Isolation and Characterization of a Novel Class II Histone Deacetylase, HDAC10*

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A novel histone deacetylase, HDAC10, was isolated from a mixed tissue human cDNA library. HDAC10 was classified as a class II subfamily member based upon similarity to HDAC6. The genomic structure of HDAC10 was found to consist of 20 exons. HDAC10 has two sequence variants, HDAC10v1 and HDAC10v2, and two transcripts were detectable by Northern blot analysis. HDAC10v1 and HDAC10v2 were found to be identical through exon 17 but diverged after this exon. HDAC10v2 has an 82-bp alternate exon that generates a frameshift and shortens the sequence by 11 amino acids. In this study, the characterization of HDAC10v1 was performed. HDAC10v1 has an N-terminal catalytic domain, two putative C-terminal retinoblastoma protein binding domains, and a nuclear hormone receptor binding motif. The HDAC10v1 enzyme was found to be catalytically active based upon its ability to deacetylate a β-H-acetylated histone H4 N-terminal peptide. Immunofluorescence detection of transfected HDAC10v1-FLAG indicated that the enzyme is a nuclear protein. Furthermore, coimmunoprecipitation experiments indicated that HDAC10v1 associated with HDAC2 and SMRT (silencing mediator for retinoid and thyroid hormone receptors). In addition, based upon the public data base, a single nucleotide polymorphism was found in the C terminus of HDAC10 which changes a Gly residue to Cys, suggesting that HDAC10 molecules containing these single nucleotide polymorphisms may be folded improperly. HDAC10 extends the HDAC superfamily and adds to a growing number of HDACs that have been found to have splice variants, suggesting that RNA processing may play a role in mediating the activity of HDACs.

There are currently 16 reported human HDAC isoforms (8–11) that are divided into three classes based upon sequence homology, intracellular localization, and association with proteins that form DNA-binding complexes. HDAC1, HDAC2, HDAC3, and HDAC8 were categorized as class I based upon their similarity to the yeast gene Rpd3 (8). HDAC 4/HDAC5, HDAC5/HDACB, HDAC6, HDAC7, and HDAC9 were designated as class II, based upon their similarity to yeast gene Hda1 (9, 10). The third class of HDACs consists of seven human genes that are similar to yeast silent information regulator gene (Sir2) (13, 14). A unique characteristic of class III HDACs is their NAD+-dependent protein deacetylase and ADP-ribosylase activity (15–17).

HDACs have been found in multiprotein complexes, implicating HDACs in transcription regulation, hormone signaling, cell cycle, differentiation, and DNA repair. Class I and II HDACs were found to be components of different complexes (8–10). HDAC1 and 2 formed a core complex with retinoblastoma protein (Rb)-associated proteins (18), and this core complex was found to be associated with either mSin3 and mSin3-associated peptides or components of the NuRD complex (19–21). HDAC1 was also recently found to suppress MyoD-mediated transcription in an Rb-dependent manner (22). In addition, HDAC1 and 2 have also been found to associate indirectly with numerous other factors that are involved in cellular signaling, transcriptional repression, and DNA repair (8). Both class I and class II HDACs were found to associate with nuclear receptor corepressor (NCoR) and silencing mediator for retinoid and thyroid hormone receptors (SMRT) (23, 24). In addition, numerous direct interactions with nuclear factors have been demonstrated for class I HDACs (8). The roles of class II HDACs were reviewed recently (9, 10). Additional reports described nuclear complexes of HDAC4 and HDAC5 or HDAC7 with MEF2, a regulator of muscle myogenesis (25, 26), and HDAC9 was also found to mediate MEF2 transcriptional repression (10). The cellular localization of HDAC4, HDAC5, and HDAC7 was determined by their phosphorylation status and whether they were associated with 14-3-3 proteins (9, 10). Complexes of class III HDACs with other proteins have yet to be identified. However, studies in yeast demonstrated that like other HDACs, yeast Sir2 is involved in silencing, cell cycle regulation, DNA repair, and meiotic checkpoint control (27–30). Furthermore, the three-dimensional structure of Sir2-Af1 complexed with the cofactor NAD revealed core domains that are conserved among Sir2 proteins and are thought to function in the binding of NCoR, nuclear receptor corepressor; SMRT, silencing mediator for retinoid and thyroid hormone receptors; TSA, trichostatin A; EST, expressed sequence tag; SNP, single nucleotide polymorphism; Rb, retinoblastoma protein.

Chromatin remodeling plays a major regulatory role in transcription and DNA replication (1, 2). One model for how chromatin remodeling occurs involves ATP-dependent displacement of histones by nucleosome remodeling complexes (3, 4) and changes in the acetylation status of histones and transcription factors catalyzed by histone acetyltransferases and histone deacetylases (HDACs) (5–7).

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† The abbreviations used are: HDAC(s), histone deacetylase(s);
NAD, histones, and potentially other substrates for deacetylation (31).

The cellular localization, expression patterns, and sensitivity to HDAC inhibitors differ among HDAC classes. Class I human HDACs are predominantly nuclear proteins that are expressed ubiquitously in many human cell lines and tissues (8), whereas class II HDACs are expressed differentially (8) and shuttle between cytoplasm and nucleus (9, 10). Class I and class II HDACs are sensitive to HDAC inhibitors, whereas class III HDACs are insensitive to HDAC inhibitor trichostatin A (TSA) (8, 9, 32).

Recent evidence suggests that classes of HDAC enzymes have specific roles. For example, a genomic approach demonstrated that yeast genes are expressed differentially in response to individual deletions of Rpd3, Sir2, and Hda1 (34). Yeast HDACs were found to play distinct roles in regulating genes that control cell cycle progression, amino acid biosynthesis, and carbohydrate transport and utilization (34). Studies performed in Dro sophila demonstrated that Rpd3 plays a regulatory role in segmentation during fly development (35).

The sequencing of the human genome has led to the identification of new HDAC family members. In this study, the histone deacetylase class II subfamily member, HDAC10v1, was characterized. This HDAC10 sequence was described in a recent review of class II HDACs, but at that time it was designated HDAC9 (9) and while this manuscript was in review, was also reported as HDAC10a (12). In addition, a splice variant of HDAC10 which is different from the reported splice variant HDAC10β was also found. The catalytically active HDAC10v1 enzyme was found to be expressed in the nucleus, has two sequence variants, and is associated with HDAC2 and SMRT.

**EXPERIMENTAL PROCEDURES**

**Construction of Pan-tissue and Dorsal Root Ganglion cDNA Libraries**—Mixed tissue and dorsal root ganglion cDNA libraries were prepared from poly(A)+ RNA that was isolated and pooled from 31 human tissues or dorsal root ganglia, respectively, using a procedure from Invitrogen (36).

**Cloning of HDAC10v1 and HDAC10β**—A novel 1,156-bp open reading frame was identified using a Hidden-Markov model that was built from HDACs 1–8. This model was used in combination with Smith-Waterman alignments of HDACs 1–8 to search the human EST data base for sequences with HDAC-like functional domains and sequence similarity, respectively. This open reading frame was used to search a data base of sequenced cDNA library clones. This data base was created to catalogue the clones that are present in cDNA libraries that were prepared in house. Two clones from a cDNA library created from pooled RNA of 31 tissues (mixed tissue) and two clones from a dorsal root ganglion cDNA library were found to contain the ORF. The mixed tissue cDNA library clone, designated HDAC10v1, was determined to be the most complete cDNA (~2 kb) and the complete HDAC10v1 sequence was reconstructed based upon the comparison of sequences from the human EST and genomic data bases. A search of the human genomic data base using the HDAC10v1 cDNA sequence identified a sequence with the GenBank accession number AL022328, which contained the HDAC10v1 sequence. A second variant of this sequence had also been predicted from this genomic sequence and was subsequently found in the data base of sequenced dorsal root ganglion library clones and designated HDAC10β.

**Identification of Splice Sites within AL022328**—To identify intron-exon junctions within HDAC10v1 and HDAC10v2 and to define the genomic structure of these genes, HDAC10v1 and HDAC10v2 cDNA sequences were aligned with the genomic sequence AL022328. The region within the AL022328 sequence which aligned with the cDNAs was then searched for the conserved nucleotides that are present at the RNA splicing sites. The identification of exon-intron junctions enabled the prediction of exons and intron lengths. Exons were then displayed as alignments with the corresponding genomic sequence, splice sites were shown as overlap of these alignments, and the intron sequences were represented as gaps with lengths defined by a number within the gap.

**Functional Domain Analysis**—HDAC10v1 and HDAC10v2 peptide sequences were analyzed for the presence of potential functional domains by searching the peptides against the protein families data base on the Swissprot 39 and SP-TrEMBL 14 protein sequence data bases. Isolation and Characterization of HDAC10

| Sample | Tissue | Sex of donor | Age range of donor | Number of samples pooled |
|--------|--------|--------------|-------------------|-------------------------|
| 1      | Brain 1 | M            | 6                  | 1                       |
| 2      | Brain 2 | F            | 16–36              | 2                       |
| 3      | Cerebellum | M          | 64                 | 1                       |
| 4      | Spinal cord | M/F      | 17–72              | 31                      |
| 5      | Fetal brain | M/F     | 20–23 wks          | 8                       |
| 6      | Heart | M            | 27                 | 1                       |
| 7      | Heart | F            | 19–50              | 12                      |
| 8      | Lung  | M            | 27                 | 1                       |
| 9      | Trachea | M            | 17–70              | 84                      |
| 10     | Liver 1 | M            | 27                 | 1                       |
| 11     | Liver 2 | M            | 15–35              | 2                       |
| 12     | Fetal liver | ?       | 15–24 wks          | ?                       |
| 13     | Stomach | M/F         | 23–61              | 15                      |
| 14     | Pancreas | M            | 17–69              | 18                      |
| 15     | Colon  | M            | 35–50              | 2                       |
| 16     | Intestine | M            | 25–80              | 2                       |
| 17     | Kidney | M            | 24–59              | 8                       |
| 18     | Bone marrow | M/F     | 18–68              | 24                      |
| 19     | Spleen | M            | 22–60              | 7                       |
| 20     | Thymus | M            | 6–45               | 9                       |
| 21     | Thyroid | M/F         | 10–46              | 4                       |
| 22     | Adrenal gland | M       | 32–50              | 6                       |
| 23     | Salivary gland | M/F   | 13–78              | 43                      |
| 24     | Mammary gland | F     | 23                  | 8                       |
| 25     | Skeletal muscle | M/F | 23–56              | 10                      |
| 26     | Testis | M            | 28–64              | 25                      |
| 27     | Prostate 1 | M       | 26–64              | 23                      |
| 28     | Prostate 2 | M       | 14–60              | 10                      |
| 29     | Uterus | F            | 15–77              | 10                      |
| 30     | Placenta | M            | 22–41              | 15                      |

* Numbers following tissues represent separate samples from the same tissue type.
* M, male; F, female.

**Preparation of HDAC10v1-FLAG**—A FLAG epitope tag sequence was added to the 3′-end of HDAC10v1 by PCR. The PCR primers were 5′-ACGGGATATACATTGGTTCTG-3′ and 5′-GGGAATTCCTATTTATTATACATCTTTTTTTATAATATCCCGGTGCGACGACACCAGGTGAGGATGCCA-3′. The FLAG-tagged HDAC10v1 was reconstructed using the EcoRV site in the first primer and subcloned into the Xbal and EcoRi sites of human expression vector pCDNA3.1(+)(Invitrogen).

**HDAC10v1 Protein Expression**—HDAC10v1-FLAG or FLAG vector were in vitro translated using 1 µg of HDAC10v1-FLAG, 2 µl of [35S]methionine, and SP6 TNT Quick Coupled Transcription/Translation System (Promega, Madison, WI) according to the manufacturers’ instructions. Proteins were separated on a 7.5% SDS-PAGE gel and visualized by a PhosphorImager.

**Northern Analysis**—The distribution of HDAC10v1 and HDAC10v2 in normal and tumor human tissues was investigated using Northern blot analysis. Probes were prepared by randomly [32P]-labeling a 750-bp EcoRV/NotI pCMV580p6-HDAC10 fragment using Redi-Prime kit.
HDAC10 has two sequence variants. Panel A, Clustal W alignment of the 5′-end cDNA sequence of HDAC10 variants (HDAC10v1 and HDAC10v2). The predicted active catalytic domain (red box), predicted gene duplication generating a partial inactive catalytic domain (red box with dashed line), and two putative Rb binding boxes (green boxes), putative LXXLL binding domain (blue box), and stop codons (*) are shown. Arrows indicate the first and last conserved catalytic amino acids, based upon the three-dimensional structure of HDLP, a bacterial HDAC-like protein (44). Panel B, schematic of the genomic structure of HDAC10v1. Exons (filled boxes) and introns (lines between boxes) are shown. Box and line lengths correspond to the lengths of exons and introns, respectively. HDAC10v1 bases 1801–2022 (the predicted end of HDAC10v1) with bases 5090–5537 of genomic sequence AL022328 are aligned. Exons are shown as cDNA sequence alignments with the corresponding genomic sequence, predicted splice sites are shown as sequence overhangs preceding and following aligned sequences, and introns are represented as gaps with lengths defined by the number within the gap. Panel C, schematic of the genomic structure of HDAC10v2. Exons (filled boxes) and introns (lines between boxes) are shown. Bases 1801–1989 (the predicted end of HDAC10v2) with bases 5090–5278 of genomic sequence AL022328 are aligned. The 82-bp alternate exon sequence is boxed.

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(Amersham Biosciences, Inc.) which did not distinguish between HDAC10 variants or a 172-bp NheI/Sfi fragment of HDAC10v2, containing 82 bp unique to HDAC10v2 and 90 bp shared by HDAC10v1 and HDAC10v2. A Northern blot containing poly(A)+ RNA from 12 normal tissues (OriGene Technologies, Rockville, MD) was hybridized at 65 °C using conventional methods (39). HDAC10 transcript levels were determined by comparison with the signals obtained with a GAPDH-specific probe.

Southern Analysis—To confirm the presence of the two HDAC10 variants in mixed tissue and dorsal root ganglion libraries and to provide evidence that HDAC10v2 is an expressed gene, a combination of PCR and Southern analysis was performed. Primers 5′-CATGTGTCATGACATGACAGTACAGACGC-3′ and 5′-CAAGCCACCAGGTGAGGATCAGTGTGAGAAGCGCCCATGCTCATA-3′ were designed to span the HDAC10v2-unique 82-bp alternate exon and to amplify ~1-kb PCR products from cDNA that was prepared from the mixed tissue and dorsal root ganglion libraries using Advantage PCR reagents (CLONTECH, Inc., Palo Alto, CA). The PCR products were separated by 1% agarose electrophoresis and transferred to a nitrocellulose membrane using a standard Southern protocol (39). The membrane was then hybridized with a 32P-labeled 172-bp NheI/Sfi fragment of the sequenced plasmid containing the HDAC10v2 clone. The probe contained the 82-bp alternate exon sequence that is unique to HDAC10v2 and 90 bp 5′-prime of this sequence that is shared with HDAC10v1.

Real Time PCR—Human cell lines, H1299 human lung carcinoma, T24 bladder carcinoma, SJRH53 muscle rhabdomyosarcoma, SJSA-1 osteosarcoma, HCT116 colon carcinoma, SW2 small-cell lung carcinoma, human fibroblasts, and A549 human lung carcinoma, were obtained from American Type Tissue Culture Collection. Total RNA from cultured cell lines was isolated with the RNeasy 96 kit according to the manufacturer’s protocol (Qiagen, Valencia, CA). RNA from human tissues was purchased (CLONTECH Inc.), and the tissue sources are listed in Tables I and II. RNAs were quantified using reverse transcription-PCR on an ABI Prism Sequence Detection System. The primers used for detection of HDAC10 were 5′-GGATCCCATCTCTCTTGAGGATGATGAC3′ (forward primer), 5′-AGAAGCGCCCAAGCTGATCATA-3′ (reverse primer), and Taqman probe 5′-AGCCGTCTCTTTACTCTCTGTGCACCG-3′ and did not distinguish between the HDAC10 variants.

The Taqman Reaction System (Eurogentec, Belgium) was used with 10 ng of total RNA in a 25-μl reaction in the proportions indicated by the manufacturer but supplemented with 0.25 units/μl reverse transcriptase (MultiScribe ABI, Perkin Elmer, Branchburg, NJ) and 0.08 units/μl RNaseOUT RNase inhibitor (Invitrogen). Computations were performed using ABI sequence detection software (version 1.6.3). The reverse transcription-PCR assays were standardized with cRNAs transcribed in vitro with the T7 RNA polymerase reaction using the MaxiScript kit (AMBION Inc., Austin, TX) according to the manufacturer’s protocol. Parallel to the reverse transcription-PCR, the total amount of RNA in each reaction was quantitated in a fluorometric assay using the RiboGreen kit (Molecular Probes, Eugene, OR) according to the manufacturer’s instructions, using mammalian ribosomal RNA provided with the kit as standard.

Transfection—1 × 106 293 cells were transfected with 10 μg of either C-terminally FLAG epitope-tagged HDAC1, HDAC3, HDAC4, HDAC6, HDAC7, or HDAC10 expression vectors and pCMV4-FLAG vector (Stratagene, La Jolla, CA) or buffer (Mock) as transfection controls by electroporation.

Immunoprecipitation Assays—Immunoprecipitations were performed as described (40). Briefly, 5 × 105 293 cells grown to 50% confluence in 15-cm dishes were transfected with 30 μg of HDAC10-FLAG or HDAC1-FLAG or pCMV4-FLAG vector using Geneporter transfection kit according to the manufacturer’s instructions (Research Genetics, Huntsville, AL). 48 h after transfection, recombinant proteins were immunoprecipitated from precleared supernatant by incubation with α-FLAG M2 agarose affinity gel (Sigma) for 16 h at 4 °C or anti-HDAC2 (Santa Cruz Biotechnology) and anti-SMRT (BD Trans-
duction Laboratories, Lexington, KY) for 16 h at 4 °C followed by incubation with Sepharose A/G beads. For immunoblot analysis, the beads were washed with MSWB buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, 1 mM EDTA, 0.1% Nonidet P-40), and the proteins were separated by SDS-PAGE. Immunoblots were performed using immunoprecipitation antibodies and anti-α-FLAG M2 (Sigma) and were detected using ECL reagents (Amersham Biosciences, Inc.). HDAC activity assays were performed as described previously (41). 

5 × 10^6 293 cells grown to 50% confluence in 15-cm dishes were transfected with 30 μg of C-terminally FLAG-tagged HDAC1, HDAC3, HDAC4, HDAC6, HDAC7, HDAC10v1, or MITR/HDRP/HDACC or pCMV4-FLAG vector using Geneporter transfection kit according to the manufacturer’s instructions (Research Genetics). HDRP/MITR/HDACC is an HDAC-related protein with sequence similarity to HDAC4 and HDAC5; it is not catalytically active but forms complexes with both HDAC1 and HDAC3 and was used as a negative control (42). Immunoprecipitations were performed by incubating the precleared lysate with α-FLAG M2 agarose affinity gel (Sigma) for 24 h at 4 °C. To measure inhibition of HDAC1 and HDAC10 activity by TSA, supernatants were incubated for 20 min at 4 °C with TSA (5 nM, 50 nM) in dimethyl sulfoxide or the solvent alone. Supernatants were incubated with 1 × 10^5 cpm of an N-terminally labeled histone H4 peptide substrate [3H]Ac(H41–24)SGRG KGGKGLGKGGAKRRHVKLRD that chemically acetylated in vitro as described previously (40). The amount of [3H]acetate released was quantitated by scintillation counting.

Cellular Localization—1 × 10^5 COS-7 or 293 cells were plated onto chamber slides. Cells were transfected on the slides with 2 μg of FLAG epitope-tagged HDAC10v1 or Enabled (Ena) or FLAG vector using Geneporter2 transfection kit according to the manufacturer’s instructions. Ena is a cytoskeleton-associated cytoplasmic protein substrate of Abl tyrosine kinase that transduces the axon-repulsive function of the Roundabout receptor during axon guidance (43, 44). The cells were fixed and permeabilized as described previously (45), incubated with 25 mg/ml anti-FLAG-fluorescein isothiocyanate conjugate (Upstate Biotechnology) for 1 h, and then photographed using light and fluorescence microscopy. In addition, other wells containing 293 cells were incubated with a combination of 25 mg/ml anti-FLAG-fluorescein isothiocyanate conjugate and 100 nM TOTO3 (Molecular Probes) as a nuclear stain and imaged by confocal fluorescence microscopy.

Identification of Single Nucleotide Polymorphisms (SNPs) in HDAC10—To determine whether there were SNPs in HDAC10, the DNA sequence spanning the coding region of HDAC10 was used to search against the SNP Consortium data base (DbSNP) and public ESTs. The public EST hits from this search were aligned, and if the number of nucleotide differences were greater than or equal to two, then the sequence flanking the SNP was downloaded. Similarly, wild type sequences were identified as exact matches to the query sequence. Wild type and SNP-containing HDAC10 sequences were then aligned using Clustal W.

RESULTS

Identification and Cloning of a Novel HDAC Gene—A combination of Hidden-Markov models to identify functional domains and iterative Smith-Waterman alignments to identify sequence similarity among HDACs 1–8 was used to define a pattern that could be used to search for new HDAC sequences in the human EST data base. An open reading frame encoding a potential novel histone deacetylase was identified using this
approach. A search of human genomic sequences using the open reading frame as a query sequence identified a genomic sequence with the GenBank accession number AL022328, along with two peptides that had been predicted from this sequence. Furthermore, the sequence within the open reading frame was used to search a database of sequenced human cDNA clones. Two clones from a mixed tissue library and two clones from a dorsal root ganglion library were found to contain this sequence. The longest cDNA sequence was found in the mixed tissue library and was designated HDAC10v1, which encodes a 673-amino acid peptide.

A variant of the HDAC10v1 sequence, HDAC10v2, was also found in the human dorsal root ganglion cDNA library and upon in silico translation was consistent with the second 662-amino acid peptide predicted from the AL022328 genomic sequence. Alignment of HDAC10v1- and HDAC10v2-predicted peptides indicated that the two sequences differed at their N termini (Fig. 1A). Alignment of HDAC10 variant cDNA sequences with the genomic sequence AL022328 was used to identify the conserved sequences at exon-intron junctions in HDAC10v1 and HDAC10v2, enabling the characterization of the genomic structure of the two variants (Fig. 1, B and C). HDAC10v1 is composed of 20 exons, and HDAC10v2 contains 18 exons. Comparison of HDAC10v1 with HDAC10v2 cDNAs with genomic sequence AL022328 demonstrated that they were identical in sequence and structure through exon 17 and then diverged. An 82-bp alternate exon that extends exon 18 in HDAC10v2 also contains the sequence within exon 19, but lacks exon 20 of HDAC10v1. The insertion of the alternate exon in HDAC10v2 generates a frameshift that shortens HDAC10v2 relative to HDAC10v1 by 11 amino acids (Fig. 1A). These results suggested that HDAC10v1 and HDAC10v2 are products of alternative splicing, making HDAC10 the fourth HDAC enzyme that has been found to have alternative splice variants (10, 11).

**Predicted Rb and Nuclear Receptor Binding Motifs in HDAC10v1**—An Rb binding motif was defined previously as the amino acid sequence LXXCE, where X can be any amino acid (47). Two putative Rb binding sequences that resemble LXXCE motifs and align with the LXXCE motif in HDAC1 were found in HDAC10v1 and HDAC10v2 (Fig. 1A, green boxes). LCLLA is located between amino acids 510 and 515, and LSCIL is located between amino acids 560 and 564. The presence of LXXCE-like motifs suggests the potential for binding to or competing with proteins that bind the AB pocket of Rb and Rb-like proteins.

LXXLL motifs in p160 coactivators were found to be important for binding to liganded hormone receptors (47, 48). A putative LXXLL motif was found in HDAC10v1 and HDAC10v2 from amino acids 467–471 (Fig. 1A, blue box). The presence of two predicted Rb binding motifs and a putative receptor binding motif in HDAC10 distinguish this family member from other known HDACs and might indicate a specific function.

**HDAC10 Is Similar in Sequence to Class I and Class II HDACs**—HDACs have been classified by catalytic domain location. Class I HDACs generally have N-terminal catalytic domains. With the exception of HDAC6, which contains both N-terminal and C-terminal catalytic domains, class II HDAC catalytic domains are C-terminal. To compare the locations of catalytic domains in HDACs, predictions of the locations of catalytic domains were made for known HDAC peptides and HDAC10v1 (Fig. 2) by searching the sequences for HDAC1–10 against the Pfam database (37). A single catalytic domain was predicted in the N terminus of HDAC10v1, spanning amino acids 4–323. Interestingly, although the average length of class I HDACs is 443 amino acids, and the average length of class II HDACs is 1,069 amino acids, HDAC10v1 is 673 amino acids in length, placing this enzyme between the average sizes of class I and class II HDACs (Fig. 2).

HDACs have also been classified by sequence similarity with yeast HDACs Rpd3, Hda1, and Sir2. Alignment of the peptide sequences of HDAC10v1, yeast HDACs Rpd3, Hda1, and Sir2 demonstrated that HDAC10v1 had the highest sequence similarity with Crl3 (23%). Alignment of HDAC10v1 with human HDAC1–8 and Sir1–7 peptide sequences demonstrated that HDAC10v1 was most similar to class II human HDAC6 (37%). Furthermore, alignment of the HDAC10v1 catalytic domain with catalytic domains of class I and class II HDACs demonstrated that HDAC6 catalytic domains 1 and 2 have the most sequence similarity, 55 and 53%, respectively, with HDAC10v1.

**HDAC10 Expression**—To investigate whether both HDAC10 splice variants are expressed, a 172-bp probe containing 82 bp that was unique to HDAC10v2 and 90 bp shared by HDAC10v1 and HDAC10v2 was used to perform Southern analysis of ~1-kb fragments of HDAC10v1 and HDAC10v2 which were PCR amplified from mixed tissue and dorsal root ganglion cDNA libraries using primers that span the region containing the 82-bp unique sequence. Two PCR products differing by ~100 bp were amplified from dorsal root ganglion cDNA, consistent with the presence of both HDAC10v1and HDAC10v2 in this library (Fig. 3A). Southern analysis using the 172-bp probe confirmed that there are two species of HDAC10 cDNA present in both cDNA libraries, although they are not visible on the ethidium bromide-stained gel of the PCR amplification from the mixed tissue library (Fig. 3B).

To investigate further the expression patterns of HDAC10 splice variants, Northern blot analysis was performed using a 750-bp probe that did not distinguish among the HDAC10 variants. This analysis demonstrated that HDAC10 variants are present in normal tissues. A 3-kb transcript was present in normal testes, stomach, spleen, small intestine, placenta, lung, liver, kidney, breast, colon, and brain (Fig. 4A), whereas a second 3.5-kb transcript was detected mainly in kidney and testes. The observation that the transcripts are larger than predicted by the nucleotide sequences may be explained by the
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HDAC10 is present as two variants in mixed tissue and dorsal root ganglion cDNA libraries. Panel A, 1-kb fragments of HDAC10v1 and HDAC10v2 were PCR amplified and electrophoresed on an ethidium bromide-stained agarose gel. Panel B, the agarose gel in panel A was Southern blotted and probed with a 32P-labeled 172-bp probe containing 82 bp that are unique to HDAC10v2 and 90 bp that are present in both HDAC10 variants.

To examine HDAC10 expression levels in normal tissues compared with cell lines, real time PCR using primers that did not distinguish among HDAC10 splice variants was also used to survey the distribution and levels of HDAC10 in tissues and tumor cell lines, relative to the levels of 18 S ribosomal RNA. HDAC10 was detected at varying levels by real time PCR in a wide range of tissues (Fig. 4B), confirming the Northern blot analysis (Fig. 4A). In normal tissues, HDAC10 was detected at the highest levels (copies/ng of total RNA) in fetal brain (18,168), thymus (11,274), and cerebellum (10,903). In tumor cell lines, HDAC10 was detected at the highest level in the SJRH30 rhabdomyosarcoma cell line (15,745). These results suggest that HDAC10 is expressed differentially in some tissues at the RNA level.

**HDAC10v1 Is a Nuclear Protein**—The translated HDAC10v1 peptide sequence predicts a 72-kDa protein that was confirmed by in vitro translation (Fig. 5A). To determine the cellular localization of HDAC10v1, FLAG epitope-tagged HDAC10v1, Enabled (Ena), or FLAG vector was transfected into COS-7 and 293 cells. The FLAG epitope was detected by fluorescence immunocytochemistry and confocal microscopy (Fig. 5, B and C). Ena is a cytoskeleton-associated cytoplasmic protein and the substrate of Abl tyrosine kinase that transduces the axon-repulsive function of the Roundabout receptor during axon guidance (46, 47). As expected, Ena was detected in the cytoplasm, whereas HDAC10v1 was detected in the nuclei of COS-7 and 293 cells. The detection of HDAC10v1 in the nuclei of both cell lines suggested that HDAC10v1 is predominantly a nuclear protein (Fig. 5B). These results were confirmed by high magnification (×80) confocal microscopy (Fig. 5C).

**SNPs in HDAC10**—An additional modification that may have functional consequences is the presence of SNPs in HDAC10. To determine whether SNPs are present in the coding sequence of HDAC10, a search of SNP data bases was performed. Sequences with differences of 2 or more bps were retrieved. A potential SNP was identified in the C terminus of HDAC10. To determine whether HDAC10v1 could form complexes with proteins that were found previously to mediate transcriptional repression, communoprecipitation experiments were performed. Interactions of transfected HDAC10v1-FLAG with endogenous HDAC2 and SMRT were analyzed, and the interaction with HDAC2 and SMRT was confirmed using two approaches. Antibodies against the endogenous proteins were used for immunoprecipitation, and immunoblots were performed with FLAG antibody. Then the reverse reaction was performed, using FLAG-conjugated beads to immunoprecipitate transfected FLAG-tagged proteins, and immunoblots were performed with antibodies against the endogenous proteins. These results suggested that HDAC10v1 can form complexes with HDAC2 and SMRT (Fig. 7).

**DISCUSSION**

HDACs are members of an expanding family of enzymes that modulate chromatin structure (8–11). The sequencing of the human genome allowed us to identify an additional HDAC isoform that was designated HDAC10. The HDAC10 sequence was identified previously as an EST on chromosome 22 (50) and was subsequently annotated in the human EST data base as a sequence similar to KIAA0901 and mouse HDA2. The HDAC10 sequence was also reported in a recent review of class II HDACs (9). Until now, there were no experimental data to demonstrate that this sequence represented a catalytically active enzyme. In this study, the EST was cloned, sequenced, and designated as HDAC10v1.

**HDAC10v1 Represents a New Member of the HDAC Superfamily**—To determine whether HDAC10v1 could deacylate the histone H4 peptide substrate at a level that was equivalent to HDAC3 and HDAC4 (Fig. 6A), although HDAC1 was more active in this assay (Fig. 6B). Furthermore, to determine whether HDAC10v1 activity was inhibited by TSA, HDAC10v1 activity was measured in the presence of 5 nM or 50 nM TSA (Fig. 6C). The deacetylase activity of HDAC10v1 was decreased to background levels at 50 nM TSA, a concentration of TSA which also abrogated HDAC1 activity (data not shown).

**HDAC10v1 Associates with HDAC2 and SMRT**—To determine whether HDAC10v1 could form complexes with proteins that were found previously to mediate transcriptional repression, communoprecipitation experiments were performed. Interactions of transfected HDAC10v1-FLAG with endogenous HDAC2 and SMRT were analyzed, and the interaction with HDAC2 and SMRT was confirmed using two approaches. Antibodies against the endogenous proteins were used for immunoprecipitation, and immunoblots were performed with FLAG antibody. Then the reverse reaction was performed, using FLAG-conjugated beads to immunoprecipitate transfected FLAG-tagged proteins, and immunoblots were performed with antibodies against the endogenous proteins. These results suggested that HDAC10v1 can form complexes with HDAC2 and SMRT (Fig. 7).

**SNPs in HDAC10**—An additional modification that may have functional consequences is the presence of SNPs in HDAC10. To determine whether SNPs are present in the coding sequence of HDAC10, a search of SNP databases was performed. Sequences with differences of 2 or more bps were retrieved. A potential SNP was identified in the C terminus of HDAC10 which led to a non-conservative amino acid change at position 588 from Gly (GGC) to Cys (TGC). Five samples that were found to have this SNP were aligned with four wild type samples that all had Gly at this position (Fig. 8).

**DISCUSSION**

HDACs are members of an expanding family of enzymes that modulate chromatin structure (8–11). The sequencing of the human genome allowed us to identify an additional HDAC isoform that was designated HDAC10. The HDAC10 sequence was identified previously as an EST on chromosome 22 (50) and was subsequently annotated in the human EST database as a sequence similar to KIAA0901 and mouse HDA2. The HDAC10 sequence was also reported in a recent review of class II HDACs (9). Until now, there were no experimental data to demonstrate that this sequence represented a catalytically active enzyme. In this study, the EST was cloned, characterized, and designated as HDAC10v1.

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class IIa (HDAC4, 5 and 7) and class IIb (HDAC6 and HDAC10) based upon homology among deacetylase domains. In this study, HDAC10 was found to be most similar to HDAC6. Furthermore, HDAC10 has undergone a partial duplication event similar to HDAC6. This is based upon the high identity of HDAC10 N-terminal catalytic domain amino acids with C-terminal amino acids that are part of a second domain that was predicted to be a partial inactive catalytic domain (12). Like other HDACs, HDAC10 N-terminal catalytic domain includes conserved residues that were found previously to make up the binding pocket and charge relay systems in the bacterial HDAC-like protein HDLP which are thought to be important for HDAC activity (49, Table I). Multiple sequence alignments of HDAC catalytic domain amino acids suggested that strict conservation of some of these amino acids may not be required. These amino acid differences that are found in subsets of HDACs might offer an additional method of classifying HDACs. HDAC10v1 was demonstrated to be catalytically active, and like other class I and class II HDACs, HDAC10v1 deacetylase activity is inhibited by TSA (Fig. 3). Although HDAC10v2 has not yet been determined to be catalytically active, HDAC10v2 contains the amino acids that are predicted to be necessary for catalytic activity, and thus, HDAC10v2 is expected to be a catalytically active splice variant.

HDACs have generally been found as single transcripts based upon Northern blot analyses (8–10). However, analysis of the genomic structure of HDAC10 and a search of the human genomic data base for sequences that are similar to HDAC10 revealed that HDAC10 had two sequence variants. The presence of HDAC10v1 and HDAC10v2 in the human EST data base and sequenced cDNA clones confirmed their existence. Further evidence for multiple HDAC10 transcripts was demonstrated by the identification of two transcripts in normal tissues by Northern blot analysis (Fig. 2) and in mixed tissue and dorsal root ganglion cDNA libraries by Southern analysis (Fig. 3). These data suggested that HDAC10 variants are products of alternative splicing. While this manuscript was in review, another group also found a splice variant of this gene called HDAC10β (12). Interestingly, HDAC10β and HDAC10v2 are different sequences, indicating that HDAC10 has multiple splice variants. Furthermore, other HDACs were also recently found to have multiple splice variants, including HDAC3, HDAC7, and HDAC9 (11), suggesting that HDACs are regulated at the level of RNA processing.

**FIG. 4.** HDAC10 is expressed ubiquitously. Panel A, Northern blot analysis of normal tissues. 12 normal tissue poly(A) RNA Northern blots were probed with a 750-bp 32P-labeled fragment of HDAC10v1 or GAPDH as a control. Panel B, real time PCR analysis of the levels of HDAC10 in poly(A) RNA from normal tissues, tumor tissues, and tumor cell lines. HDAC10 levels were determined relative to 18 S ribosomal RNA in poly(A) RNA from normal human tissues and human tumor cell lines (see Table I and Table II). RNA levels were normalized to the levels of HDAC10 and 18 S ribosomal RNA. Samples with one donor are indicated by black bars, and samples with >1 donor are shown by gray bars.
Previous studies using Northern blot analysis suggested that class I HDACs are expressed ubiquitously, whereas class II HDACs have a more limited tissue distribution (8, 9). In this study, HDAC10 was found to be expressed ubiquitously (Fig. 5A). However, differential expression was found for the two HDAC10 transcripts, and increased RNA levels were observed in certain tissues. The ubiquitous expression of HDAC10 was confirmed by real time PCR (Fig. 5B). In addition, a real time PCR survey of HDAC 1, 3, 4, 5, 6, 7, 8 and 10 RNA levels in normal tissues and cell lines (data not shown) suggests that HDACs can be classified by their expression patterns in tissues relative to cell lines and demonstrated that HDAC10 is present at varying levels in a wide range of normal tissues and tumor cell lines. The expression pattern of HDAC10v1 was found to be more similar to...
mSin3A complexes with HDAC1, HDAC2, and Rb-like proteins were found to be important modulators of cellular proliferation, G1 cell cycle arrest as a result of DNA damage, cellular senescence, and contact inhibition (51–54). Rb represses E2F transcription in an HDAC1- and HDAC2-dependent manner through the interaction of LXCXE motifs in HDAC1 and HDAC2 with the LXCXE motif in Rb and/or other A/B pocket proteins (55, 56). The two LXCXE-like motifs found in the C terminus of HDAC10v1 and HDAC10v2 may suggest that HDAC10 variants can bind Rb and other LXCXE or EIXC motif-containing proteins (for review, see Ref. 56).

The structurally similar nuclear receptor corepressors SMRT and NCoR were also previously found to play a role in mSin3A-mediated repression and to associate with HDACs (8, 22, 23, 51, 57–60). HDAC3, 4, 5, and 7 associate directly through SMRT repressor domains RD2 and RD3, and HDAC1 and HDAC2 interact indirectly through the SMRT RD1 repressor domain that binds mSin3A (58, 59). HDAC3-, 4-, and 5-mediated repression occurs through contact with corepressors NCoR and SMRT (9, 10). These corepressors mediate the repression of a diverse array of signaling pathways of unliganded DNA-binding nuclear hormone receptors, including thyroid hormone receptor, retinoic acid receptor, orphan nuclear receptors, and antagonist-bound progesterone and estrogen receptors (61). NCoR and SMRT also associate with non-nuclear receptor proteins, such as homeodomain proteins Rpx2 and Pit (62). Aberrant recruitment of the NCoR-HDAC complex by retinoid receptors was found to play a role in the pathogenesis of acute promyelocytic leukemia (63). Co-immunoprecipitation experiments demonstrated that HDAC10v1 associated with SMRT. Furthermore, HDAC10v1 and HDAC10v2 contain a putative LXXLL motif that might be involved in binding to hormone receptors (47, 48). Although the flanking sequences around these motifs have been found to be loosely conserved, the flanking sequences in HDAC10 seem to be very different from other known LXXLL motifs. However, HDAC10v1 was found to be associated with HDAC2 and SMRT in complexes and thus, may...
play a role in transcriptional repression mediated by these complexes.

SNPs (for review, see Ref. 33) have been found to account for most of the genetic variation among individuals. The SNPs that are thought to be most likely to alter gene function are changes in regulatory and coding regions that generate non-conservative amino acid changes. SNPs are being evaluated as disease markers by associating higher than expected incidences of SNPs in disease-associated genes. Automated alignment and comparison of EST sequences with SNPs databases are accepted and efficient approaches to SNP identification. The search for SNPs in the coding region of HDAC10 led to the identification of a variable C terminus that may represent a SNP. A nonconservative amino acid change was found at position 588 which causes a change from Gly (GGC) to Cys (TGC) in five samples compared with four wild type samples that all contained Gly at this position. Although the SNP occurs outside of the catalytic domain, catalytic activity and/or protein-protein interactions of a catalytically active novel HDAC (HDAC10). The existence of multiple splice variants and RNA transcripts that contains Gly at this position. Although the SNP occurs outside of the catalytic domain, catalytic activity and/or protein-protein interactions of a catalytically active novel HDAC (HDAC10). The existence of multiple splice variants and RNA transcripts that contains Gly at this position. Although the SNP occurs outside of the catalytic domain, catalytic activity and/or protein-protein interactions of a catalytically active novel HDAC (HDAC10).

In summary, this study characterized the genomic structure, tissue distribution, cellular localization, and protein interactions of a catalytically active novel HDAC (HDAC10). The existence of multiple splice variants and RNA transcripts that display different tissue distributions might indicate a potential mechanism for HDAC10 specificity. Furthermore, the association of HDAC10 with HDAC2 and SMRT will enable further studies on the specific function of this HDAC isoform.

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Addendum—While this manuscript was in review, another report characterized HDAC10 (12).

REFERENCES
1. Krude, T., and Keller, C. (2001) Cell. Mol. Life Sci. 58, 665–672
2. Dave, J. R., and Spencer, V. A. J. (1999) Cell Biochem. Biophys. Suppl. 32-33, 141-148
3. Wu, J., and Gurnstein, M. (2000) Trends Biochem. Sci. 25, 619–623
4. Jones, K. A., and Kadonaga, J. T. (2000) Genes Dev. 14, 1992-1996
5. Kuzminov, A., and Reinberg, D. (2001) Curr. Top. Microbiol. Immunol. 254, 35-58
6. Sterner, D. E., and Berger, S. L. (2000) Microbiol. Mol. Biol. Rev. 64, 435-459
7. van Holde, K. E. (1988) “Biological Modification.” Springer, New York
8. Gray S. G., and Ekstrom, T. D. (2003) Exp. Cell. Res. 292, 75-83
9. Bertos, R. Wang, A. H., and Yang, X.-J. (2001) Biochem. Cell Biol. 79, 243–252
10. Fischle, W., Kiermer, V., Dequiedt, F., and Verdine, E. (2001) Biochem. Cell Biol. 79, 337–348
11. Zhou, X., Marks, P. A., Rifkind, R. A., and Richon, V. M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 145–145
12. Kao, H.-Y., Lee, C.-H., Komarov, A., Han, C. C., and Evans, R. M. (2002) J. Biol. Chem. 277, 187–193
13. Aftab, G., and Murnane, J. P. (1999) Gene (Amst.) 234, 161–168
14. Frye, R. A. (1999) Biochem. Biophys. Res. Commun. 260, 273–279
15. Frye, R. A. (2000) Biochem. Biophys. Res. Commun. 273, 793–798
16. Imai, S., Armstrong, C. M., Kaebelstein, M., and Guarrante, L. (2000) Nature 403, 785–800
17. Landry, S., Silman, J. A., and Sternagran, R. (2000) Biochem. Biophys. Res. Commun. 278, 685–690
18. Tomoy, J. C., and Mouded, M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 145–145
19. Zhang, Y., Iratni, R., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (1997) Cell 89, 357–364
20. Kasten, M. M., Dorland, S., and Stillman, D. J. (1990) Mol. Cell. Biol. 10, 4528–4538
21. Zhang, Y., Sun, Z. W., Iratni, R., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (1998) Mol. Cell. 1, 1021–1031
22. Puri, P. L., Lenz, S., Steigler, P., Tschler T.-T., Schlitz, R. L., Muscat, G. E. O., Giordano, A., Kedes, L., Wang, J. Y. J., and Sartoretti, V. (2001) Mol. Cell, 885–897
23. Zhang, Y., Ng, H. H., Erdjument-Bromage, H., Tempst, P., Bird, A., and Reinberg, D. (1999) Genes Dev. 13, 1924–1935
24. Kao, H. Y., Mansfield, D., Mendez, D., and Evans, R. M. (2000) Genes Dev. 14, 55–66
25. Alland, L., Huhle, R. H., Potes, J., Chin, L., Schreiber-Agus, N., and DePinho, R. A. (1997) Nature 387, 49–55
26. Lu, J., McKinsey, T. A., Zhang, C. L., and Olson, E. N. (2000) Mol. Cell, 233–244
27. Dressel, U., Bailey, P. J., Wang, S. C., Downes, M., Evans, R. M., and Muscat, G. E. (2001) J. Biol. Chem. 276, 17007–17013
28. Li, Y. C., Cheng, T. H., and Gartenberg, M. R. (2001) Science 291, 650–653
29. Tsukamoto, Y., Kato, J., and Ikeda, H. (1997) Nature 388, 900–903
30. San-Segundo, P. A., and Roeder, G. S. (1999) Cell 97, 313–324
31. Landry, J., Sutton, A., Tafrov, S. T., Hellen, C. U. J., and Stillman, D. J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 5807–5811
32. Min, J., Landry, J., Sternglanz, R., and Xu, R.-M. (2001) Cell 105, 269–279
33. Isakson, A., Landegren, U., Syvanen, A.-C., Bork, P., Stein, C., Ortigao, F., and Brookes, A. J. (2000) Eur. J. Hum. Genet. 8, 154–158
34. Mannervik, M., and Levine, M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6797–6801
35. Gerard, G. F., Schmidt, B. J., Ketwitz, M. L., and Campbell, J. H. (1992) Focus 14, 91–93
36. Broman, A., Birney, E., Durbin, R., Eddy, S. R., Finn, R. D., and Sonnhammer, E. L. L. (1999) Nucleic Acids Res. 27, 260–262
37. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1993) Curr. Protocols Mol. Biol. 23, (Suppl. 1) 4.0.1–4.9
38. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1993) Curr. Protocols Mol. Biol. 26, (Suppl. 1) 2.9.1–2.9.2
39. Grozinger, C. M., Haseg, C. A., and Schierle, S. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4983–4988
40. Emiliani, S., Fischle, W., Van Lint, C. A., and Verdin, E. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2785–2780
41. Zhou, X., Richon, V. M., Rifkind, R. A., and Marks, P. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1056–1061
42. Grozinger, C. M., Haseg, C. A., and Schierle, S. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 275–283
43. Bernier, M. R., Kalkhoven, E., Haare, S., and Parker, M. G. (2001) Nature 403, 733–736
44. McNeary, E. M., Rose, D. W., Flynn, S. E., Westin, S. Mullen, T. M., Pollen, A., Inoustra, J., Torchia, J., Noble, R. T., Assa-Munt, N., Millburn, M. V.,
Glass, C. K., and Rosenfeld, M. G. (1998) *Genes Dev.* **12**, 3357–3368

49. Finnin, M. S., Donigian, J. R., Cohen, A., Richon, V. M., Rifkind, R. A., Marks, P. A., Breslow, R., and Pavletich, N. P. (1999) *Nature* **401**, 188–193

50. Dunham, I., Shimizu, N., Roe, B. A., Chissoe, S., Hunt, A. R., Collins, J. E., Bruskiewich, R., Beare, D. M., Clamp, M., Smink, I. J., Ainscough, R., Almeida, J. P., Babbage, A., Bagguley, C., Bailey, J., Barlow, K., Bates, K. N., Beasley, O., Bird, C. P., Blakey, S., Bridgeman, A. M., Buck, D., Burgess, J., Burrell, W. D., O'Brien, K. P., et al. (1999) *Nature* **402**, 489–495

51. Hassig, C. A., Fleischer, T. C., Billin, A. N., Schreiber, S. L., and Ayer, D. E. (1997) *Cell* **89**, 341–347

52. Luo, R. X., Postigo, A. A., and Dean, D. C. (1998) *Cell* **92**, 463–473

53. Magnaghi-Jaulin, L., Groisman, R., Naguibneva, I., Robin, P., Lorain, S., Le Villain, J. P., Trealen, F., Trouche, D., and Harel-Bellan, A. (1998) *Nature* **391**, 601–605

54. Brehm, A., Miska, E. A., McCance, D. J., Reid, J. L., Bannister, A. J., and Kouzarides, T. (1998) *Nature* **391**, 597–601

55. Dahiya, A., Gavin, M. R., Luo, R. X., and Dean, D. C. (2001) *Mol. Cell. Biol.* **20**, 6799–6805

56. Ayer, D. E. (1999) *Trends Cell Biol.* **9**, 193–198

57. Heinzel, T., Lavinsky, R. M., Mullen, T. M., Soderstrom, M., Laherty, C. D., Torchia, J., Yang, W. M., Brzoz, G., Ngo, S. D., Davie, J. R., Seto E., Eisenman, R. N., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1997) *Nature* **387**, 43–48

58. Nagy, L., Kao, H. Y., Chakravarti, D., Lin, R. J., Hassig, C. A., Ayer, D. E., Schreiber, S. L., and Evans, R. M. (1997) *Cell* **89**, 373–380

59. Huang, E. Y., Zhang, J., Miska, E. A., Guenther, M. G., Kouzarides, T., and Lazar, M. A. (2000) *Genes Dev.* **14**, 45–54

60. Wen, Y. D., Perisic, V., Stasewski, L. M., Yang, W. M., Krones, A., Glass, C. K., Rosenfeld, M. G., and Sets, E. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 7292–7297

61. Aranda, A., and Pascual, A. (2001) *Physiol. Rev.* **81**, 1269–1304

62. Xu, L., Lavinsky, R. M., Dasen, J. S., Flynn, S. E., McInerney, E. M., Mullen, T. M., Heinzel, T., Szeto, D., Korzus, E., Kurokawa, R., Aggarwal, A. K., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1998) *Nature* **395**, 301–306

63. Minucci, S., and Pelicci, P. G. (1999) *Semin. Cell Dev. Biol.* **10**, 215–225
Isolation and Characterization of a Novel Class II Histone Deacetylase, HDAC10
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