Histone deacetylases are the catalytic subunits of multiprotein complexes that are targeted to specific promoters through their interaction with sequence-specific DNA-binding factors. We have cloned and characterized a new human cDNA, HDAC-A, with homology to the yeast HDA1 family of histone deacetylases. Analysis of the predicted amino acid sequence of HDAC-A revealed an open reading frame of 967 amino acids containing two domains: a NH2-terminal domain with no homology to known proteins and a COOH-terminal domain with homology to known histone deacetylases (42% similarity to RPD3, 80% similarity to HDA1). Three additional human cDNAs with high homology to HDAC-A were identified in sequence data bases, indicating that HDAC-A itself is a member of a new family of human histone deacetylases. The mRNA encoding HDAC-A was differentially expressed in a variety of human tissues. The expressed protein, HDAC-Ap, exhibited histone deacetylase activity and this activity mapped to the COOH-terminal region (amino acids 495–967) with homology to HDA1p. In immunoprecipitation experiments, HDAC-A interacted specifically with several cellular proteins, indicating that it might be part of a larger multiprotein complex.

Acetylation of core histones, first described by Allfrey and co-workers (1), has been correlated with transcription, chromatin assembly, DNA repair, and recombinational events (2–7). Transfer of an acetyl group from acetyl-CoA onto the e-amino group of different lysines residues in the NH2-terminal tail of core histones is a ubiquitous modification found in all eukaryotic species examined. Histone acetylation levels are controlled by the competing activities of histone acetyltransferases and histone deacetylases.

Cloning of the first histone acetyltransferase (8) and the first histone deacetylase (9) has led to the identification of a growing number of proteins with similar enzymatic activities (reviewed in Refs. 7 and 10). The characterization of the first histone acetyltransferase and the first histone deacetylase as homologues of Saccharomyces cerevisiae GCN5 and S. cerevisiae RPD3, respectively, two factors previously described genetically as transcriptional regulators, confirmed the long speculated role of histone modification in euakaryotic transcriptional regulation. Together with the demonstration that acetylation levels of nucleosomal histones change in discrete regions associated with certain promoters (11–13), these results established that chromatin is not only a structural scaffold responsible for DNA compaction in the euakaryotic nucleus but is also an active and dynamic participant in transcriptional regulatory mechanisms.

This model has been recently validated by the demonstration that the enzymatic activity of the yeast histone acetyltransferase GCN5 is necessary for the transactivation activity of this factor (14, 15). Similarly, mutation of amino acids critical for the histone deacetylase activity of RPD3 or HDAC1 reduced partially or totally their repressor activity (16, 17). Additional evidence for the involvement of histone acetylation in transcriptional regulation has come from studies with fungal toxins, such as trichostatin A and trapoxin, that specifically inhibit histone deacetylases. These inhibitors shift the dynamic equilibrium between histone acetylation and deacetylation toward the hyperacetylated state (18). Several reports established a correlation between hyperacetylation of histones and transcriptional activation; for example, both trichostatin A and trapoxin increase the transcriptional activity of the human immunodeficiency virus type 1 promoter in vivo and in vitro (19, 20). However, other studies have shown that histone deacetylases can also play a significant role in transcriptional silencing. In mammalian cells, inhibition of histone deacetylases by trichostatin A or trapoxin activates or represses a small fraction of cellular genes (21) and disruption of RPD3 and SIR3 in S. cerevisiae showed that both genes are required to fully activate or repress specific promoters (22–24).

The histone deacetylases identified can be grouped into three families: S. cerevisiae RPD3 and RPD3-related proteins in higher organisms, such as HDAC1, 2, and 3 (9, 25–28); S. cerevisiae HDA1 and the related S. cerevisiae HOS1, 2, and 3 (29, 30); and HD2 isolated from Zea mays (31), which presents no homology to the other two families. Biochemical and molecular biological studies in different systems have established that histone deacetylases are components of large multiprotein complexes that are targeted to promoter sites through their interaction with sequence-specific transcription factors (reviewed in Refs. 32 and 33).

In this report, we describe the identification and cloning of a human cDNA with a region of homology to yeast HDA1. The
Human Histone Deacetylase HDAC-A

FIG. 1. HDAC-A encodes a putative new histone deacetylase. A, the nucleotide sequence and the deduced amino acid sequence corresponding to the long ORF representing HDAC-A are given in percent. Different regions of each protein are indicated by the correspondingly boxed, underlined (absolutely conserved); conserved residues are in gray. B, schematic representations of the histone deacetylase core region. B, alignment of the HDAC-A, NY-CO-9 (HDAC-B), HDA1, and RPD3 proteins. The sequence of NY-CO-9 is not complete. Proteins were aligned with PIMA (version 1.4) and printed with BOXSHADE (version 3.21). Identical residues are in black; conserved residues are in gray. C, schematic representations of HDAC-A, NY-CO-9 (HDAC-B), HDA1, and RPD3 are aligned. Homology values refer to HDAC-A that represent additional members of this new family of human histone deacetylases.

MATERIALS AND METHODS

Reagents and Cell Lines—293 cells were grown in Opti-MEM medium (Life Technologies) supplemented with 2% fetal bovine serum (HyClone), 50 units/ml penicillin, 50 µg/ml streptomycin, and 2 µg/ml glutamine at 37 °C in a humidified 95% air, 5% CO2 atmosphere. HeLa cells were cultured on glass coverslips in Dulbecco’s modified Eagle’s medium (Life Technologies) supplemented with 2% fetal bovine serum containing 50 units/ml penicillin, 50 µg/ml streptomycin, 50 µg/ml amphotericin B, 10 µg/ml Gentamycin, 20 µg/ml cholecystokinin, and 5 µg/ml Transferrin. HeLa cells were transfected by electroporation (ElectroMax DH10B cells, Life Technologies). Transfection and electroporation were performed using the standard protocol (35, 36).

Plasmids—To generate COOH-terminal epitope-tagged constructs of different histone deacetylases, we used polymerase chain reaction amplification with a reverse primer containing the sequence for the FLAG peptide. First the following two primers were used to amplify the HDAC-A cDNA corresponding to the human glyceraldehyde-3-phosphate dehydrogenase cDNA to control for the relative amount of mRNA loaded in each lane. Relative expression levels were quantitated with a Fujix BAS1000 PhosphorImager system and Mac Bas software (Fuji Photo Film Co., Ltd.).

The abbreviations used are: kb, kilobase pair(s); PCR, polymerase chain reaction; ORF, open reading frame.
AAGCACC-3', backward, 5'-CGGAATTCCACGCTGTCATACGAG-GCC-3'; HDAC-A495–967 forward, 5'-CGGAATTCCACGCTGTCATACGAG-GCC-3'; HDAC-A544–967 forward, 5'-CGGAATTCCACGCTGTCATACGAG-GCC-3'.

Subcellular Localization of HDAC-A—HeLa cells growing on coverslips were transfected using LipofectAMINE (Life Technologies). After 48–72 h, cells were fixed with 1.0% paraformaldehyde in phosphate-buffered saline for 5 min at room temperature. Cells were permeabilized with 0.5% Triton X-100 in phosphate-buffered saline and stained as described (37). The primary antibody, anti-FLAG (M2, Sigma), was used at a 1:1000 dilution. Imaging was done on a Leica DM-R epifluorescence microscope with a 100 x or a 63 x 1.4 N.A. PlanApo lens. Images were collected digitally with a cooled 12-bit CCD (Princeton MicroMax). Digital deconvolution was performed as described previously (37).

Immunoprecipitation: Histone Deacetylase Assays—293 cells (5 x 10^7 cells) were transfected with LipofectAMINE. After 36 h, the cells were harvested and lysed in low stringency lysis buffer (50 mM Tris-HCl, pH 7.5, 120 mM NaCl, 0.5 mM EDTA, 0.5% Nonidet P-40) in the presence of a protease inhibitor mixture (Boehringer Mannheim). Protein concentrations of extracts were normalized with a modified Lowry assay (Bio-Rad). To control for expression of different constructs, Western blot analysis was performed with the enhanced chemiluminescence procedure (Amersham) as described (28). Protein G–Sepharose was preincubated with bovine serum albumin at 10 mg/ml to reduce nonspecific
binding, and extracts were precleared for immunoprecipitation as described (28). Precleared lysates were immunoprecipitated by incubation with the M2 anti-FLAG antibody (Sigma) at 10 mg/ml overnight at 4 °C. As a control, immunoprecipitations were also performed with the M2 anti-FLAG antibody after a 2-h preincubation at room temperature with a synthetic peptide corresponding to the FLAG epitope (100-fold molar excess). Immune complexes were recovered by adding 20 µl of the preblocked 50% protein G-Sepharose slurry for 4 h at 4 °C and washing

![Tissue-specific expression of HDAC-A. A, Northern blot analysis of HDAC-A was performed with mRNA isolated from different human tissues. Hybridization to human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown as control. The arrow indicates the HDAC-A mRNA; an additional band in testis and a series of weaker bands possibly corresponding to a degradation product are also indicated. Molecular weights are shown on the left. B, quantitative Northern dot blot analysis for the expression of HDAC-A. Expression levels are relative to the expression level in the tissue with maximum expression (skeletal muscle).](image)

**Fig. 2.** Tissue-specific expression of HDAC-A. A, Northern blot analysis of HDAC-A was performed with mRNA isolated from different human tissues. Hybridization to human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown as control. The arrow indicates the HDAC-A mRNA; an additional band in testis and a series of weaker bands possibly corresponding to a degradation product are also indicated. Molecular weights are shown on the left. B, quantitative Northern dot blot analysis for the expression of HDAC-A. Expression levels are relative to the expression level in the tissue with maximum expression (skeletal muscle).

**FIG. 3.** HDAC-Ap is a predominantly nuclear protein with a distinct subnuclear localization. A, a field of randomly growing HeLa cells that were transfected with an expression vector encoding FLAG-tagged HDAC-A is shown. Panel A1 shows staining with the anti-FLAG antibody. Panel A2 shows staining of DNA using DAPI. Panel A3 shows the corresponding DIC image. The cell at the bottom is in mitosis and exhibits a diffuse cellular distribution of HDAC-A–FLAG. The upper two cells are in interphase and show predominantly nuclear staining. The scale bar represents 10 µm. B, HDAC-A–FLAG accumulates in discrete foci within the interchromatin space. The nucleus of a HeLa cell transiently expressing HDAC-A–FLAG was imaged by collecting serial z-sections at 0.4-µm intervals. A single optical section near the center of the cell nucleus is shown. Panel B1 shows anti-FLAG staining before digital deconvolution. Panel B2 shows the corresponding digitally deconvolved optical section. Panel B3 shows the corresponding digitally deconvolved DAPI section. Panel B4 shows a composite image in which DAPI is green and HDAC-A–FLAG is red. The scale bar represents 10 µm.

**TABLE I**

| Synonym | Genomic location | Accession no. | Genomic sequence | dbEST |
|---------|-----------------|--------------|-----------------|-------|
| HDAC-A  | KIAA0288        | AB006626     | AA101045        | N27858 |
|         |                 |              | AA3274322       |       |
| HDAC-B  | NY-CO-9         | Chr. 17      | AF039691        | AC004150 |
|         | KIAA0600        | AB011172     | AA194873        | AA116072 |
|         |                 |              | AA428724        |       |
| HDAC-C  | Chr. 7p15-p21   |              | AC004994        | AA405905 |
|         |                 |              | AC004744        | AA287983 |
|         |                 |              | AC002410        |       |
| HDAC-D  | Chr. 12q31      |              | AC004466        | W04418 |
|         |                 |              | H09529          | N64095 |

**Human Histone Deacetylase HDAC-A**

Human sequences with homology to HDAC-A were identified using BLAST and TBLASTN algorithms searching nonredundant nucleic acid databases on the GenomNet WWW server (Human Genome Center, University of Tokyo). Accession numbers for cDNA sequences, genomic sequences, and representative EST clones are given. Sequence alignment for genomic clones of HDAC-C and HDAC-D were performed and manually aligned by using the HDAC-A sequence as template.
three times with low stringency lysis buffer, twice with lysis buffer containing 0.5 mM NaCl, and twice with histone deacetylase buffer (10 mM Tris-HCl, pH 8.0, 10 mM NaCl, 10% glycerol). For inhibition studies, the immunoprecipitated complexes were preincubated with trichostatin A (400 nM) in histone deacetylase buffer for 30 min at 4°C. Beads were resuspended in 30 μl of histone deacetylase buffer containing 20,000 cpm of an acetylated H4 peptide. Histone deacetylase activity was determined after incubation for 2 h at 37°C, as described (28).

Coimmunoprecipitation—Twenty-four hours after transfection, 293 cells (2 × 10^5) were metabolically labeled with [35S]Met, [35S]Cys. Cells were starved by a 20-min incubation in methionine- and cysteine-free Dulbecco’s modified Eagle’s medium (Life Technologies). Labeling was done in methionine- and cysteine-free 90% Dulbecco’s modified Eagle’s medium and 10% Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum containing 0.15 mCi/ml radiolabeled amino acids ([35S]Met, [35S]Cys). Cells were harvested after 16 h and lysed as described above. Lysates were divided after preclearing and immunoprecipitated with M2 antibody or preblocked M2 antibody (Sigma) for 2 h at 4°C. Protein G-Sepharose slurry (50%, 20 μl) was added for 1 h at 4°C, and immunoprecipitates were washed three times with low stringency lysis buffer, three times with the same buffer supplemented with 500 mM NaCl and 0.5% sodium deoxycholate, and once with phosphate-buffered saline. Immune complexes were eluted from the beads with phosphate-buffered saline, containing 1% SDS for 15 min at room temperature and analyzed by SDS–polyacrylamide gel electrophoresis on 10% gels. After electrophoresis, the gels were fixed, enhanced with Amplify (Amersham), dried, and exposed for autoradiography.

RESULTS

Cloning of a Novel Human cDNA Encoding a Protein with Similarity to the S. cerevisiae HDA1p—A cloning strategy was developed to identify new long cDNAs (34). Random sequencing of a size-selected (>9 kb) human brain cDNA library identified a new cDNA encoding a putative protein with striking homology to a yeast histone deacetylase, HDA1 (see Fig. 1, A and B). Sequence analysis of this cDNA, referred to as HDAC-A hereafter, revealed an open reading frame (ORF) of 2,901 base pairs encoding a putative protein of 967 amino acids (Fig. 1A). This ORF is preceded by a 1134-nucleotide untranslated 5′ region and followed by a 4415-nucleotide untranslated domain. The molecular mass of the derived protein is 105 kDa, and the calculated isoelectric point is 6.7. A stop codon (TAA) is located in-frame immediately upstream of the presumed start codon. Although, several potential initiation sites are located in the 5′-untranslated region upstream of the putative translation initiation site (data not shown), the resulting ORFs are probably too short to yield a protein in vivo. Since the deduced ORF encodes a functional protein, this mRNA belongs to a growing number of transcripts that do not use the first ATG codon to initiate translation (38).

Alignment of HDAC-Ap with other histone deacetylases, such as HDA1p and RPD3p, showed that HDAC-Ap contains two distinct domains. The COOH-terminal domain (amino acids 495–967) displayed homology to known histone deacetylases, whereas the NH2-terminal domain (amino acids 1–494) exhibited no homology to known proteins (Fig. 1B). Comparison of HDAC-Ap with HDA1p and RPD3p revealed a high degree of homology to HDA1p in the catalytical core region (43% identity and 60% similarity), whereas the homology to RPD3p in a similar region was significantly lower (25% identity and 43% homology) (Fig. 1C).

HDAC-A Belongs to a Larger Family of Similar Sequences—Using the BLAST and TBLASTN algorithms to search for other sequences with homology to HDAC-A, we identified another human cDNA, NY-CO-9. This cDNA was recently cloned as an autoantigen from serum from patients with colon cancer (39). The deduced amino acid sequence of this cDNA, which we propose to call HDAC-B, showed high overall homology (58% identity, 67% similarity) to the HDAC-A protein, particularly in the conserved catalytic histone deacetylase domain. The cDNA clone described by Scanlan and co-workers (independently isolated by us –KIAA0600) is truncated at the 5′ and 3′ extremities in comparison to HDAC-A. However, the genomic sequence of HDAC-B was identified on chromosome 17 (Human Genome Project, GenBank accession no. AC004150) and regions corresponding to the 5′ and 3′ extremities of the HDAC-A ORF were found. Therefore, we expect the full-length HDAC-B protein to be similar in size to HDAC-A. Two additional genomic sequences located on human chromosomes 7p15-7p21 and 12q31, respectively, also showed high sequence similarity to HDAC-A (Table I). Expressed sequence tags corresponding to these genomic clones were identified (Table I), and it is therefore likely that these encode additional HDAC-A homologues. We propose to call these new cDNAs HDAC-C and HDAC-D.

Differential Expression of the HDAC-A Transcript in Different Human Tissues—To explore the expression of HDAC-A in different tissues, we performed Northern blot analysis (Fig. 2A) with a radiolabeled probe corresponding to the HDAC-A cDNA. A single species of 8.4 kb was detected in all tissues examined except testis, where a smaller species of 3.4 kb was present as well. The size of this transcript is in good agreement with the size of the cloned cDNA (8459 nucleotides). A weaker band corresponding to 4.2 kb in many tissues examined might represent a degradation product of the HDAC-A mRNA. To compare HDAC-A expression in a wider array of tissues and to obtain more information on transcript abundance, we used normalized mRNA master dot blots (CLONTECH). Quantification of the dot blots showed that HDAC-A was highly expressed in the colon tissue.
in skeletal muscle, thymus, and small intestine. Heart, colon, brain, ovary, peripheral blood leukocytes, prostate, pancreas, spleen, and lung showed intermediate expression, while liver, placenta, and kidney showed very low expression (Fig. 2B).

**HDAC-A Is a Predominantly Nuclear Protein with a Distinct Subnuclear Localization**—To examine the subcellular localization of the HDAC-A protein, HeLa cells were transfected with a HDAC-A-FLAG fusion construct, and indirect immunofluorescence was performed with the anti-FLAG M2 antibody, as described (37). HDAC-Ap was found predominantly in the interphase cell nucleus (Fig. 3A). As expected, HDAC-Ap shows diffuse localization during mitosis, when the nuclear compartment is disintegrated (bottom of Fig. 3A1). In the interphase cell nucleus, HDAC-Ap is excluded from nucleoli and accumulates in discrete foci (Fig. 3B). Similar chromatin-depleted foci have been reported for the localization of endogenous HDAC1p (37). Like HDAC1p, HDAC-Ap is depleted near the periphery of the cell nucleus and excluded from heterochromatic territories.

**HDAC-A Is a Histone Deacetylase**—To determine whether HDAC-Ap possesses intrinsic histone deacetylase activity, plasmids expressing FLAG-tagged fusion proteins were transfected into 293 cells. After transfection, a new protein of approximately 120 kDa was detected by Western blots with the anti-FLAG antibody (Fig. 4B). We used the anti-FLAG antibody to immunoprecipitate HDAC-Ap and assayed for histone deacetylase activity with a synthetic peptide corresponding to the NH2-terminal tail of histone H4 as a substrate (28). Similar immunoprecipitations were performed after transfection of plasmids encoding FLAG-tagged versions of HDAC1, mHDAC2, and HDAC3 as controls. Material immunoprecipitated with the anti-FLAG antibody in cells transfected with the HDAC-A–FLAG plasmid demonstrated histone deacetylase activity (Fig. 4A). In contrast, immunoprecipitated material from cells transfected with the corresponding empty vector plasmid yielded no detectable histone deacetylase activity. No histone deacetylase activity was detected when immunoprecipitation was performed with the anti-FLAG antibody preincubated with an excess of a synthetic peptide corresponding to the FLAG epitope. As reported for other histone deacetylases, the enzymatic activity of HDAC-Ap was inhibited by trichostatin A. These results demonstrate that HDAC-Ap is a functional enzyme with histone deacetylase activity in vivo.

To map the catalytical domain of HDAC-A, we generated deletion constructs of HDAC-A either from the NH2 or COOH terminus guided by the homology to other histone deacetylases, especially HDA1 (Figs. 1A and 5A). A fragment corresponding to amino acids 495–967 of HDAC-Ap, the region homologous to HDA1p, exhibited significant histone deacetylase activity, which was reproducibly higher than the activity of the full-length protein (Fig. 5B). A fragment of HDAC-Ap corresponding to amino acids 545–967 exhibited no histone deacetylase activity, indicating that the short region between amino acids 495 and 545 is critical for enzymatic activity. As expected, the amino-terminal portion of HDAC-Ap, amino acids 1–544, had no histone deacetylase activity. The higher histone deacetylase

**Fig. 5.** Histone deacetylase activity maps to a COOH-terminal domain of HDAC-A. A, schematic representation of different constructs used to map different domains of HDAC-A. B, transfections and histone deacetylase immunoprecipitation analysis was performed as described in the legend to Fig. 4A. C, Western blot analysis of cellular extracts with anti-FLAG antibody after transfection of FLAG-tagged HDAC-A constructs. TSA, trichostatin A.
activity of the fragment containing amino acids 495–967 in comparison to the full-length protein is consistent with the possibility that the amino-terminal region of HDAC-Ap contains a negative regulatory element.

**HDAC-A Is Part of a Multiprotein Complex**—Both HDAC1p and HDAC2p are part of a large multiprotein complex, and the *S. cerevisiae* histone deacetylase HDA1p represents the enzymatic component of the ~350-kDa HDA complex in yeast (29, 30). To determine whether HDAC-Ap is also part of a multiprotein complex, we performed coimmunoprecipitation experiments after transfection of a HDAC-A–FLAG fusion construct into 293 cells. Analysis of immunoprecipitated material by SDS–polyacrylamide gel electrophoresis identified a predominant 119-kDa band, in agreement with the predicted molecular mass of HDAC-Ap of 105 kDa. Several cellular proteins were found to coprecipitate with HDAC-Ap. Preincubation of the anti-FLAG antiserum with the corresponding FLAG peptide before immunoprecipitation showed that HDAC-Ap specifically interacts with proteins with molecular masses of 290, 137, 64, 50, 27, 25, 24, and 20 kDa, (Fig. 6). The band corresponding to the amino-terminal region of HDAC-Ap contains a negative regulatory element.

**DISCUSSION**

We have cloned and analyzed a cDNA encoding the first member of a new family of human histone deacetylases. The members of this new family are more closely related to the *S. cerevisiae* protein HDA1p than to RPD3p. To distinguish this new family from a previously identified group of human histone deacetylases with highest homology to RPD3, HDAC1, 2, and 3 (9, 25–28), we propose to call the newly identified cDNAs HDAC-A, B, C, and D. HDAC-Ap is expressed in different tissues and is predominantly localized to the cell nucleus. HDAC-Ap exhibits histone deacetylase activity in vitro that maps to a COOH-terminal region of the protein.

HDAC1p and HDAC2p are thought to exert their effects as part of large multiprotein complexes. These complexes contain mSin3 (22, 40–42), the corepressors N-Cor and/or SMRT (43–45), RbpAp48 and RbpAp46 (9), SAP30 and SAP18 (41, 46, 47), and Mi-2 (48–50). They can be recruited to promoters by sequence-specific DNA-binding proteins such as Mad-Max (40, 41, 47), the unliganded nuclear hormone receptors (43–45), DP1-E2F and the Rh family of transcription factors (13, 51–53), transcriptional repressors containing a BTB/POZ domain (54, 55), Cbf1/Rbp-Rb, the mammalian homolog of Drosophila Suppressor of Hairless (56), CtBP (57), homeodomain-containing repressors like Rpx, POU domain proteins such as Pit-1 (58), and MeCP2, a methyllysine-binding protein involved in DNA methylation (reviewed in Ref. 59). The transcription factor YY1 interacts directly with HDAC2p but also with a recently identified new human RPD3 ortholog, HDAC3p (26). Despite its high degree of homology to HDAC1 and 2, HDAC3p coimmunoprecipitates with distinct factors and appears to participate in a different multiprotein complex. In yeast, two different histone deacetylase complexes (HDA and HDB) have been defined biochemically (29, 30). The ~600-kDa HDB complex is the yeast equivalent of the human HDAC1/HDAC2-containing complex, and these two complexes share several subunits, including RPD3, RbpAp48, Sin3, and SAP30 (22, 46).

HDAC-A is part of a multiprotein complex in vivo. Preliminary experiments using gel filtration and density gradient centrifugation analysis to fractionate cellular extracts indicated that HDAC-Ap segregates as a higher molecular mass species (~220 kDa on sucrose gradients and ~600 kDa or gel filtration) than predicted based on its molecular mass. These observations are consistent with the possibility that HDAC-Ap is part of a multiprotein complex in the cell nucleus.
associated with distinct biological functions (for review, see Ref. 32). The growing list of nonhistone proteins that are also regulated by acetylation, such as p53 (60, 61), EKLF (62), TFII E, and TFII F (63), also raises the possibility that so-called “histone” deacetylases might target nonhistone proteins for deacetylation. However, the nuclear localization of HDAC-Ap and its targeting to similar subnuclear granules, as observed for HDAC1, 2, and 3 (37), suggests that these proteins might be functionally related.

Future effort will be devoted to defining the factors that interact with HDAC-Ap and the enzymatic specificity of HDAC-Ap in comparison to HDAC1, 2, and 3. It is anticipated that these studies will increase our emerging understanding of the role of histone deacetylation in transcriptional regulation.

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Note Added in Proof—After this manuscript was accepted for publication, Verdel and Khochbin (64) reported the identification of related cDNAs in mice. Their cDNA called mHDA1 is highly homologous to HDAC-B reported in this manuscript, whereas their cDNA called mHDA2 does not correspond to either HDAC-A, -B, -C, or -D.

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