Acetylation of histone core particles plays an important role in modulating chromatin structure and gene expression. The acetylation status of the histone tails is determined by two opposing enzymatic activities, histone acetyltransferases and histone deacetylases (HDACs). Here we describe the isolation and characterization of HDAC10, a novel class II histone deacetylase. Molecular cloning and Northern blot analyses reveal that the HDAC10 transcript is widely expressed and subjected to alternative splicing. HDAC10 is both nuclear and cytoplasmic, a feature reminiscent of HDACs 4, 5, and 7. Distinct from other family members, HDAC10 harbors an amino-terminal catalytic domain and a carboxyl pseudo-repeat that shares significant homology with its catalytic domain. Mutational analysis reveals that transcriptional repression by HDAC10 requires its intrinsic histone deacetylase activity. Taken together, HDAC10 represents a distinct HDAC that may play a role in transcription regulation.

The ability to modify chromatin structure, either locally or globally, is a critical feature of signal transduction pathways and gene regulation. Accordingly, the amino-terminal tails of histones are targets for several modifications including methylation, phosphorylation, and acetylation (1, 2). Recent studies suggest that the amino-terminal tails of histones play a critical role in modulating chromatin structure and hence, regulation of gene expression (3–7). Acetylation of histone tails is thought to create an open chromatin structure, thereby promoting the accessibility of transcription factors (7). Consistent with this view, histone acetylation at specific lysines is associated with transcriptional activation, whereas deacetylated histones are found in transcriptionally silent regions. The acetylation status of the histone tails is determined by the interplay between histone acetyltransferases (HATs) and histone deacetylases (HDACs). The identification of HATs and HDACs represents a critical step toward our understanding of transcription regulation.

To date, three classes of HDACs exist that can be distinguished by size, catalytic domain, subcellular localization, and mode of action. Mammalian class I HDACs including HDACs 1, 2, 3, and 8 resemble the yeast global transcriptional regulator Rpd3. Class II HDACs (HDACs 4–7) are similar to yeast Hda1. HDACs 4, 5, and 7 harbor a highly conserved carboxyl-terminal catalytic domain and constitute a subfamily, whereas HDAC6 is distinct in that it contains a duplicated catalytic domain (8, 9). The prototype of class III HDACs is the yeast Sir2 protein whose enzymatic reaction appears to be unique and requires the cofactor, NAD+ (10). The enzymatic activity of class I and class II HDACs can be efficiently blocked by the treatment of trichostatin A, whereas that of class III HDACs is trichostatin A-insensitive (10).

Both class I and class II HDACs function, in part, through direct or indirect association with transcriptional corepressors, such as SMRT, nuclear receptor corepressor, and mSin3A (11–17). In several instances, HDACs have been shown to directly interact with sequence-specific DNA-binding transcription factors and repress their activity (18). The class I HDACs are nuclear proteins that are generally small in size ranging from 40 to 55 kDa and are expressed ubiquitously. In contrast, class II HDACs are larger in size ranging from 100 to 130 kDa and can shuttle between the nucleus and cytoplasm (19–21). The nuclear export of class II HDACs depends on highly conserved serine residues among class II HDACs and their association with 14-3-3 proteins (22–26). Furthermore, the expression patterns of HDACs 4, 5, and 7 are tissue-specific with heart, lung, and skeletal muscle showing the greatest abundance of mRNA (8, 9, 17, 27, 28). Indeed, class II HDACs have been shown to regulate the activity of myocyte enhancer factor 2, a protein family involved in muscle differentiation as well as heart development (19, 21, 29–32). These three HDACs also interact with Fox virus and zinc finger domain-containing factors, such as BCL6 and PLZF, and may mediate their repression activity (33).

Here we describe the isolation of HDAC10 and characterization of its biochemical properties. Molecular cloning and North-
ern blot analyses reveal that human HDAC10 is subjected to alternative splicing, generating at least two spliced isoforms, HDAC10/H9251 and HDAC10/H9252. We find that the tissue distribution of HDAC10 is distinct from that of other HDACs and shows by fluorescence microscopy that HDAC10/H9251, similar to HDACs 4, 5, and 7, can be nuclear or cytoplasmic. HDAC10/H9251 harbors intrinsic HDAC activity, which is essential for its associated repression activity. Taken together, we conclude that HDAC10 is a novel histone deacetylase, which plays a role in transcriptional regulation.

EXPERIMENTAL PROCEDURES

Cell Cultures—HEK293, NIH-3T3, and A549 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum along with penicillin-streptomycin antibiotics.

Isolation of HDAC10 cDNAs and Plasmid Construction—Human HDAC10 was isolated by PCR reactions using 5’-primer (5’-CATGAATTCGGTACCATGGGGACCGCTTGTGTACC-3’) and 3’-primer (5’-CATTCTAGAGTCGACTCAAGCCACCAGGTGAGGATGGC-3’) according to the putative HDAC10 sequence deposited in GenBank (GenBank™ accession number CAB63048). A cDNA library derived from human hepatocellular carcinoma cell line HepG cells was used as a template for PCR amplification (Bioline). PCR products were digested with EcoRI/SalI and ligated with EcoRI/SalI-linearized pVP16 (Promega). The DNA sequence was confirmed by the dideoxy-sequencing method. Full-length HDAC10 was isolated from pVP16-HDAC10 by EcoRI/SalI digestion and subcloned into CMX-FLAG, CMX-Gal4, and CMX-1Y to create CMX-FLAG-HDAC10, CMX-Gal4-HDAC10, and CMX-1Y-HDAC10. Truncation, deletion, and mutation constructs were generated by PCR and subcloned into vectors, CMX-Gal4 (a mammalian expression vector harboring the yeast Gal4 DNA-binding domain) (17) and CMX-1Y, a yellow fluorescence protein (YFP) expression vector (17). Site-directed mutagenesis was carried out with the QuickChange mutagenesis kit according to instructions by the manufacturer (Stratagene). The resulting mutants were confirmed by sequencing. Sequence analyses were performed using the DNASTAR program and the BLAST program from NCBI. Mouse HDAC10 EST clone (GenBank™ accession number AI323102) was requested from Genome System, Inc. Sequence analyses and the prediction of open reading frames were carried out using the DNASTAR program. CMX-HA-HDAC10, CMX-HA-HDAC7, and CMX-FLAG-HDAC1 have been reported previously (34). HDAC4 was generated by a PCR reaction using pBJ5-HDAC4-FLAG, a gift from Dr. Schreiber, as a template and cloned into a HA-tagged vector, CMX-1H, to create CMX-HA-HDAC4.

RNA Isolation and Northern Blot Analyses—Mouse tissues were from 3-month-old C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME), and RNA was isolated using TRIzol reagent (Invitrogen). All

**FIG. 1.** HDAC10 is a new member of class II HDACs. A, amino acid sequence alignment between human HDAC10α (GenBank™ accession number AF407272), human HDAC6, and Drosophila HDAC6. Conserved residues are shaded. The missing 20 amino acids (253–272) in HDAC10β are indicated by broken lines. The partially duplicated regions (rep-1 and rep-2) of HDAC10α are flanked by arrows. Putative nuclear receptor interacting motifs are underlined. Putative nuclear export sequences (NES) are indicated by dotted lines on top of the sequences. B, sequence alignment between partially duplicated regions, rep-1 and rep-2. The identical amino acids between repeats 1 and 2 are shown. C, schematic representation of class II HDACs. Histone deacetylase domain is shown in gray. The degree of identity and similarity between HDAC10 (100%) and individual class II HDACs are shown. D, sequence alignment between partially duplicated regions, rep-1 and rep-2. The identical amino acids between repeats 1 and 2 are shown.
samples were separated on 1% agarose gels. Hybridization was carried out at 42 °C with 50% formamide. Human tissue and cancer cell blots were purchased from CLONTECH. Northern blots were probed with a full-length HDAC10 cDNA. An EcoRI fragment containing 1.6-kb mouse HDAC10 cDNA was used as a probe to analyze the mouse tissue blot. Northern blot analyses were performed using hybridization and washing protocols according to a previously published report (35).

Green Fluorescence Microscopy—NIH-3T3 cells were plated onto 2-well chamber slides (Nunc) and transfected using Lipofectin (Invitrogen). After 36 h, cells were washed in 1/100 phosphate-buffered saline and fixed in 3% paraformaldehyde. Cells were washed three times with 1/100 phosphate-buffered saline. DAPI was applied to the samples after the final wash to visualize nuclei. Images were visualized using a LEICA fluorescence microscope equipped with a camera.

Transient Transfection—A549 cells (60–70% confluence, 48-well plate) were cotransfected with 16.6–66.6 ng of pCMX-GAL4 and pCMX-GAL4-HDAC10 constructs, 100 ng of pMH100-TK-luciferase, and 100 ng of pCMX-LacZ in 200 µl of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum by the DOTAP-mediated procedure (17). After 24 h, the medium was replaced. Cells were harvested and assayed for luciferase activity 36–48 h after transfection. The luciferase activity was normalized to the level of β-galactosidase activity. Each transfection was performed in triplicate and repeated at least three times.

Coimmunoprecipitation—Coimmunoprecipitation was carried out according to a published protocol (17). 10-cm plates of HEK293 cells were transfected with 10 µg of the appropriate plasmids using Targenfect F1 (Targeting Systems, San Diego, CA). Cells were harvested 48 h later and lysed in NET-N buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 10% glycerol, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitors mixture (Roche Molecular Biochemicals)) for 15 min at 4 °C, scraped, and centrifuged for 15 min at 13,000 rpm. The supernatant was kept as whole cell extract. After preclearing by incubation with protein A/G-agarose (Santa Cruz Biotechnologies), immunoprecipitations were carried out using either HA-agarose (Santa Cruz Biotechnologies) or M2-agarose (Sigma) and were allowed to proceed for 2 h at 4 °C. Beads were washed 3–4 times in lysis buffer without Triton X-100 for histone deacetylase assays and in 1/100 phosphate-buffered saline with 0.1% Nonidet P-40 for coimmunoprecipitations. For coimmunoprecipitations, samples were heat denatured in SDS loading buffer, separated on SDS-PAGE gels, transferred to a nitrocellulose membrane, and probed with appropriate antibodies.

Histone Deacetylase Assays—Histone deacetylase assays were performed according to a described protocol (17). Briefly, 60,000 cpm of [3H]-histones was incubated with FLAG antibody immunoprecipitates for 2 h at 37 °C. Reactions were stopped by the addition of acetic acid/HCl to a final concentration of 0.12 mol/l.072 mol and extracted with two volumes of ethyl acetate. Samples were centrifuged, and supernatants were analyzed in a scintillation counter. Each reaction contained approximately one-third of lysates harvested from 10-cm plates of cells.

RESULTS

Isolation of Human and Mouse cDNAs Encoding HDAC10—To identify novel histone deacetylases, we carried
out BLAST analyses against the histone deacetylase domain of mouse HDAC7. This analysis led to the identification of a putative human HDAC (GenBank™ accession number CAB63048) encoding a predicted 673 amino acid protein with an amino-terminal catalytic domain. Full-length cDNA was isolated from a cDNA library derived from human hepatocel-

**Fig. 2.** Tissue distribution of HDAC10. A, human HDAC10 is highly expressed in liver, spleen, and kidney. The human tissue blot was probed with a $^{32}$P-labeled full-length HDAC10 cDNA fragment. Transcripts of 2.8 and 3.5 kb were detected in most tissues. In some tissues, such as skeletal muscle, a larger transcript was also observed. B, a cancer cell line blot was examined together with a human tissue blot. Cancer cell lines, including HL60, S3, K-562, Raji, and SW480, predominantly express a single transcript. C, tissue distribution of mouse HDAC10. The mouse blots were probed using an EcoRI fragment from the mouse HDAC10 EST clone as described under “Experimental Procedures.”

**Fig. 3.** HDAC10 is both nuclear and cytoplasmic in NIH-3T3 cells. A, putative NESs in HDAC10a. The conserved leucine residues are underlined. B, expression constructs of YFP-HDAC10 were transiently transfected into NIH-3T3 cells. 36 h later, the subcellular localization of HDAC10 was examined by fluorescence microscopy. Left panel, full-length HDAC10; middle panel, HDAC10 (1-349); right panel, HDAC10 (339–669). Top panels are YFP-HDAC10. Bottom panels are DAPI staining. C, HDAC10 does not associate with HDACs 4, 5, or 7. FLAG-HDAC10 and HA-HDACs 4, 5, and 7 were expressed either alone or together in HEK293 cells. After 48 h, whole cell extracts were prepared and fractionated on SDS-PAGE followed by Western blot analyses with either anti-FLAG (lanes 15–21) or anti-HA (lanes 22–28) antibodies. Alternatively, whole cell extracts were incubated with anti-FLAG antibody-conjugated beads, and bound fractions were washed and resolved on SDS-PAGE followed by Western blot analyses probed with anti-FLAG (lanes 1–7) or anti-HA (lanes 8–14) antibodies.
Novel Class II Histone Deacetylase HDAC10

Tissue Distribution of HDAC10—Unlike class I HDACs, the expression of class II members appears to be tissue-specific (8, 9, 17, 27, 28). Northern blot analyses revealed human HDAC10 to be highly expressed in the liver, spleen, and kidney (Fig. 2A) with two transcripts of 2.8 and 3.5 kb observed in many tissues. HDAC10 was also expressed in most of the cancer cell lines examined (Fig. 2B). The mouse homologue was similarly expressed (Fig. 2C), showing at least two transcripts.

HDAC10 Is Localized in the Nucleus and Cytoplasm—Whereas class I HDACs are primarily nuclear, class II HDACs have been found to shuttle between the nucleus and cytoplasm. The fact that HDAC10 contains four copies of putative NESs suggests that HDAC10 may shuttle between the nucleus and cytoplasm (Fig. 3A). To examine the subcellular localization of HDAC10, we generated a construct encoding HDAC10 fused to the carboxyl terminus of YFP. YFP-HDAC10 was expressed in NIH-3T3 cells, and its subcellular localization was examined under fluorescence microscopy. Interestingly, HDAC10 was localized in both the nucleus and cytoplasm (Fig. 3B, left panel). This observation is reminiscent of the subcellular localization of HDACs 4, 5, and 7. Furthermore, the amino-terminal catalytic domain (1–349) (middle panel) and the carboxyl-terminal region (339–669) (right panel) of HDAC10 were also localized in both the nucleus and cytoplasm.

The similar subcellular localization of HDAC10 prompted us to examine its potential association with HDACs 4, 5, or 7. FLAG-HDAC10 and HA-HDACs 4, 5, or 7 were either singly expressed or coexpressed in HEK293 cells, and immunoprecipitation experiments were carried out using anti-FLAG antibody-conjugated agarose beads. As shown in Fig. 3C, whereas FLAG-HDAC10 was efficiently precipitated by anti-FLAG antibody-conjugated beads (lanes 4–7), HDACs 4, 5, or 7 were not coprecipitated (11–14). As controls, Western blots of whole cell extracts showed good expression of all HDACs tested (lanes 15–28). Based on these results, we conclude that HDAC10 does not associate with HDACs 4, 5, or 7.

Deacetylase Activity—To determine whether HDAC10 harbors intrinsic histone deacetylase activity, FLAG-HDAC1 and FLAG-HDAC10 were transiently transfected into HEK293 cells. Whole cell extracts were prepared and immunoprecipitated with FLAG antibody-conjugated agarose beads. Western blot analyses were conducted to confirm the presence of FLAG-HDAC1 and HDAC10 in whole cell extracts (Fig. 4A, lanes 2 and 3) and in the immunoprecipitates (lanes 4 and 5). As a control, a mock transfection with the vector alone was processed in parallel with the HDAC1 and HDAC10 samples (lanes 1 and 4). The immunoprecipitates were incubated with purified 3H-acetate-labeled histones, and the amount of released 3H-acetate was measured by scintillation counting. Whereas the control extracts contained background levels of HDAC activity, the HDAC10 immunocomplex exhibited a 9-fold higher activity than the control (Fig. 4B, lanes 1 and 3). The FLAG-HDAC1 immunocomplex exhibited even higher HDAC activity. We concluded that HDAC10 harbors intrinsic HDAC activity (lane 2).

Transcriptional Repression—Both class I and class II HDACs are able to repress basal transcription in transient transfection assays, presumably through their associated HDAC activities. HDAC10 was fused to the DNA-binding domain of Gal4 to determine repressor activity. A reporter plas-
HDAC10 may be ubiquitous with the highest expression in the liver, spleen, and kidney. Intriguingly, many cancer cell lines predominantly express a 2.8-kb transcript. Further investigation is underway to explore the significance of this observation. Sequence analyses of a mouse EST clone revealed a possible additional isoform of mammalian HDAC10 that lacks an intact deacetylase domain. This clone contains seven putative open reading frames and therefore may be a pseudogene. As with the human isoform, mouse HDAC10 is widely expressed in many tissues.

Transcriptional repression by nuclear hormone receptors is mediated through their association with SMRT and nuclear receptor corepressor, which recruit histone deacetylase complexes containing both class I and class II HDACs (17). The association of nuclear hormone receptors with corepressors is mediated through the signature motif (I/L)XX(I/V)X, which is similar to the (IV/L)XX(I/V)X(I/V)L motif in HDAC10. However, yeast two-hybrid assays detect no interaction between HDAC10 and the ligand binding domains of the retinoic acid receptor and thyroid hormone receptor (data not shown). Nonetheless, this does not rule out the possibility that HDAC10 may interact with other nuclear receptors.

Fluorescence microscopy studies indicated that HDAC10 can be nuclear and/or cytoplasmic, a feature similar to that of HDACs 4, 5, and 7. Our sequence analyses suggested that two copies of putative NESs are present in the amino terminus (1–349) and carboxyl terminus (339–669) of HDAC10, respectively. This finding is inconsistent with our observation that these two fragments can also localize in the nucleus and cytoplasm. It has been suggested that HDACs function in the nucleus and that nuclear export is a simple yet effective way to inactivate HDAC (28). However, the functional roles for HDACs in the cytoplasm can not be excluded.

Using transient transfection assays, we showed that an HDAC10 fusion potently represses basal transcription from a Gal4-thymidine kinase promoter construct. The amino-terminal HDAC domain alone is able to repress transcription, although the carboxyl-terminal domain can repress approximately 3–4-fold. Alanine substitutions of several residues conserved in other HDACs (corresponding to amino acids Asp-170, Asp-172, and His-195 of HDAC10) eliminate the amino-terminal repression activity (34, 45). These observations reveal that both the carboxyl and amino terminii are required for maximal repression.

HDACs are often found as part of multi-component complexes. HDAC1 and HDAC2 copurify with mSin3A and Mi-2 (14, 15, 46–48), and HDAC3 associates with transcriptional corepressors and possibly with HDAC4 and HDAC5 (28, 49–52). In an attempt to examine interactions between HDAC10 and other class II HDACs, we found no evidence of an association between HDAC10 and HDACs 4, 5, or 7. This observation suggests that HDAC10 has distinct protein association properties and thus may participate in new ways to control transcription.

Acknowledgments—We thank the sequencing facilities at the Salk Institute, Drs. David Samols, Cheng-Ming Chiang, and Ruth Yu for critical comments on the manuscript.

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**DISCUSSION**

In this study, we described the isolation of HDAC10, a novel mammalian class II histone deacetylase with transcriptional repression activity. Human HDAC10 encodes at least two and possibly more differentially spliced isoforms. The largest HDAC10 isoform encodes a protein of 669 amino acids and contains an amino-terminal histone deacetylase catalytic domain. Similar to other class II HDACs, HDAC10 can localize to either the nucleus or cytoplasm.

Northern blot analyses indicated that the expression of
