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*

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Antibodies to histone deacetylases (HDACs) have been used to immuno-isolate deacetylase complexes from HeLa cell extracts. Complexes shown to contain HDAC1, HDAC3, HDAC6, and HDAC1+2 as their catalytic subunits have been used in an antibody-based assay that detects deacetylation of whole histones at defined lysines. The class II deacetylase HDAC6 was inactive in this assay, but the three class I enzymes deacetylated all histone lysines tested, although with varying efficiency. In comparison to HDAC1, HDAC3 preferentially deacetylated lysines 5 and 12 of H4 and lysine 5 of H2A. H4 tails in purified mononucleosomes were refractory to deacetylation by both HDAC1 and HDAC3, unless ATP was added to the reaction mix. Surprisingly, ATP also consistently enhanced cleavage of free, non-nucleosomal histones, but not small peptides, by both enzyme complexes. We found no evidence that ATP operates by phosphorylation of components of the HDAC complex, but have shown that HDACs 1, 2, and 3 all co-immunoprecipitate with the ATP-dependent chaperone protein Hsp70. Another common ATP-dependent chaperone, Hsp90, was absent from all HDAC complexes tested, whereas Hsp60 associated with HDAC1 only. We suggest that Hsp chaperone proteins enhance the deacetylase activity of HDAC complexes by ATP-dependent manipulation of protein substrates.

Enzyme-catalyzed acetylation of selected lysines in the histone N-terminal tail domains has crucial effects on chromatin structure and function (1–4). Chromatin function is regulated not only by the overall level of histone acetylation, but also by targeted acetylation of individual histones and even of specific lysine residues. The first examples of this were found in Drosophila and yeast, but there are now several examples in mammals of situations where patterns of acetylation of individual histones (usually H3 and H4) in defined chromatin domains vary independently (5, 6). It is now also clear that acetylation often does not function in isolation, but forms part of a wider, coordinated pattern of post-translational modification, including serine phosphorylation and methylation of lysines and arginines (7–9). Maintenance of such complex regional patterns of histone-specific and residue-specific modification, requires targeting of the appropriate enzymes (10). It also requires that the catalytic specificity of these enzymes is such that they modify only selected residues, even when several copies of the same residue (e.g. lysine) are present in the same region of the histone tail.

Levels of acetylation are established and maintained by the complementary activities of two families of enzymes, the histone acetyltransferases and the histone deacetylases (HDACs)1 (11, 12). The histone deacetylases have been placed in three groups on the basis of sequence homologies. The mammalian class I enzymes (HDACs 1–3 and 8) are orthologues of the yeast (Saccharomyces cerevisiae) deacetylase and transcriptional regulator Rpd3, whereas the class II enzymes (HDACs 4–7 and 9) are orthologues of yeast Hda1 (13–20). A third, unrelated, deacetylase family is homologous to the yeast silencing regulator Sir2 (21, 22). The Sir2 deacetylases are completely dependent on NAD for catalytic activity (21).

Both histone acetyltransferases and HDACs are frequently found as components of multiprotein complexes (11, 12). HDAC1 and HDAC2 are found together in three separate complexes, distinguished by their subunits. The first of these contains DNA-binding transcriptional repressors (e.g. the methyl DNA-binding protein McCP2), the histone-binding proteins RbAp48/RbAp46, and the co-repressors Sin3A/B (12, 23). The Sin3 complex can interact with N-CoR and SMRT, two silencing mediators for unliganded retinoid and thyroid hormone receptors (24–26). The second complex contains, in addition to HDAC1/2 and RbAp48/46, metastasis-associated protein 2 (MTA2) and the dermatomysotis autoantigen Mi-2 (27–30). These (and possibly other) subunits confer both nucleosome remodeling and deacetylase activities, and it is referred to as the NuRD complex. A third complex contains HDAC12, the transcriptional co-repressor CoREST and additional proteins, but no RbAp48/46 (31). Additionally, HDAC3 has been shown to interact directly with N-CoR and SMRT (16) and to co-purify with the class II enzymes HDAC4 and HDAC5 (18). Both class I and class II enzymes have recently been shown to co-localize in microscopically defined nuclear bodies, though whether this is a result of physical association remains uncertain (32).

The acetylation of defined lysines in the histone tail domains must be a result of the selective action of histone acetyltrans-

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1 The abbreviations used are: HDAC, histone deacetylase; WCE, whole cell extract; ATPγS, adenosine 5’-O-(thiotriphosphate); PAS, protein A-Sepharose.
ferases or HDACs or both. In the present study, we explore the catalytic specificities of human HDACs, using antibodies to HDAC1, HDAC2, HDAC3, and HDAC6 to immuno-isolate endogenous complexes containing these catalytic components. We have used a Western blotting assay to show that the class I HDACs can deacetylate all lysines tested in free histones, but with variable efficiency. The enzymes show no deacetylation of nucleosomal histones, unless ATP is added to the reaction. Surprisingly, ATP also significantly and consistently enhances the deacetylation of free histones by all three class I enzymes. We show that the ATP-dependent chaperone proteins Hsp60 and Hsp70 are associated with HDAC complexes, providing circumstantial evidence that the stimulatory effect of ATP on HDAC catalytic activity operates through these proteins.

MATERIALS AND METHODS

Antibodies—Rabbit polyclonal antisera were prepared against components of deacetylase complexes using the following synthetic peptides: HDAC1, EEPKAEKGYEKEEVL; HDAC2, GERIDTGRKQEQLSVP; HDAC3, APNFTDDGDHNDKDESVDEI; HDAC6, AHGQGEGEAPIH4, ENIYNEDPEGSVDPEGQGS. Preparation and specificities of rabbit antisera and purification of antibodies were as described previously (33). All antisera for the immunoprecipitation procedures were column-purified for total IgG using protein A-Sepharose as described by the manufacturer (Amersham Biosciences, Inc.). Antibodies against Sin3A and N-CoR were obtained commercially (Santa Cruz). A mouse monoclonal antibody against HDAC3 (that also recognizes HDAC1 and HDAC2) was purchased from BD Transduction Laboratories. Mouse monoclonal antibodies to Hsp60, Hsp70, and Hsp90 were obtained from Santa Cruz. The specificities of all antisera employed for the experiments described here were tested by Western blotting (see below), and all were shown to detect just a single major protein band (or explicable doublet) of the appropriate molecular weight.

SDS-PAGE and Western Blotting—SDS-containing polyacrylamide gels were prepared and run as described (34). Western blots used standard blocking buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% (v/v) Tween 20) containing 5% nonfat powdered dried milk (Marvel™) (34). In general, 50% saturated ammonium sulfate cuts of rabbit polyclonal antisera against either acetylated isoforms of histones H2A, H2B, and H4, or HDAC1, -2, -3, and -6, were used for immunodetection, with final dilutions of between 200 and 1000. A peroxidase-conjugated protein A-Sepharose as described by the manufacturer (Amersham Biosciences, Inc.) was used to purify total IgG using protein A-Sepharose for Western blotting experiments, filters were reversibly stained with Ponceau S dye (Sigma) to confirm equality of loading.

RESULTS

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Catalytic Components of HDAC Complexes—Co-immunoprecipitation experiments were performed to determine whether the different class I HDACs are contained in separate complexes and whether they associate with the class II enzymes HDACs and HDAC6. Illustrative results are shown in Fig. 1. HDAC immunocomplexes, prepared with antibodies to HDAC1, HDAC3, HDAC4 (Fig. 1, lanes 1, 3, and 4), and HDAC6 (data not shown), are free of significant contamination with other HDAC subunits. In contrast, immunocomplexes prepared with antibodies to HDAC2 (Fig. 1, lane 2) contain significant amounts of HDAC1, detectable both with a polyclonal antiserum specific for HDAC1 (top panel) and with a monoclonal antibody recognizing all three class I HDACs (third panel). As expected, antibodies to HDAC1, -2, and -3 all pre-
Catalytic Specificity of HDAC Immunocomplexes against Free Histones—HDAC1, HDAC2, HDAC3, and HDAC6 immunocomplexes were tested for their ability to cleave specific acetylated lysine residues in hyperacetylated histones using a Western blot approach. Our panel of site-specific antibodies recognize the following acetylated lysine residues: Lys-14 and Lys-9/18 in H3; Lys-12/15 in H2B; Lys-5 in H2A; Lys-16, Lys-8, and Lys-5 in H4. These antibodies were used to probe Western blots of core histones before and after treatment with deacetylase complexes. Deacetylation is seen as diminution of a specific histone band. To facilitate comparison of the different immunocomplexes, each assay contained both the same amount of core histone and the same amount of deacetylase activity, measured using the radiolabeled peptide assay.

HDAC1, HDAC2, and HDAC3 immunocomplexes give at least partial deacetylation of all lysines tested in H2A, H2B, H3, and H4 (Fig. 2, A and B, top three panels). In contrast, HDAC6 immunocomplexes show a partial deacetylation only of H4Ac5 and H4Ac8 (Fig. 2, A and B, bottom panels). The activities of the HDAC1 and HDAC2 complexes are always comparable, both giving complete deacetylation of H3Ac14 and all H4 lysines, but incomplete deacetylation of H2A and H2B. This identity may be due in part to the presence of HDAC1 in the anti-HDAC2 immunoprecipitates (Fig. 1). The specificity of HDAC3 seems rather different, with complete deacetylation of H2A, H4Ac5, and H4Ac12, but only partial deacetylation of H3, H2B, H4Ac8, and H4Ac16.

The differences between the specificities of HDAC1 and HDAC3 were confirmed by a kinetic analysis in which we monitored deacetylation of H2A, H2B, and H4 by differing amounts of HDAC1 and HDAC3 immunocomplexes. The results are shown graphically in Fig. 3A. They show that HDAC1 deacetylates all four H4 lysines at a similar rate but deacetylases H2A more slowly and H2B more rapidly (Fig. 3A). HDAC3 deacetylates H4 Lys-8 and Lys-16, and H2B, at exactly the same rate as HDAC1, but deacetylates H4 Lys-5 and Lys-12, and H2A Lys-5, much more rapidly (Fig. 3A).

Deacetylation of Nucleosomal Histones by HDAC1 and HDAC3 Immunocomplexes—The specificities of immunocomplexes containing HDAC1 and HDAC3 were tested against a mononucleosome substrate (Fig. 4). Mononucleosomes were extensively purified by sucrose density gradient centrifugation, followed by inactivation of endogenous deacetylase activity, before use in the Western blot-based assay. HDAC1 and HDAC3 immunocomplexes were adjusted to the same level of deacetylase activity, as determined by the conventional peptide assay.
assay, to allow comparison of their ability to deacetylate histones in chromatin. All three sites tested in mononucleosomes (H4 Lys-16, Lys-8, and Lys-5) were refractory to deacetylation by either enzyme (Fig. 4).

HDAC1 is an integral component of the NuRD chromatin remodeling complex (27–29), so ATP-dependent remodeling activity is likely to be present in material immunoprecipitated with anti-HDAC1. We hypothesized that ATP-dependent chromatin remodeling might facilitate HDAC-catalyzed deacetylation, and as a first test of this, 0.5 mM ATP was included in the deacetylation reaction. Under these conditions, HDAC1 gave detectable deacetylation at H4 Lys-5, Lys-8, and Lys-16, whereas HDAC3 gave almost complete deacetylation of H4 Lys-5, partial deacetylation of H4 Lys-16, but no detectable deacetylation of H4 Lys-8 (Fig. 4).

ATP Enhances Deacetylation of Free Histones by HDAC Immunocomplexes—Initially as a control for the experiments on nucleosomal histones, we tested the effects of ATP on deacetylation of free histones. Surprisingly, we detected a significant and consistent enhancement of activity. To explore this further, experiments on the kinetics of deacetylation of free histones, using varying concentrations of HDAC immunocomplex, were carried out in the presence of 0.5 mM ATP. The results are shown in Fig. 3B. ATP enhances cleavage of H2A, H2B, and H4 lysines by both HDAC1 and HDAC3. (Compare the rates of cleavage in Fig. 3B with those of the same samples assayed in parallel in the absence of ATP, in Fig. 3A.) There are quantitative, histone-specific differences between the two enzymes, in the degree of stimulation by ATP. Cleavage of H2A Lys-5 by HDAC3 shows only a small increase in the presence of ATP.
FIG. 4. Deacetylation of nucleosomal H4 lysines by HDAC1 and HDAC3 immunocomplexes in the presence and absence of ATP. Hyperacetylated mononucleosomes purified on sucrose gradients from butyrate-treated HeLa cells were incubated with HDAC1 or HDAC3 immunocomplexes in the presence or absence of 0.5 mM ATP as indicated. Levels of acetylation of H4 lysines 5, 8, and 16 in untreated and treated samples were assayed by Western blotting. Control samples were untreated (control) or treated with material immunoprecipitated with pre-immune serum (pre-imm.). The assays without ATP were run in duplicate to confirm the reproducibility of the detection system. The blot to detect H4 Ac16 (top panel) was stripped and reprobed to detect H4 Ac5 (second panel). The bottom panel shows protein loading after Western transfer, but before immunostaining, as detected by Ponceau S dye.

compared with HDAC1, whereas cleavage of H4 Lys-8 and H2B by HDAC1 shows only a small enhancement compared with HDAC3 (Fig. 3, A and B). The enhancement of activity by ATP is always most pronounced at low concentrations of enzyme. Levels of deacetylation converge at higher enzyme concentrations as the reaction nears completion.

The effect of ATP on deacetylase activity was tested in an alternative assay using hyperacetylated core histones labeled in vivo with [3H]acetate. Deacetylation by HDAC1 and HDAC3 immunocomplexes over a 2-h time course is shown in Fig. 5. ATP strongly enhances activity of both enzymes at the earlier time points. The same assay, at a fixed time of 30 min, was used to determine the concentration of ATP required for optimum deacetylase activity. As shown in Fig. 6, both HDAC1 and HDAC3 immunocomplexes are maximally stimulated at or below 0.1 mM ATP. The analogue ATPγS, which is hydrolyzed much more slowly than ATP, gives only a small stimulation of deacetylation over the same range of concentrations (Fig. 6), showing that the stimulatory effect requires ATP hydrolysis. Neither GTP nor ADP increases rates of deacetylation (data not shown). In contrast to its effects on deacetylation of whole histones, ATP at concentrations ranging from 0.1 to 1.0 mM has no effect at all on the deacetylation, by HDAC1 or HDAC3, of the chemically acetylated H3 and H4 peptides used for routine HDAC assays (results not shown).

Class I HDACs Co-immunoprecipitate with Hsp60 and Hsp70 Chaperone Proteins—We have explored two possible mechanisms for the stimulation of HDAC activity by ATP. The first is that a protein kinase activity present in the immunocomplexes, phosphorylates one or more components of the complex and thereby stimulates activity. There are precedents for this. Evidence has been presented that HDAC1 is phosphorylated in vivo at sites within −100 residues of the C-terminal end (41), and we have shown that treatment with alkaline phosphatase abolishes the HDAC activity of preparations from Xenopus oocytes (37). Additionally, HDAC4 has been shown to associate with extracellular signal-regulated kinases 1 and 2 and can be phosphorylated in vitro by these enzymes (42, 43), whereas the homologous HDAC5 is phosphorylated at specific serines by the calcium/calmodulin-dependent protein kinase (44). However, we have, as yet, been unable to find evidence that protein phosphorylation plays a role in the stimulation of HDAC activity by ATP in our in vitro assays. Alkaline phosphatase treatment of immunoprecipitates is without effect, whereas assays carried out in the presence of high levels of [γ-32P]ATP, failed to produce any radiolabeled proteins detectable by electrohoresis and phosphorimaging (results not shown).

The second possible mechanism proposes a role for ATP-dependent chaperone proteins in the positioning of the histone substrate. Such proteins are crucial mediators of protein folding and assembly of multiprotein complexes and are involved in the recognition of damaged, partially denatured proteins and their degradation by intracellular proteases. The heat shock proteins Hsp60, Hsp70, and Hsp90 are abundant, ATP-dependent molecular chaperones in eukaryotic cells, mediating the roles of many key, regulatory proteins (45). As a first test of the HDAC/chaperone hypothesis, we asked whether these proteins were present in HDAC immunoprecipitates.

Mouse monoclonal antibodies to human Hsp60, Hsp70, and Hsp90 were used to test HDAC immunoprecipitates by Western blotting. The results are shown in Fig. 7A. We find Hsp70 in material precipitated by antibodies to HDAC1, HDAC2, and HDAC3 and Hsp60 in anti-HDAC1 immunoprecipitates. In contrast, none of the immunoprecipitates so far examined contains detectable amounts of Hsp90, even after prolonged exposure of the labeled filters. To test the significance of these results, we asked whether antibodies to heat shock proteins could immunoprecipitate HDACs. As shown in Fig. 7B, antibodies to Hsp70 precipitated easily detectable amounts of HDAC1 and HDAC2, whereas antibodies to Hsp90 did not. (The antibody to Hsp60 has proved unsuitable for immunoprecipitation with either protein A- or protein G-Sepharose). Further, material precipitated with antibodies to Hsp70, but not with antibodies to Hsp90, contained significant HDAC activity (Fig. 7C).

DISCUSSION

By immunoprecipitation, we have been able to separate active deacetylase complexes from HeLa cell extracts containing, as their catalytic subunits, HDAC1, HDAC2, HDAC3, or HDAC6. Of all the antibodies tested, only anti-HDAC2 precipitated more than one catalytic subunit (i.e. HDAC1 and HDAC2), suggesting that some deacetylase complexes contain both these enzymes. This has been noted previously (12, 27). The finding that material precipitated with antibodies to HDAC1 contained only the HDAC1 catalytic subunit confirms that complexes with just this catalytic component do exist, but also raises the question why this antibody does not also precipitate complexes containing both HDAC1 and HDAC2. Perhaps HDAC2 in the complex obscures the epitope recognized by the anti-HDAC1 antibody.

Catalytic Specificity of Class I HDACs—Using an assay based on Western blotting and immunostaining with antisera specific for particular acetylated lysine residues on defined histones, we show that HDAC1 and HDAC3 can deacetylate all four core histones at all lysines tested, although with varying degrees of efficiency. In contrast, HDAC6 showed weak deacetylation only of H4 Lys-5 and Lys-8, although the enzyme clearly retained the ability to deacetylate the chemically acetylated H3 and H4 peptides used in the standard HDAC assay. It has been shown previously that epitope-tagged HDAC6 immunoprecipitated from transfected cells efficiently deacetylates...
FIG. 5. Effect of ATP on deacetylation of radiolabeled core histones by HDAC1 and HDAC3 immunocomplexes. Core histones radiolabeled in vivo with [3H]acetate were incubated with HDAC1 and HDAC3 immunocomplexes, as indicated, and acetate release measured by scintillation counting. Immunocomplexes used in each experiment contained the same level of deacetylase activity, releasing 1.60 nmol of acetate in 2 h from a mixture of H3 and H4 peptides in the standard assay. Reactions were performed in the absence () or presence (●) of 0.5 mM ATP over a time course of 0–120 min. Values are averages from three separate experiments, with error bars indicating standard deviation.

FIG. 6. Deacetylation of radiolabeled histones by HDAC1 and HDAC3 in the presence of ATP or ATP·S. HeLa histones radiolabeled in vivo with [3H]acetate were incubated for 30 min with HDAC1 (●) or HDAC3 (○) immunocomplexes in the presence of variable concentrations of ATP (solid symbols) or ATP·S (open symbols). Rates of deacetylation are expressed as picomoles acetate released per minute.

free core histones (17, 18). In fact, we have shown that V5-tagged Drosophila HDAC6, immunoprecipitated from stably transfected SL2 cells, is capable of efficient deacetylation of whole core histones using exactly the same assay as that employed here (39). It is possible that these divergent findings represent a real difference between endogenous HDAC6 complexes and those containing the epitope-tagged protein isolated from overexpressing, transfected cells. However, we consider it equally likely that the lack of activity of the endogenous immunocomplexes is because of an inhibitory effect of the bound antibody. Although the peptide used to raise this antibody is located at the extreme C-terminal end of the protein (residues 1201–1212), binding of an IgG molecule to it may still be able to disrupt the correct positioning of intact histones. Binding to a more distal epitope tag apparently has no such effect. Until this is resolved, it remains possible that the present results are not a true reflection of the ability of endogenous HDAC6 complexes to deacetylate intact histones.

The Western blotting assay used here was developed to study the specificity of HDAC1/HDAC2 in the NuRD complex (27). Using free histones, it was shown that the NuRD complex could deacetylate all lysines of H4 and efficiently deacetylated H3 (detected with a non-lysine-specific antisera). These results are in good agreement with our assays of HDAC1/HDAC2 immunoprecipitates, which we assume contain a mixture of the Sin3 and NuRD complexes and possibly others. However, although our results show that HDAC1, HDAC2 (probably), and HDAC3 can deacetylate all histone lysines tested so far, analysis of the reaction using varying enzyme concentrations revealed differential rates of deacetylation. For example, HDAC1 deacetylated H2A Lys-5 more slowly than any other histone, whereas HDAC3 showed preferential deacetylation of H4 Lys-5 and Lys-12 and H2A Lys-5. (This shared specificity may reflect the close homology between the first 9 residues of H2A and H4). Class I deacetylases in other organisms have specificities resembling that of human HDAC3. The maize enzyme HD1-B has been reported preferentially to deacetylate Lys-5 and Lys-12 of H4 in free histones (46), whereas, in S. cerevisiae, mutant strains lacking functional Rpd3 show increased acetylation, specifically of H4 Lys-5 at the promoters of certain repressed genes (47).

The Role of ATP in Deacetylation by HDAC Complexes—None of the immunoprecipitated enzyme complexes used here gave significant deacetylation of purified mononucleosomes, unless ATP was added to the reaction mix. More surprisingly, ATP also stimulated deacetylation of free histones by HDAC1 and HDAC3. The stimulatory effect of ATP was seen with all histones and lysines tested, although the degree of stimulation varied. Stimulation by ATP was also seen when histones radiolabeled in vivo were used as substrate in an alternative assay. In clear contrast, ATP had no effect on cleavage of peptide substrates. Previous studies have reported that ATP does not appear to facilitate the deacetylation of non-nucleosomal histone substrates (29, 30). The explanation for this discrepancy is likely to be technical. In previous studies, end point deacetylation assays were used to show that an equal amount of product ([3H]acetate) accumulated over the course of the experiment. However, in neither of the previous studies was the rate of deacetylation measured, so neither study addresses our observation that, although the total amount of product generated may be little changed in the presence of ATP (as the reaction nears completion), the rate of substrate utilization is increased.

We have explored two possible mechanisms by which ATP might stimulate the catalytic activity of HDAC complexes. The first is that activity is enhanced by phosphorylation of one or more components of the complex by an endogenous protein kinase. In testing this, we have found no evidence that the activity of HDAC complexes is influenced by protein phosphate groups, or that components of immunopurified HDAC complexes are subject to ATP-dependent phosphorylation in vitro. Although phosphorylation may well be an important regulator of HDAC function in vivo, we have no evidence that it makes a major contribution to the ATP effects described here. A second possible mechanism is that cleavage of protein substrates requires the action of an ATP-dependent chaperone protein. In
ATP Enhances Histone Deacetylase Activity

Fig. 7. Co-immunoprecipitation of Hsp chaperones and HDACs. A, material immunoprecipitated (IP) from HeLa WCE with rabbit polyclonal antibodies to HDAC1, HDAC2, or HDAC3, as indicated at the top of the figure, was run out on SDS-PAGE gels, Western blotted, and immunostained with mouse monoclonal antibodies to Hsp60, Hsp70, and Hsp90, as indicated to the left of each panel. Cognate protein bands are indicated by arrows. The strongly staining, faster moving band in some tracks is rabbit IgG H-chain, cross-reacting with the anti-mouse IgG second antibody. B, material immunoprecipitated from HeLa WCE with mouse monoclonal antibodies to Hsp70 and Hsp90 was run out on SDS-PAGE gels, Western blotted, and immunostained with rabbit polyclonal antibodies to HDAC1 and HDAC2. Cognate protein bands are indicated by arrows. The faster moving bands are mouse IgG H-chain cross-reacting with the anti-rabbit IgG second antibody. C, HDAC activity (radiolabeled peptide assay) of material immunoprecipitated by antibodies to Hsp70 and Hsp90. Assays contained either 50 μl (black bars) or 25 μl (gray bars) of immunoprecipitate.

Testing this, we have shown that HDAC immunocomplexes are associated with the ATP-dependent chaperone proteins Hsp60 and Hsp70. This finding strongly supports, but does not prove, the hypothesis that chaperone proteins are responsible for the observed effects of ATP.

Possible Roles of ATP-dependent Chaperone Proteins in Histone Deacetylation—Hsp60 and Hsp70 chaperone proteins both bind exposed, hydrophobic regions of non-native proteins to protect them from aggregation and/or to facilitate their (re)folding into the native state (45). Both use ATP binding and cleavage to regulate the handling of target proteins, and both require additional proteins (co-chaperones) to optimize the different stages of the processing reaction. However, the co-chaperones involved and the mechanisms that link ATP binding and hydrolysis with substrate manipulation are quite different. For example, the ATPase activity of Hsp70 (often rate-limiting) is stimulated by members of the DnaJ family and release of ADP by members of the GrpE family (48, 49). The bacterial Hsp60 homologue GroEL associates with the essential co-chaperone GroES in giant macromolecular assemblies (50). It will be of interest to establish which, if any, co-chaperones are also present in HDAC complexes.

The mechanism by which Hsp chaperone proteins might stimulate the catalytic activity of HDAC complexes remains speculative, as does the manner in which they might interact with histones. The interaction between histones and the well known histone chaperones nucleoplasmin and N1, provides few clues, being mainly charge-mediated and still not completely understood (51). It is possible that Hsp proteins orientate the histone substrate, such that the appropriate region of the tail domain is positioned adjacent to the catalytic pocket of the HDAC. However, the histone tail domains themselves are unlikely targets for Hsp chaperones. Although they are likely to be at least partly unfolded, and lack the acidic amino acids that inhibit chaperone binding, they are also poor in runs of three or more hydrophobic residues that favor such binding (45). Additionally, the finding that deacetylation of chemically acetylated, 20-amino acid, H3 and H4 tail peptides was not stimulated by ATP argues that chaperones are not required for positioning of the tails themselves. It is possible that Hsp chaperones associate with the globular core of the histones, where hydrophobic residues are more common. However, if this is the mechanism by which HDAC-associated chaperones operate when histones are packaged into nucleosomes, there must also be a mechanism that allows them to access the nucleosome core. This requires DNA displacement and de facto nucleosome remodeling. Thus, the stimulatory effect of ATP on deacetylation of mononucleosomes in vitro could be because of ATP-dependent remodeling or chaperone activity or both. In this respect, it is interesting that deacetylation of mononucleosomes by HDAC3, a deacetylase not known to associate with a nucleosome remodeling complex, was also stimulated by ATP.

ATP, Chaperones, and HDAC Specificity—Our results raise the interesting possibility that different HDACs are associated with different chaperone proteins. Although Hsp70 was found associated with all three class I HDACs, and Hsp90 with none, Hsp60 associated only with HDAC1 complexes. It is puzzling that there is no Hsp60 in the material immunoprecipitated by anti-HDAC2, given that this material also contains significant amounts of HDAC1. It may be that the association of HDAC2 and Hsp60 with HDAC1 are mutually exclusive. The presence of different chaperone proteins may explain why ATP stimulates the activity of different enzymes to differing extents. For example, deacetylation of H2B by HDAC1 is stimulated only slightly by ATP, whereas cleavage of the same histone by HDAC3 is increased severalfold. Conversely, ATP has only a small effect on cleavage of H2A by HDAC3, but causes a severalfold increase in its cleavage by HDAC1. It is, of course, important to consider that many proteins other than histones are acetylated in vivo (52, 53). HDAC complexes presumably act on these proteins to maintain appropriate levels of acetylation, and must therefore be able to associate with, and modify, a wide range of structurally diverse proteins. ATP-dependent chaperone proteins may make an important contribution to meeting this requirement.

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