HDA1 and HDA3 Are Components of a Yeast Histone Deacetylase (HDA) Complex*

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Histone acetylation is maintained through the action of histone acetyltransferases and deacetylases and has been correlated with increased gene activity. To investigate the functional role of these enzymes in the regulation of transcription, we have purified from Saccharomyces cerevisiae two histone deacetylase activities, HDA and HDB, with molecular masses of 350 and 600 kDa, respectively. In vitro, the HDA activity deacetylates all four core histones, has a preference for histone H3, and is strongly inhibited by trichostatin A (a specific inhibitor of histone deacetylases). HDB is considerably less sensitive to trichostatin A. We report the extensive purification of the HDA activity and the identification of peptides (p75, p73, p72, and p71) whose presence correlates with deacetylase activity on native polyacrylamide gels. An antibody to p75 immunoprecipitates peptides with molecular masses similar to those in the 350-kDa complex. Additionally, antibodies to p75 and p71 specifically precipitate histone deacetylase activity and co-immunoprecipitate each other. Gene disruptions of p75 (HDA1) or p71 (HDA3) cause the loss of the 350-kDa (but not the 600-kDa) activity from our chromatography profiles. These data argue strongly that HDA1 and HDA3 are subunits of the HDA complex, which is structurally distinct from the second, HDB complex.

Nucleosomes can have both positive and negative effects on transcription (1–6). The dynamic effects of nucleosomes on transcription may be due in part, to the acetylation of lysine residues present in the hydrophilic amino-terminal portions (tails) of the core histones (7). This acetylated state is maintained through the competing activities of histone acetyltransferases and deacetylases. Histone acetyltransferases transfer the acetate from acetyl-CoA to neutralize the positively charged ε-amino group of an unacetylated lysine residue. In contrast, deacetylation is achieved through the hydrolysis of the acetyl moiety, restoring a positive charge. Numerous studies have demonstrated the colocalization of nucleosomes containing hyperacetylated histones with active or potentially active genomic domains. Conversely, histone hypoacetylation has been correlated with heterochromatic regions that are predominantly silent (7–10).

Lysine acetylation occurs on all four core histones, with H2A modified on Lys-5; H2B on Lys-5, Lys-12, Lys-15, and Lys-20; H3 on Lys-9, Lys-14, Lys-18, Lys-23, and Lys-27; and H4 on Lys-5, Lys-8, Lys-12, and Lys-16 in many eukaryotes (11, 12). The acetylation of these highly conserved residues is not random (12, 13). Newly synthesized histone H4 in Drosophila and HeLa cells is acetylated cytoplasmically at Lys-5 and Lys-12 prior to deposition (14, 15). Also, the hyperactive X chromosome of Drosophila larvae contains histone H4 that is preferentially acetylated at Lys-16 (9, 16). Conversely, most of the sites of H4 acetylation are hypoacetylated in silent heterochromatic regions such as the inactive X chromosome in mammals (10) and the yeast silent mating-type loci and yeast telomeres (17).

Despite these correlations, it is still not known whether the acetylated state of histones has a causal effect on histone deposition or gene activity. To understand the physiological effects of histone acetylation and deacetylation, it will be necessary to identify the protein subunits of the appropriate enzymes and to obtain loss-of-function mutations in their genes, whose effects on gene activity may then be monitored in vivo. Partial purification and characterization of histone acetyltransferases and deacetylases have led to the conclusion that there are several different enzymes within the cell with different substrate specificities (18–20). Recently, a cytoplasmic yeast histone acetyltransferase subunit with specificity for H4 Lys-12 in vitro has been found (21). In addition, an active acetyltransferase (55 kDa) subunit from Tetrahymena macronuclei with a homologue in yeast (GCN5) has also been identified (22, 23).

In contrast to histone acetyltransferases, less is known about deacetylases. Partial purification studies have detected activities in yeast (24, 25) and higher eukaryotes (26–29). Sanchez Del Pino et al. (25) reported the partial purification from yeast whole cell extracts of two forms of deacetylase that were able to deacetylase histones. Other researchers have demonstrated that histone deacetylase activity is tightly associated with the nuclear matrix (29–31). A deacetylase activity is also associated with yeast nuclei (24). Sodium butyrate inhibits histone deacetylation in yeast spheroplasts (24) and higher eukaryotes (32). However, sodium butyrate is neither a very potent inhibitor (requiring millimolar concentrations) of the deacetylase nor specific in its actions (33, 34). In contrast, trichostatin A (TSA) is a very specific and potent inhibitor (demonstrating inhibition in the nanomolar range) of histone deacetylases (25, 28).

In this report, we describe the identification of two distinct nuclear-associated histone deacetylases: HDA, a 350-kDa activity that is highly sensitive to TSA; and HDB, a 600-kDa activity that is insensitive to TSA.

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§The abbreviations used are: TSA, trichostatin A; HPLC, high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; TEMED, N,N,N′,N′-tetramethylethylenediamine; GST, glutathione S-transferase.
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### Yeast Histone Deacetylase HDA

#### Enzyme Properties of HDA

**Histone Specificity and Km**—The HDA complex can deacetylate all core histones, but has a preference for H3 in vitro. Using purified H3 as the substrate the Km values were determined to be 2.3 and 10.5 nM for a mixture of total histones. These are extremely low values for a Km and suggest that this enzyme has an exceptionally strong substrate affinity.

**pH Optimum**—The pH optimum for the enzyme was 7.0 (at 250 mM NaCl with 0.25 pH unit steps), with approximately one-third of the activity remaining at pH 5 or 8. Bio-Rad Rotophor analysis determined that the pl of the protein was 7.1.

**Ionic Strength Optimum**—The optimum NaCl concentration for the enzyme was found to be 0.275 M (at pH 7 with 50 mM steps). The enzyme was found to be stable between 50 and 500 mM NaCl with total activity restored upon dialysis or dilution with additional NaCl.

**Metal Ions—Enzyme activity is not enhanced by any metal ion cofactors; however, many metal ions inhibit the deacetylase, including Zn²⁺, Fe³⁺, Cu²⁺, Co²⁺, and Cr³⁺.** Zn²⁺ was particularly potent inhibitor, reducing the activity of the enzyme by >95% at 0.5 mM. Although not as extensively purified, the activity described earlier did not require metal ion cofactors and was similarly inhibited by Zn²⁺ and Cu²⁺ (24).

**Sodium Butyrate**—Sodium acetate—Sodium acetate and sodium butyrate were found to be mild inhibitors of HDA. Sodium acetate completely eliminated activity at 25 mM and was found to be a superior inhibitor compared with sodium butyrate (which reduced activity by 70% at this concentration). This result suggests that acetate, which is released by the deacetylation of histones, may act as a feedback inhibitor of the enzyme.

**N-Acetyllyspermidine**—No activity was detected for this substrate even upon prolonged incubations.

**Polypeptides Containing Chemically Acetylated Lysine**—Poly-L-lysine or poly(Lys, Ala) (1:1), when chemically acetylated, reduced deacetylase activity to <1% when present in 20-fold excess (w/w) for the competitor/histone (Table I). The unacetylated forms of these polyamino acids were 30-fold less effective at reducing the measured activity at the same concentrations. Acetylated forms of histone H3 and H4 tails reduced measured enzyme activity to <1% when present in 20-fold excess, similar to the acetylated polyamino acids. However, we found that the unacetylated forms of these histone tail peptides had the ability to inhibit the enzyme independent of acetylation. Therefore, acetylated polypeptides are strong competitors for enzyme activity, but the enzyme may also recognize other sequences within the histone tails.

### RESULTS

Purification of Histone Deacetylases HDA and HDB—Yeast histones are neither efficiently labeled nor easily purified in large quantities. Therefore, in vivo radiolabeled HeLa histones were used to follow the release of [³H]acetate to assay the activity of the histone deacetylase enzyme, as described under “Materials and Methods.” To obtain the deacetylase, highly purified yeast nuclei were prepared as described (see “Materials and Methods”), and nuclear proteins were extracted using 450 mM NaCl. The unusually high ionic strength of the low salt wash (175 mM NaCl) helped to remove many contaminating proteins that were loosely associated with the nuclei. This nuclear extract was precipitated at 50% ammonium sulfate saturation. After dialysis, the preparation was step-eluted in series from DEAE-Sepharose and S-Sepharose columns. The S-Sepharose column resolved the deacetylase activity into two forms: the 300 mL eluting HDA activity, which was not extensively purified in this paper, and the higher salt eluting HDB activity (see Fig. 2). To further fractionate the HDA activity, we employed a Mono Q HR 10/10 column (Fig. 1A). This strong anion-exchange column yielded a single sharp peak of enzyme activity between 290 and 320 mM NaCl. This activity was further fractionated on a Mono-P Prep hydroxyapatite column into a single peak of activity that eluted between 265 and 333 mM sodium phosphate (Fig. 1B). The HDA activity was size-fractionated as a 350-kDa peak on a Superdex 200 column (Fig. 1C). To minimize aggregation, gel filtration chromatography was performed at 350 mM NaCl. Detergents and these higher salt conditions did not alter the molecular mass of the HDA activity, but ionic strengths above 400 mM NaCl did tend to reduce overall HDA activity even with subsequent dialysis. In contrast, the HDB activity was retained even in the 1.0 M NaCl step elution and could be directly rechromatographed, revealing a 600-kDa complex on Superdex 200 chromatography (Fig. 2). Fractions from the gel filtration column containing peak HDA activity were chromatographed using a Mono S HR 5/5 column, yielding a single broad peak of enzyme activity (Fig. 1D) over a range of 190–290 mM NaCl. Mono S chromatography offers only a slight purification of the deacetylase as measured.

### Table I

| Competitor                  | HDA activity (% activity of 100%) |
|-----------------------------|----------------------------------|
|                             | S-Fdd excess | 20-Fdd excess |
| H3 tail                     | 17           | 4.4           |
| H3 tail, acetylated         | 17           | 0             |
| H4 tail                     | 16           | 1.0           |
| H4 tail, acetylated         | 15           | 0.9           |
| Poly-Lys                    | 41           | 25            |
| Poly-Lys, acetylated        | 33           | 0.4           |
| Poly(Lys, Ala)              | 48           | 29            |
| Poly(Lys, Ala), acetylated  | 41           | 0.9           |
| N'-Acetyl-Lys               | 100          | 100           |
| N'-Acetyl-Lys               | 100          | 100           |

Values are the percent activity 5- or 20-fold excess competitor/([³H]acetylated histones (ww) compared with the same assay done with no competitor. The deacetylase assay was performed as described under “Materials and Methods” for a purified HDA fraction and with a 30-min incubation. The assays were performed as indicated under “Materials and Methods.”
Yeast Histone Deacetylase HDA

Fig. 1. Column chromatography of HDA histone deacetylase. A, Mono Q HR 10/10 chromatography fractions were assayed for deacetylase activity as described under "Materials and Methods." 25 microliters of each 7.5-ml fraction were assayed for activity; total counts/minutes released for a 30-min assay are indicated (○). B, fractions 7–9 were pooled, dialyzed, and loaded onto a hydroxypatite column (10 mm × 12 cm). 25 microliters of each 4.0-ml fraction were assayed as described above, and fractions 14–18 were pooled and dialyzed. C, shown is the Superdex 200 gel filtration chromatography (10 mm × 46 cm) profile with activity for 10 μl of each 1.0-ml fraction. Peak activity fractions 6–8 were pooled and dialyzed. Protein molecular mass standards (in kilodaltons) are indicated by arrows. D, shown is the Mono S HR 5/5 chromatography profile and activity measured from 25 μl of each 1.0-ml fraction.

Fig. 2. Purification of yeast histone deacetylases HDA and HDB. HDA was extensively purified as indicated and described under "Materials and Methods." but has the ability to further separate the HDA complex into a broad peak of activity whose peptides may be gel-purified (see below). In summary, we have purified a 350-kDa histone deacetylase activity to near homogeneity, which we term HDA, and have shown that it is distinct from a second, 600-kDa activity, termed HDB.

As described under "Materials and Methods," we have characterized certain other features of the HDA complex. It has an exceptionally high substrate affinity for histones (K_m = 2.3 nM for histone H3 and 10.5 nM for total core histones), a pH optimum of ~7.0, and an NaCl optimum of 275 mM and is strongly inhibited by Zn^{2+} and by trichostatin A and less so by sodium butyrate. Additionally, the HDA enzyme does not deacetylate acetylserpin, but is specifically inhibited by polypeptides containing chemically acetylated lysine and both acetylated and unacylated histone H3 and H4 N termini. This latter result suggests that it may also interact with the regions around the acetylated lysine residues at the histone N termini.

The enzyme activity present in crude lysates or nuclear fractions prior to the ammonium sulfate precipitation is much lower than after the ammonium sulfate cut, suggesting the presence of an inhibitor in these preparations (Table II). Due to this inhibition, the -fold purification from the initial crude lysate cannot be accurately determined. Chromatography on both DEAE-Sepharose and S-Sepharose increases the amount of activity present overall, suggesting the removal of inhibitors. The amount of activity after the S-Sepharose column chromatography is especially enhanced when one considers that approximately one-third of the starting activity is diverted into the HDB 1.0 M NaCl salt step. We have not characterized the inhibition, but it may be caused by unlabeled substrates within the crude preparations, allosteric regulators present in the whole cell lysate, or possible enzyme trapping within nuclear structures since histone deacetylases are tightly associated with the nuclear matrix (29, 30).

HDA Histone Deacetylase Activity Is Strongly Inhibited by Trichostatin A—TSA is a potent and specific inhibitor of both mammalian and yeast histone deacetylase activities in vitro (25, 28, 36). We have also found that TSA causes histone hyperacetylation in yeast spheroplasts, but we have had to use concentrations (10.0 μM) 5-fold higher than those used previously (Ref. 25 and data not shown). The presence of 10.0 μM TSA increases the overall levels of acetate incorporated by 3-fold compared with the wild-type control. Additionally, we observed on Triton-acid-urea gels proportionally more diacetylated H4; increases in acetylated forms of H2B, H3, and H2A were also evident (data not shown).

TSA was also found to be a potent noncompetitive inhibitor of the HDA activity in vitro as shown by a Lineweaver-Burk plot (Fig. 3). This plot demonstrates that the K_m for the enzyme is unaffected, but the rate of reaction is reduced with increasing concentrations of TSA. Replotting this data on a Dixon plot (Fig. 3, inset) determined that TSA has a K_i of 9.7 nM for HDA. While our calculated value for K_i is exceptionally low for an inhibitor, its value is ~3-fold higher than the value reported by Yoshida et al. (28) for the mammalian deacetylase. It is interesting to note that the K_i for TSA is very similar to the K_m for total histones that we calculated at 10.5 nM, suggesting that the HDA affinity for both TSA and histones is similar. In contrast to HDA, the HDB activity was found to be less sensitive to TSA, with a K_i at least 10-fold greater. We also found that the peak HDA activity (Mono S fraction 12) was inhibited by 80% at 10 nM TSA at 50–100 μg/ml histone, whereas the HDB activity (Mono S fraction 25) was inhibited by <20% (Fig.
4). This suggests that the HDA and HDB activities are distinct from each other in their sensitivity to TSA.

HDA Activity Co-chromatographs with Four Peptides (p75, p73, p72, and p71)—Four peptides with relative molecular masses of 75, 73, 72, and 71 kDa co-migrate with enzyme activity in the Mono S fractions on SDS-PAGE (Fig. 5). Sequence analysis of gel-isolated proteins has confirmed that p75, p73, and p71 are distinct from each other. We have not yet confirmed that p73 and p72 have different primary structures. It is interesting to note that the band representing the 73-kDa peptide is less obvious in the earlier Mono S fractions (Fig. 5, fraction 10). While the decrease in the p73 band intensity does not destroy enzyme activity, we did notice that loss of p73 in fraction 10 is inversely proportional to the appearance of a peptide in the earlier Mono S fractions (lane 3) having similar electrophoretic (SDS-PAGE) migration compared with p75, p73, and p71. This is presumably due to the presence of the very rare HDA1 (p75) and HDA3 (p71) proteins, but many additional peptides. This is presumably due to the presence of abundant cross-reactive proteins in the crude mixture, as revealed by silver staining (data not shown). Therefore, a partially purified extract of higher activity and purity was used as described under “Materials and Methods.” Immunoprecipitation with the anti-HDA1 antibody (Fig. 6A) from partially purified extracts (lane 5; input) demonstrates the presence of peptides (lane 3) having similar electrophoretic (SDS-PAGE) migration compared with p75, p73, p72, and p71 from highly purified Mono S fractions chromatographed on Mono Q (lanes 1 and 2) and Mono S fractions 12–14 and 9–11, respectively. This contrasts with bands precipitated by the preimmune serum (lane 4). Note that the immunoprecipitated material (lane 3) contains relatively less of the p73 species as compared with fractions 12–14 (lane 1). This may be due to the lability of p73 as described above. We also observed a faint additional band present between the p75 and p73 proteins that is present in lanes 1–3. This band is found in fractions across

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**Table II**

| Fraction        | Total 1000 cpm of [3H]acetate (fmol) | Purification Yield |
|-----------------|--------------------------------------|--------------------|
| Nuclear extract | 198 350                               | 0.56 (1.0) (100)    |
| DEAE-Sepharose  | 223 30.5                               | 7.3                |
| S-Sepharose     | 198 11.3                               | 17.5               |
| Mono Q HR 10/10 | 98 1.65                                | 59.1               |
| Hydroxyapatite  | 34 0.22                                | 153.6              |
| Superdex 200    | 30 0.07                                | 428.6              |
| Mono S HR 5/5   | 28 0.041                               | 682.9              |

*1 Unit is defined as the activity required to release 1000 cpm of [3H]acetate from the acetylated histone substrate under standard histone deacetylase assay conditions per min (see “Materials and Methods”).*
immunodepleted a significant portion of the total activity (80% for anti-HDA3). Both antibodies for HDA1 and HDA3 also cant amount of activity in the pellets (28% for anti-HDA1 and 21% for anti-HDA3). Both antibodies for HDA1 and HDA3 also precipitating both proteins, although we observed only slightly stained fractions; the assay was performed as described for Fig. 1 and under "Materials and Methods."

In addition, as shown in Table III, antibodies directed against both HDA1 (p75) and HDA3 (p71) recovered a significant amount of activity in the pellets (28% for anti-HDA1 and 21% for anti-HDA3). Both antibodies for HDA1 and HDA3 also immunodepleted a significant portion of the total activity (80% for anti-HDA1 and 67% for anti-HDA3), whereas incubation with preimmune serum or an antisera directed against an unrelated protein (enolase 1) led to no immunodepletion. The residual activity either may be cross-contaminating HDB or may be due to incomplete immunoprecipitation. These data suggest a modified form of p75 as we have obtained seemingly identical proteolytic peptide analysis of the two proteins (data not shown).

To assay for the specificity of the antibodies and to determine if p75 and p71 co-immunoprecipitate, the precipitates were assayed by Western blotting (Fig. 6B). As shown in Fig. 6B, the HDA1 antibody detects a 75-kDa band (lane 1), while the HDA3 antibody detects only the 71-kDa species (lane 10), showing little if any cross-reaction between the antibodies. Lanes 3 and 8 contain immunoprecipitations using the HDA1 antibody. It is evident that the HDA1 antibody immunoprecipitates both p75 (lane 3) and p71 (lane 8). Conversely, the HDA3 antibody precipitates both p71 (lane 6) and p75 (lane 5). We observed that the HDA1 antibody is more effective at immunoprecipitating both proteins, although we observed only slightly less activity present in the anti-HDA3 immunoprecipitate. We have also found that Western blot analysis using these antibodies demonstrates staining across HDA activity profiles in our chromatographic analyses, but not against fractions containing only purified HDB activities (data not shown).

In addition, as shown in Table III, antibodies directed against both HDA1 (p75) and HDA3 (p71) recovered a significant amount of activity in the pellets (28% for anti-HDA1 and 21% for anti-HDA3). Both antibodies for HDA1 and HDA3 also immunodepleted a significant portion of the total activity (80% for anti-HDA1 and 67% for anti-HDA3), whereas incubation with preimmune serum or an antisera directed against an unrelated protein (enolase 1) led to no immunodepletion. The residual activity either may be cross-contaminating HDB or may be due to incomplete immunoprecipitation. These data...
argue strongly that HDA1 (p75) and HDA3 (p71) are members of the same active histone deacetylase HDA complex.

HDA Activity Is Selectively Disrupted by Deletions in the Genes Coding For Either p75 or p71—If the HDA and HDB activities result from the assembly of different protein subunits, one would predict that deletions of subunits in the HDA complex would disrupt the HDA activity, but not the HDB activity. A Superdex 200 profile (on a preparation that retains both HDA and HDB activities) (Fig. 7A) separated two deacetylase activities, revealing a higher molecular mass complex (600-kDa HDB activity; fraction 5) and a smaller HDA complex (fraction 7). Deletions in either the p75 (HDA1) or p71 (HDA3) genes disrupt the smaller HDA activity, yet leave the larger HDB complex intact (Fig. 7B). This is especially evident when the peak Superdex fractions 5–7 were pooled and rechromatographed on a Mono S column. This illustrates the elimination of the HDA peak and the retention of the HDB peak in either hda1 or hda3 mutant strains (Fig. 7B). These data demonstrate that HDA1 (p75) and HDA3 (p71) components are required for HDA deacetylase activity on these chromatography columns, but not for HDB enzyme activity.

**DISCUSSION**

We have characterized two histone deacetylase activities, HDA (350 kDa) and HDB (600 kDa), from yeast. Several lines of evidence suggest that the activities are functionally distinct from each other and that we have identified at least two peptides (p75 and p71) that are associated with HDA enzyme activity. The HDA activity is highly sensitive to the specific histone deacetylase inhibitor TSA. The HDB activity is only modestly sensitive over a wide range of substrate concentrations. Estimates of the abundance of the HDA deacetylase complex from our purifications suggest that the complex is not very abundant and is represented by <100 copies/cell. However, the $K_m$ for this enzyme is exceptionally low (10.5 nM) for a heterogeneous acetylated histone substrate in vitro, suggesting very high substrate specificity.

Purification of the HDA activity demonstrates four main bands on SDS-PAGE representing peptides p75, p73, p72, and p71 that correlate with activity. On native gels, a slice containing the HDA activity contains almost exclusively peptides with the molecular masses of p75, p73, p72, and p71 when re-electrophoresed by SDS-PAGE. Antibodies to p75 co-immunoprecipitate a complex that contains peptides with molecular masses similar to those in the HDA peak. Western blot analysis confirms that antibodies to p75 co-immunoprecipitate p71 and vice versa. Finally, disruptions of the genes coding for either p75 or p71 cause the loss of the HDA activity from our chromatography columns. These disruptions do not cause the loss of the HDB activity. These data argue strongly that p75 (HDA1) and p71 (HDA3) are components of HDA histone deacetylase activity. We do not yet know genetically whether p73 and p72 are distinct from each other or whether they are required for HDA enzyme activity.

Previously, the partial purification of both a high mass (500 kDa) and a low mass (150 kDa) deacetylase activity has been reported (25). This work showed that the high mass activity was less sensitive to TSA than the low mass activity. Additionally, the high mass form was lost at higher ionic strengths as the low mass form increased. We did not find a 150-kDa activity within our crude nuclear preparations. However, either it may have been removed during the preparation, or our isolation conditions were not conducive to generating the smaller product. It is likely that the activity reported previously as high mass is the same as HDB. Why two histone deacetylase activities exist with different sensitivities to TSA is unclear. The HDA activity is specific for histones in vitro since it will not deacetylate acethylspermidine, and it is strongly inhibited by polyacetates containing chemically acetylated lysine or by histone H3 and H4 N-terminal peptides, whether or not they are acetylated (see “Materials and Methods”). We do not yet know the specific activity of the HDB activity.

Most of the HDA activity is directed toward histone H3 in vitro. However, this result must be interpreted with caution regarding in vivo specificity. The HDA activity is capable of
deacetyllating all four histones, and we have no evidence as yet for the presence of other histone deacetylases that have specificity for histones other than H3 in vitro. It is certainly possible that the apparent specificity for H3 reflects the in vitro assay used. H3La cells grown in high concentrations of butyrate possess H3 acetylated at Lys-27. However, this is only in the penta-acetylated form, which is also the least abundant even in the presence of the histone deacetylase inhibitor sodium butyrate, composing <1% of the H3 in H3La cells (11). It is possible that the turnover rate of H3 Lys-27 acetylation is more rapid than that of other lysines in H3 in the presence of butyrate, resulting in disproportional labeling of this site. The yeast deacetylase may then reverse this reaction (of Lys-27 or other high turnover sites), rapidly resulting in the apparent specificity for H3. In vivo, we find that when the yeast deacetylase activity is inhibited, the acetylation state of all the histones increases.

While this report is the first to identify the individual subunits of a histone deacetylase, numerous questions still need to be addressed. As of yet, we do not know the function of the individual subunits of the HDA complex and whether either HDA1 or HDA3 is the catalytic subunit. Moreover, histone deacetylase may require the targeting of the complex to specific chromosomal domains. It is possible that one of the subunits identified has this role. While the HDB complex is distinct in its sensitivity to TSA and its response to HDA-specific gene disruptions, we do not know whether it is functionally distinct, histone-specific, and/or redundant in vivo. We have also not eliminated the possibility that these two complexes share a common catalytic subunit that is not removed by gene disruptions to HDA1 or HDA3. The differential inhibition by TSA (between HDA and HDB) may then be due to the modulation of its sensitivity due to the altered associations within the two complexes. Finally, we do not yet know whether these two activities are in fact important for gene regulation in yeast. By using both enzyme purification and genetic analysis, it is our hope that these questions will soon be answered.

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