. Additionally, phosphorylation of HDAC2 associated with Sp1/Sp3 augments deacetylase activity recruited to the estrogen-regulated promoters.

An interesting outcome from the study by Tsai and Seto [2002] was that in addition to HDAC1 and HDAC2, the remaining class I HDACs (HDAC3 and HDAC8) could also be phosphorylated. HDAC3 and HDAC8 are phosphorylated in vitro by CK2 and PKA, respectively. A recent study confirmed that HDAC8 was phosphorylated in vitro and in vivo by PKA [Lee et al., 2004], and that phosphorylation of HDAC8 differs from other class I members in two significant points. First, the site of phosphorylation is at a non-conserved residue, Ser^{39}, located at the N-terminus of HDAC8. Second, phosphorylation of HDAC8 by PKA drastically reduced enzymatic activity, which was reflected in the hyperacetylation of histones H3 and H4.

Numerous studies have shown convincingly that the functions of class II HDACs are regulated by phosphorylation. For example, HDAC4 and HDAC5 can block myogenesis by associating with and inhibiting the activity of the MEF2 transcription factor, and calcium/calmodulin-dependent kinase (CaMK) signaling prevents this inhibition by dissociating MEF2–HDAC complexes [Lu et al., 2000a,b]. Although HDAC4 and HDAC5 can be phosphorylated by CaMK in vitro [McKinsey et al., 2000a,b], a subsequent study indicated that the class II HDAC kinase was not inhibited by compounds that inhibit CaMK and was not recognized by an anti-CaMKIV antibody [Zhang et al., 2002]. In a different study, HDAC4 was found to associate with extracellular signal-regulated kinases 1 and 2 (ERK1/2) [Zhou et al., 2000]. In both instances, phosphorylation of

class II HDACs does not appear directly to influence the enzymatic activity of these proteins; rather, it modulates their subcellular localization.

While most studies on HDAC phosphorylation have focused on the identification and characterization of HDAC kinases, the involvement of phosphatases in regulating HDAC activity may be equally important. Based on the range of OA concentrations required to increase HDAC phosphorylation and the differential sensitivities of HDACs to different phosphatases, it was concluded that the phosphorylation status of HDAC1 and HDAC2 most likely are regulated by PP1 [Galasinski et al., 2002]. In another study, HDAC1 was found to associate with PP1 and promote dephosphorylation of CREB, although it is not known whether HDAC1 serves as a substrate for PP1 in this stable complex [Canettieri et al., 2003]. Using microcystin affinity chromatography to isolate protein phosphatase complexes, Brush et al. [2004] found that HDAC1, HDAC6, and HDAC10 (but not HDAC2, 3, 4, and 5) are components of active serine/threonine phosphatase complexes. Far-Western analysis showed that HDAC6 binds the catalytic subunit of PP1, but not PP2A. HDAC4 did not bind PP1 under identical conditions confirming the selectivity of the HDAC–PP1 interaction. Interestingly, the HDAC inhibitor TSA was able to disrupt the interaction of HDAC6 with PP1. The importance of PP1 in the regulation of HDACs was reinforced in an earlier unrelated study to identify PP1 binding proteins. Using a labeled PP1 protein probe, Ajuh et al. [2000] screened a human cDNA expression library, and out of 12 cDNAs that encoded PP1-interacting proteins, two of them contained HDAC6. Taken together, it is fair to conclude that PP1 most likely is a major phosphatase that regulates the state of phosphorylation of at least several human HDACs.

Sumoylation

Besides phosphorylation, another post-translational modification that has been shown to regulate HDAC activity and function is the conjugation of small ubiquitin-related modifier (SUMO-1). Unlike the protein degradation effects of ubiquitination, SUMO-1 modification exerts varied effects on the target protein, including subcellular localization, protein–protein interaction, and enzymatic activity modulation. Two independent studies [Colombo et al., 2002; David et al., 2002] identified HDAC1 as a substrate for SUMO-1 modification in vitro and in vivo at Lys^{444} and Lys^{476}. However, these two studies differed in the functional relevance of sumoylation on HDAC1 activity. Abrogation of sumoylation by mutating the target lysines markedly reduced HDAC1-mediated transcriptional repression in one of the studies [David et al., 2002], while the repressor and enzymatic activity of the same mutant remained unchanged in the other study [Colombo et al., 2002]. In a separate study by Kirsh et al. [2002], class II HDAC4 was found to be modified by SUMO-1, and a sumoylation-deficient HDAC4 mutant displayed reduced repressor and deacetylase activity. More interestingly, this study linked sumoylation to nuclear import by showing that (i) nuclear localization is a prerequisite for HDAC4 sumoylation, (ii) nuclear pore complex protein RanBP2 catalyzes SUMO-1 modification, and (iii) signaling pathways like CaMK, which induce nuclear export, abrogate SUMO-1 modification. Among other class II HDACs, HDAC6 and MITR (a variant of HDAC9) have been shown to be SUMO-1 modified in vitro [Kirsh et al., 2002]. It is worth noting that the reduced repressor activity of sumoylation-deficient HDAC1 and HDAC4 was found to be independent of their ability to associate with known binding proteins including mSin3A and N-CoR. However, several recent studies have determined that sumoylation of some transcription repressors regulates their association with HDACs and, consequently, their capacity to repress transcription [Girdwood et al., 2003; Ling et al., 2004; Yang and Sharrocks, 2004].

REGULATION BY SUBCELLULAR LOCALIZATION

In order to deacetylate histones and to repress transcription, HDACs must reside in the nucleus. Therefore, signals that enhance HDAC nuclear localization positively regulate HDAC activities. In contrast, signals that increase cytoplasmic localization of HDACs negatively regulate their activities. HDAC1, 2, and 8 are predominantly nuclear proteins, and at this time, it appears that these three class I HDACs are not regulated by subcellular localization. In contrast, HDAC3 can be found both in the nucleus and cytoplasm and the nuclear/cyto-

plasmic ratio depends on cell types and stimuli. In response to IL-1β signaling, the N-CoR/TAB2/HDAC3 corepressor complex undergoes nuclear to cytoplasmic translocation, resulting in derepression of a specific subset of NF-κB-regulated genes [Baek et al., 2002].

Class II HDACs 4, 5, 7, and 9 shuttle between the nucleus and the cytoplasm [Miska et al., 1999; Grozinger and Schreiber, 2000; McKinsey et al., 2000a; Wang et al., 2000; Kao et al., 2001; Zhang et al., 2001], and they associate with 14-3-3 proteins. The binding of HDACs to 14-3-3 is absolutely dependent on phosphorylation of conserved N-terminal serine residues of HDACs, and the association results in sequestration of HDACs to the cytoplasm. Derepression of myocyte enhancer factor (MEF2)-dependent transcription by sequestration of HDACs is well-characterized. Mutation of the conserved N-terminal serine residues of HDAC4 and HDAC5 abolishes the HDAC4/5-14-3-3 association and enhances repression of MEF2A-dependent transcription. The phosphorylated serines at the N-terminus of class II HDACs closely resembles the consensus phosphorylation sites for CaMK protein kinases, and studies have shown that CaMK-mediated phosphorylation of HDACs 4, 5, 7, and 9 promotes their association with 14-3-3 proteins resulting in increased retention of HDACs in the cytoplasm.

While 14-3-3 proteins negatively regulate class II HDACs by excluding them from the nucleus, additional sequences located at the C-terminal of HDAC4 function as a nuclear export signal [McKinsey et al., 2001; Wang and Yang, 2001]. Both 14-3-3 binding and nuclear export are required for the cytoplasmic retention of HDAC4. Also, a conserved nuclear import signal has been mapped to the N-terminal of HDACs 4, 5, 7, and 9, and binding of 14-3-3 has been suggested to mask the nuclear localization signal, thereby inhibiting nuclear targeting [Wang and Yang, 2001]. 14-3-3 binding also interferes with the association of importin-α with HDAC4 [Grozinger and Schreiber, 2000]. The nuclear export sequences of HDACs 4, 5, and 7 are signal-responsive and are activated upon CaMK-dependent binding of 14-3-3 proteins to the N-terminal phospho-serine residues of class II HDACs [McKinsey et al., 2001; Wang and Yang, 2001]. As discussed earlier, experiments in cardiomyocytes suggest the existence of an unknown stress responsive kinase that targets class II HDACs with substrate specificity similar to CaM kinase [Zhang et al., 2002]. Furthermore, HDAC4 was shown to associate with components of the Ras-MAPK signal transduction pathway, ERK1/2, and such activation resulted in increased nuclear localization of HDAC4 [Zhou et al., 2000]. However, whether this increased nuclear localization stimulates transcriptional repression is yet to be determined.

HDAC6, present predominantly in the cytoplasm, is capable of nucleo-cytoplasmic shuttling like most class II HDACs. Although HDAC6 does not bind 14-3-3, the subcellular localization of HDAC6 also appears to be regulated, as cell-cycle arrest results in partial translocation of the protein into the nucleus [Verdel et al., 2000]. It is important to note that the primary physiological substrate of HDAC6 and SIRT2 are the cytoplasmic α-tubulin protein and not histones [Hubbert et al., 2002; Matsuyama et al., 2002; North et al., 2003; Zhang et al., 2003]. Thus, signals or molecules that positively regulate HDAC6 and SIRT2 promote cytoplasmic localization of the proteins.

REGULATION BY CHANGE IN GENE EXPRESSION

The mouse *HDAC1* gene originally was isolated as an interleukin-2-inducible gene in a differential mRNA display experiment [Bartl et al., 1997]. Mouse HDAC1 mRNA expression was reported to be low in G_0 but increased at the G_1/S boundary after growth stimulation. In addition, transcription of mouse *HDAC1* is strongly induced by anisomycin and the HDAC inhibitor TSA [Hauser et al., 2002]. The expression of mouse *HDAC1* is autoregulated by recruitment of the mouse HDAC1 protein to its own promoter via the transcription factors NF-Y and Sp1 [Schuettengruber et al., 2003]. Interestingly, HDAC2 and HDAC3 protein levels increase in HDAC1-deficient ES cells, suggesting that HDAC1 regulates not only its own expression but also that of other class I HDACs [Lagger et al., 2002].

The expression of human *HDAC3* mRNA is activated by PHA, PMA, and α-CD3; but repressed by GMCSF [Dangond et al., 1998]. Different isoforms of human HDAC3 mRNA may exist, although the mechanisms that regulate the expression of these different HDAC3

isoforms are not known at this time [Yang et al., 1997]. A splice variant of the HDAC3 transcript in which exon 3 is alternatively spliced from the mRNA, has been reported [Gray et al., 2003]. Expression of this novel HDAC3 splice variant is regulated by many different extracellular stimuli, and, curiously, human and mouse HDAC3 splice variants are regulated by different signals. Besides HDAC3, splice variants exist for HDAC9 and HDAC10 [Fischer et al., 2001; Guardiola and Yao, 2001; Zhou et al., 2001; Kao et al., 2002; Tong et al., 2002; Petrie et al., 2003].

REGULATION BY AVAILABILITY OF METABOLIC COFACTORS

Unlike class I and II HDACs, the SIR2-like enzymes that comprise class III HDACs require the coenzyme NAD^+ for catalytic activity. In this reaction, nicotinamide is liberated from NAD^+ while the acetyl group of the substrate is transferred to cleaved NAD^+, generating O-acetyl-ADP-ribose. The exact physiological regulator of the SIR2 enzymes has not been confirmed as yet. However, studies have suggested two alternative models of SIR2 activation: (i) increasing the NAD^+/NADH ratio by increasing NAD^+ or by reducing the level of NADH, a competitive inhibitor of SIR2 [Lin et al., 2004], or (ii) decreasing the level of nicotinamide, an inhibitory product of SIR2 [Bitterman et al., 2002; Anderson et al., 2003]. Whatever the case, the requirement of NAD^+ for SIR2 activity provides a unique mechanism for regulating class III HDACs in response to the metabolic status of the cell.

REGULATION BY PROTEOLYTIC PROCESSING

One of the human SIR2 homologs, hSIRT3, is synthesized as an inactive precursor, which then is imported into the mitochondria and subsequently cleaved by matrix processing peptidase to yield the enzymatically active form [Schwer et al., 2002]. Similarly, maize deacetylase Hda1 is converted to an enzymatically active form by proteolytic processing of a precursor [Pipal et al., 2003]. Furthermore, the HDAC1/mSin3A corepressor complex can be depleted by targeting HDAC1 for proteosome-mediated degradation during steroid-induced preadipocyte differentiation [Wiper-Bergeron et al., 2003]. The HDAC3-associated protein N-CoR can be targeted for proteosomal degradation by mSiah2 in a cell type-specific manner [Zhang et al., 1998a]. Since N-CoR plays a role in activating HDAC3 activity, targeted proteolysis of N-CoR may represent a potential indirect mechanism for controlling HDAC3 activity.

SUMMARY AND PERSPECTIVES

In the past 8 years, tremendous progress has been made in the identification and functional characterization of HDACs. Results from many studies overwhelmingly dictate that HDACs are key regulators of gene expression. However, the means by which HDACs themselves are regulated remain to be completely defined and the gap in our knowledge in this area presents a challenge to those of us in this field. Here, we summarize the key findings from many different laboratories over the years (Fig. 1).

The best-studied mechanism of HDAC regulation is regulation by protein complex formation. Many class I HDAC complexes have been isolated, thoroughly analyzed, and have pro-

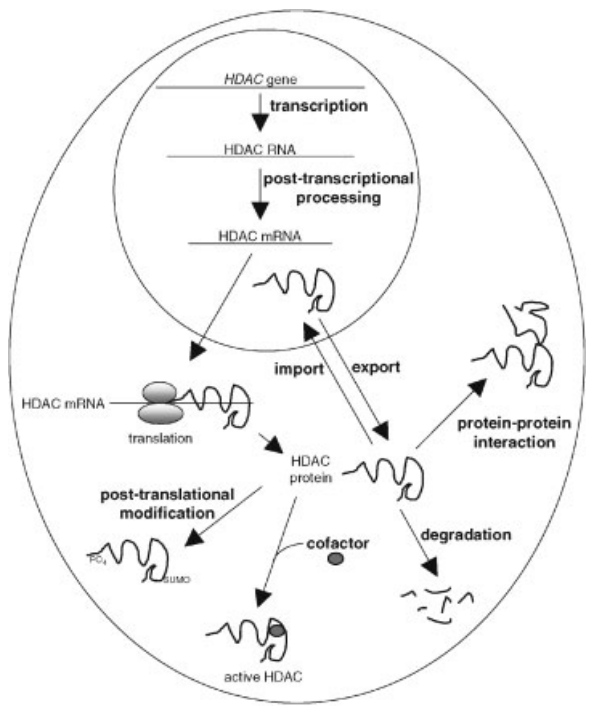

Fig. 1. A schematic illustration showing the multiple different mechanisms that could regulate the activities of Histone deacetylases (HDACs). All known processes that regulate HDACs are marked in bold. For simplicity, post-translational modifications and protein–protein interactions are shown in the cytoplasmic compartment; but in reality, many HDAC post-translational modifications and protein–protein interactions occur in the nucleus of the cell.

vided remarkable insights into how some class I HDAC activities could be activated. To further our understanding of how HDACs are regulated, there are several obvious questions that need to be addressed. First, do additional HDAC1/2 or HDAC3 complexes exist and remain to be discovered? Second, if stable multi-subunit class II, or class III HDAC complexes exist, we must rigorously isolate all the endogenous complexes and ask what comprises each of these complexes and how they might regulate the activities of HDACs within each of the complexes. Third, many different cellular proteins interact with HDACs without participating in stable multi-subunit complexes, and we need to continue to identify novel HDAC-interacting partners and systematically determine their functions.

The discovery of HDAC regulation by phosphorylation/dephosphorylation is only the beginning. Besides CK2, PKA, CaMK, ERK1/2, and PP1, there are almost certain to be additional kinases and phosphatases that regulate the phosphorylation status of HDACs and consequently, the activities of HDAC enzymes. Furthermore, in addition to phosphorylation and sumoylation, there are many more potential post-translational modifications that can exist for each HDACs and potential cross-talk can take place between the different modifications. Just as an example, in HDAC8 a potential *N*-glycosylation site is present at Asn^{136}, and it is conceivable that phosphorylation of Ser^{39} may affect glycosylation of Asn^{136} and vice versa. Future experiments to decipher and confirm additional modifications of HDACs and learn how they might affect HDAC activities will guide us closer to a complete understanding of regulation of the HDAC enzymes.

Generally speaking, class I HDACs are ubiquitously expressed while the expression of many class II enzymes are tissue-specific. A natural question, then, is what are the signals and mechanisms that regulate the tissue-specific expression of some HDACs? With the availability of cDNAs and antibodies to all class II HDACs, the stage is set to intensively pursue the answers to these critical questions.

Although the different mechanisms of HDAC regulation are separated into different sections in this article for clarity, they actually are interconnected. For instance, phosphorylation of HDACs affects their ability to form protein complexes. Reciprocally, protein–protein interactions can affect phosphorylation of HDACs. Similarly, regulation of HDACs by subcellular localization is heavily dependent on phosphorylation, and where HDACs are localized will no doubt affect whether they will be phosphorylated or dephosphorylated. This inherent complexity of regulatory networks presents additional unique challenges that will require some time to discern.

One of the chief motivations for studying HDAC regulation is the expectation that such understanding will contribute to our overall knowledge of the biology of HDACs, which in turn will increase our understanding of gene regulation in eukaryotic cells. However, equally important is the urgent need to take what we learn and apply it in the context of deacetylases in health and disease. HDAC proteins are vital regulators of fundamental cellular events, and extensive connections between HDACs and cancer exist. HDAC inhibitors are currently in clinical trials for the treatment of leukemia and solid tumors [Marks et al., 2001a,b,c, 2003; Johnstone, 2002; Johnstone and Licht, 2003]. Additionally, drugs that target HDACs potentially may be useful against malaria and toxoplasmosis, and for treatment of Huntington's disease [Darkin-Rattray et al., 1996; Steffan et al., 2001]. Knowledge gained from understanding how HDACs are regulated will provide invaluable insight into better approaches for developing HDAC inhibitors. A thorough understanding of HDAC regulation is required, therefore, not merely for the sake of interest in chromatin structure and gene regulation alone, but because HDACs are intimately involved in a myriad of normal and abnormal cellular processes that impact human health.

ACKNOWLEDGMENTS

We apologize to all investigators whose works were not cited in this article due to space limitation. We also thank Heehyoung Lee and Mary Zhang for their comments and suggestions. ES is supported by a generous endowment from the Kaul Foundation.

REFERENCES

Ajuh PM, Browne GJ, Hawkes NA, Cohen PT, Roberts SG, Lamond AI. 2000. Association of a protein phosphatase 1 activity with the human factor C1 (HCF) complex. Nucleic Acids Res 28:678–686.

Alland L, Muhle R, Hou H, Jr., Potes J, Chin L, Schreiber-Agus N, DePinho RA. 1997. Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. Nature 387:49–55.

Alland L, David G, Shen-Li H, Potes J, Muhle R, Lee HC, Hou H, Jr., Chen K, DePinho RA. 2002. Identification of mammalian Sds3 as an integral component of the Sin3/histone deacetylase corepressor complex. Mol Cell Biol 22:2743–2750.

Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Sinclair DA. 2003. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae. Nature 423:181–185.

Ayer DE. 1999. Histone deacetylases: Transcriptional repression with SINers and NuRDs. Trends Cell Biol 9: 193–198.

Baek SH, Ohgi KA, Rose DW, Koo EH, Glass CK, Rosenfeld MG. 2002. Exchange of N-CoR corepressor and Tip60 coactivator complexes links gene expression by NF-kappaB and beta-amyloid precursor protein. Cell 110:55–67.

Bartl S, Taplick J, Lagger G, Khier H, Kuchler K, Seiser C. 1997. Identification of mouse histone deacetylase 1 as a growth factor-inducible gene. Mol Cell Biol 17:5033–5043.

Becker PB, Horz W. 2002. ATP-dependent nucleosome remodeling. Annu Rev Biochem 71:247–273.

Berger SL. 2002. Histone modifications in transcriptional regulation. Curr Opin Genet Dev 12:142–148.

Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA. 2002. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. J Biol Chem 277:45099–45107.

Brosch G, Georgieva EI, Lopez-Rodas G, Lindner H, Loidl P. 1992. Specificity of Zea mays histone deacetylase is regulated by phosphorylation. J Biol Chem 267:20561–20564.

Brush MH, Guardiola A, Connor JH, Yao TP, Shenolikar S. 2004. Deactylase inhibitors disrupt cellular complexes containing protein phosphatases and deacetylases. J Biol Chem 279:7685–7691.

Cai R, Kwon P, Yan-Neale Y, Sambuccetti L, Fischer D, Cohen D. 2001. Mammalian histone deacetylase 1 protein is posttranslationally modified by phosphorylation. Biochem Biophys Res Commun 283:445–453.

Canettieri G, Morantte I, Guzman E, Asahara H, Herzig S, Anderson SD, Yates JR, Montminy M. 2003. Attenuation of a phosphorylation-dependent activator by an HDAC-PP1 complex. Nat Struct Biol 10:175–181.

Carmen AA, Griffin PR, Calaycay JR, Rundlett SE, Suka Y, Grunstein M. 1999. Yeast HOS3 forms a novel trichostatin A-insensitive homodimer with intrinsic histone deacetylase activity. Proc Natl Acad Sci USA 96:12356–12361.

Colombo R, Boggio R, Seiser C, Draetta GF, Chiocca S. 2002. The adenovirus protein Gam1 interferes with sumoylation of histone deacetylase 1. EMBO Rep 3: 1062–1068.

Cress WD, Seto E. 2000. Histone deacetylases, transcriptional control, and cancer. J Cell Physiol 184:1–16.

Dangond F, Hafler DA, Tong JK, Randall J, Kojima R, Utku N, Gullans SR. 1998. Differential display cloning of a novel human histone deacetylase (HDAC3) cDNA from PHA-activated immune cells. Biochem Biophys Res Commun 242:648–652.

Darkin-Rattray SJ, Gurnett AM, Myers RW, Dulski PM, Crumley TM, Allocco JJ, Cannova C, Meinke PT, Colletti SL, Bednarek MA, Singh SB, Goetz MA, Dombrowski AW, Polishook JD, Schmatz DM. 1996. Apicidin: A novel antiprotozoal agent that inhibits parasite histone deacetylase. Proc Natl Acad Sci USA 93:13143–13147.

David G, Neptune MA, DePinho RA. 2002. SUMO-1 modification of histone deacetylase 1 (HDAC1) modulates its biological activities. J Biol Chem 277:23658–23663.

Fischer DD, Cai R, Bhatia U, Asselbergs FA, Song C, Terry R, Trogani N, Widmer R, Atadja P, Cohen D. 2001. Isolation and characterization of a novel class II histone deacetylase, HDAC10. J Biol Chem 277:6656–6666.

Fischle W, Dequiedt F, Fillion M, Hendzel MJ, Voelter W, Verdin E. 2001. Human HDAC7 histone deacetylase activity is associated with HDAC3 in vivo. J Biol Chem 276:35826–35835.

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.

Galasinski SC, Resing KA, Goodrich JA, Ahn NG. 2002. Phosphatase inhibition leads to histone deacetylases 1 and 2 phosphorylation and disruption of corepressor interactions. J Biol Chem 277:19618–19626.

Girdwood D, Bumpass D, Vaughan OA, Thain A, Anderson LA, Snowden AW, Garcia-Wilson E, Perkins ND, Hay RT. 2003. p300 transcriptional repression is mediated by SUMO modification. Mol Cell 11:1043–1054.

Gray SG, Iglesias AH, Teh BT, Dangond F. 2003. Modulation of splicing events in histone deacetylase 3 by various extracellular and signal transduction pathways. Gene Expr 11:13–21.

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.

Grozinger CM, Schreiber SL. 2002. Deacetylase enzymes: Biological functions and the use of small-molecule inhibitors. Chem Biol 9:3–16.

Guardiola AR, Yao TP. 2001. Molecular cloning and characterization of a novel histone deacetylase HDAC10. J Biol Chem 277:3350–3356.

Guenther MG, Barak O, Lazar MA. 2001. The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. Mol Cell Biol 21:6091–6101.

Guenther MG, Yu J, Kao GD, Yen TJ, Lazar MA. 2002. Assembly of the SMRT-histone deacetylase 3 repression complex requires the TCP-1 ring complex. Genes Dev 16:3130–3135.

Hassig CA, Fleischer TC, Billin AN, Schreiber SL, Ayer DE. 1997. Histone deacetylase activity is required for full transcriptional repression by mSin3A. Cell 89:341–347.

Hauser C, Schuettengruber B, Bartl S, Lagger G, Seiser C. 2002. Activation of the mouse histone deacetylase 1 gene by cooperative histone phosphorylation and acetylation. Mol Cell Biol 22:7820–7830.

Heinzel T, Lavinsky RM, Mullen TM, Soderstrom M, Laherty CD, Torchia J, Yang WM, Brard G, Ngo SD, Davie JR, Seto E, Eisenman RN, Rose DW, Glass CK, Rosenfeld MG. 1997. A complex containing N-CoR, mSin3, and histone deacetylase mediates transcriptional repression. Nature 387:43–48.

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.

Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang XF, Yao TP. 2002. HDAC6 is a microtubule-associated deacetylase. Nature 417: 455–458.

Humphrey GW, Wang Y, Russanova VR, Hirai T, Qin J, Nakatani Y, Howard BH. 2001. Stable histone deacetylase complexes distinguished by the presence of SANT domain proteins CoREST/kiaa0071 and Mta-L1. J Biol Chem 276:6817–6824.

Johnson CA, White DA, Lavender JS, O'Neill LP, Turner BM. 2002. Human class I histone deacetylase complexes show enhanced catalytic activity in the presence of ATP and co-immunoprecipitate with the ATP-dependent chaperone protein Hsp70. J Biol Chem 277: 9590–9597.

Johnstone RW. 2002. Histone-deacetylase inhibitors: Novel drugs for the treatment of cancer. Nat Rev Drug Discov 1:287–299.

Johnstone RW, Licht JD. 2003. Histone deacetylase inhibitors in cancer therapy: Is transcription the primary target? Cancer Cell 4:13–18.

Kao HY, Verdel A, Tsai CC, Simon C, Juguilon H, Khochbin S. 2001. Mechanism for nucleocytoplasmic shuttling of histone deacetylase 7. J Biol Chem 276:47496–47507.

Kao HY, Lee CH, Komarov A, Han CC, Evans RM. 2002. Isolation and characterization of mammalian HDAC10, a novel histone deacetylase. J Biol Chem 277:187–193.

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.

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.

Laherty CD, Yang WM, Sun JM, Davie JR, Seto E, Eisenman RN. 1997. Histone deacetylases associated with the mSin3 corepressor mediate mad transcriptional repression. Cell 89:349–356.

Lechner T, Carrozza MJ, Yu Y, Grant PA, Eberharter A, Vannier D, Brosch G, Stillman DJ, Shore D, Workman JL. 2000. Sds3 (suppressor of defective silencing 3) is an integral component of the yeast Sin3·Rpd3 histone deacetylase complex and is required for histone deacetylase activity. J Biol Chem 275:40961–40966.

Lee H, Rezai-Zadeh N, Seto E. 2004. Negative regulation of histone deacetylase 8 activity by cyclic AMP-dependent protein kinase A. Mol Cell Biol 24:765–773.

Lin SJ, Ford E, Haigis M, Liszt G, Guarente L. 2004. Calorie restriction extends yeast life span by lowering the level of NADH. Genes Dev 18:12–16.

Ling Y, Sankpal UT, Robertson AK, McNally JG, Karpova T, Robertson KD. 2004. Modification of de novo DNA methyltransferase 3a (Dnmt3a) by SUMO-1 modulates its interaction with histone deacetylases (HDACs) and its capacity to repress transcription. Nucleic Acids Res 32: 598–610.

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

Lu J, McKinsey TA, Zhang CL, Olson EN. 2000b. Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. Mol Cell 6:233–244.

Lusser A, Kadonaga JT. 2003. Chromatin remodeling by ATP-dependent molecular machines. Bioessays 25:1192–1200.

Lusser A, Brosch G, Loidl A, Haas H, Loidl P. 1997. Identification of maize histone deacetylase HD2 as an acidic nucleolar phosphoprotein. Science 277:88–91.

Marks PA, Richon VM, Breslow R, Rifkind RA. 2001a. Histone deacetylase inhibitors as new cancer drugs. Curr Opin Oncol 13:477–483.

Marks PA, Rifkind RA, Richon VM, Breslow R. 2001b. Inhibitors of histone deacetylase are potentially effective anticancer agents. Clin Cancer Res 7:759–760.

Marks PA, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. 2001c. Histone deacetylases and cancer: Causes and therapies. Nat Rev Cancer 1:194–202.

Marks PA, Miller T, Richon VM. 2003. Histone deacetylases. Curr Opin Pharmacol 3:344–351.

Matsuyama A, Shimazu T, Sumida Y, Saito A, Yoshimatsu Y, Seigneurin-Berny D, Osada H, Komatsu Y, Nishino N, Khochbin S, Horinouchi S, Yoshida M. 2002. In vivo destabilization of dynamic microtubules by HDAC6-mediated deacetylation. EMBO J 21:6820–6831.

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

McKinsey TA, Zhang CL, Olson EN. 2000b. 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.

McLaughlin F, Finn P, La Thangue NB. 2003. The cell cycle, chromatin and cancer: Mechanism-based therapeutics come of age. Drug Discov Today 8:793–802.

Melnick A, Licht JD. 2002. Histone deacetylases as therapeutic targets in hematologic malignancies. Curr Opin Hematol 9:322–332.

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.

Ng HH, Bird A. 2000. Histone deacetylases: Silencers for hire. Trends Biochem Sci 25:121–126.

North BJ, Marshall BL, Borra MT, Denu JM, Verdin E. 2003. The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. Mol Cell 11:437–444.

Petrie K, Guidez F, Howell L, Healy L, Waxman S, Greaves M, Zelent A. 2003. The histone deacetylase 9 gene encodes multiple protein isoforms. J Biol Chem 278: 16059–16072.

Pflum MK, Tong JK, Lane WS, Schreiber SL. 2001. Histone deacetylase 1 phosphorylation promotes enzymatic activity and complex formation. J Biol Chem 276:47733–47741.

Pipal A, Goralik-Schramel M, Lusser A, Lanzanova C, Sarg B, Loidl A, Lindner H, Rossi V, Loidl P. 2003. Regulation and processing of maize histone deacetylase Hda1 by limited proteolysis. Plant Cell 15:1904–1917.

Roth SY, Denu JM, Allis CD. 2001. Histone acetyltransferases. Annu Rev Biochem 70:81–120.

Schuettengruber B, Simboeck E, Khier H, Seiser C. 2003. Autoregulation of mouse histone deacetylase 1 expression. Mol Cell Biol 23:6993–7004.

Schwer B, North BJ, Frye RA, Ott M, Verdin E. 2002. The human silent information regulator (Sir)2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase. J Cell Biol 158: 647–657.

Secrist JP, Zhou X, Richon VM. 2003. HDAC inhibitors for the treatment of cancer. Curr Opin Investig Drugs 4: 1422–1427.

Steffan JS, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol BL, Kazantsev A, Schmidt E, Zhu YZ, Greenwald M, Kurokawa R, Housman DE, Jackson GR, Marsh JL, Thompson LM. 2001. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. Nature 413:739–743.

Strahl BD, Allis CD. 2000. The language of covalent histone modifications. Nature 403:41–45.

Sun JM, Chen HY, Moniwa M, Litchfield DW, Seto E, Davie JR. 2002. The transcriptional repressor Sp3 is associated with CK2-phosphorylated histone deacetylase 2. J Biol Chem 277:35783–35786.

Thiagalingam S, Cheng KH, Lee HJ, Mineva N, Thiagalingam A, Ponte JF. 2003. Histone deacetylases: Unique players in shaping the epigenetic histone code. Ann NY Acad Sci 983:84–100.

Tong JK, Hassig CA, Schnitzler GR, Kingston RE, Schreiber SL. 1998. Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex. Nature 395:917–921.

Tong JJ, Liu J, Bertos NR, Yang XJ. 2002. Identification of HDAC10, a novel class II human histone deacetylase containing a leucine-rich domain. Nucleic Acids Res 30: 1114–1123.

Tsai SC, Seto E. 2002. Regulation of histone deacetylase 2 by protein kinase CK2. J Biol Chem 277:31826–31833.

Verdel A, Curtet S, Brocard MP, Rousseaux S, Lemercier C, Yoshida M, Khochbin S. 2000. Active maintenance of mHDA2/mHDAC6 histone-deacetylase in the cytoplasm. Curr Biol 10:747–749.

Verdin E, Dequiedt F, Kasler HG. 2003. Class II histone deacetylases: Versatile regulators. Trends Genet 19:286–293.

Wang AH, Yang XJ. 2001. Histone deacetylase 4 possesses intrinsic nuclear import and export signals. Mol Cell Biol 21:5992–6005.

Wang AH, Kruhlak MJ, Wu J, Bertos NR, Vezmar M, Posner BI, Bazett-Jones DP, Yang XJ. 2000. Regulation of histone deacetylase 4 by binding of 14-3-3 proteins. Mol Cell Biol 20:6904–6912.

Wen YD, Perissi V, Staszewski LM, Yang WM, Krones A, Glass CK, Rosenfeld MG, Seto E. 2000. The histone deacetylase-3 complex contains nuclear receptor corepressors. Proc Natl Acad Sci USA 97:7202–7207.

Wiper-Bergeron N, Wu D, Pope L, Schild-Poulter C, Hache RJ. 2003. Stimulation of preadipocyte differentiation by steroid through targeting of an HDAC1 complex. EMBO J 22:2135–2145.

Yang XJ, Seto E. 2003. Collaborative spirit of histone deacetylases in regulating chromatin structure and gene expression. Curr Opin Genet Dev 13:143–153.

Yang SH, Sharrocks AD. 2004. SUMO promotes HDAC-mediated transcriptional repression. Mol Cell 13:611–617.

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.

Yoshida M, Shimazu T, Matsuyama A. 2003. Protein deacetylases: Enzymes with functional diversity as novel therapeutic targets. Prog Cell Cycle Res 5:269–278.

You A, Tong JK, Grozinger CM, Schreiber SL. 2001. CoREST is an integral component of the CoREST-human histone deacetylase complex. Proc Natl Acad Sci USA 98: 1454–1458.

Yu J, Li Y, Ishizuka T, Guenther MG, Lazar MA. 2003. A SANT motif in the SMRT corepressor interprets the histone code and promotes histone deacetylation. EMBO J 22:3403–3410.

Zhang Y, Reinberg D. 2001. Transcription regulation by histone methylation: Interplay between different covalent modifications of the core histone tails. Genes Dev 15:2343–2360.

Zhang Y, Iratni R, Erdjument-Bromage H, Tempst P, Reinberg D. 1997. Histone deacetylases and SAP18, a novel polypeptide, are components of a human sin3 complex. Cell 89:357–364.

Zhang J, Guenther MG, Carthew RW, Lazar MA. 1998a. Proteasomal regulation of nuclear receptor corepressor-mediated repression. Genes Dev 12:1775–1780.

Zhang Y, LeRoy G, Seelig HP, Lane WS, Reinberg D. 1998b. The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. Cell 95:279–289.

Zhang Y, Ng HH, Erdjument-Bromage H, Tempst P, Bird A, Reinberg D. 1999. Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. Genes Dev 13:1924–1935.

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 J, Kalkum M, Chait BT, Roeder RG. 2002. The N-Cor-HDAC3 nuclear receptor corepressor complex inhibits the JNK pathway through the integral subunit GPS2. Mol Cell 9:611–623.

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

Zhang Y, Li N, Caron C, Matthias G, Hess D, Khochbin S, Matthias P. 2003. HDAC-6 interacts with and deacetylates tubulin and microtubules in vivo. EMBO J 22:1168–1179.

Zhou X, Richon VM, Wang AH, Yang XJ, Rifkind RA, Marks PA. 2000. Histone deacetylase 4 associates with extracellular signal-regulated kinases 1 and 2, and its cellular localization is regulated by oncogenic Ras. Proc Natl Acad Sci USA 97:14329–14333.

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.