



Histone Deacetylase 1 Phosphorylation Promotes Enzymatic Activity and Complex Formation

Citation

Pflum, Mary Kay H., Jeffrey K. Tong, William S. Lane, and Stuart L. Schreiber. 2001. "Histone Deacetylase 1 Phosphorylation Promotes Enzymatic Activity and Complex Formation." *Journal of Biological Chemistry* 276 (50): 47733–41. <https://doi.org/10.1074/jbc.m105590200>.

Permanent link

<http://nrs.harvard.edu/urn-3:HUL.InstRepos:41467390>

Terms of Use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA>

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. [Submit a story](#).

[Accessibility](#)

Histone Deacetylase 1 Phosphorylation Promotes Enzymatic Activity and Complex Formation*

Received for publication, June 18, 2001, and in revised form, September 28, 2001
Published, JBC Papers in Press, October 15, 2001, DOI 10.1074/jbc.M105590200

Mary Kay H. Pflum^{‡§}, Jeffrey K. Tong[‡], William S. Lane[¶], and Stuart L. Schreiber^{‡||}

From the [‡]Department of Chemistry and Chemical Biology and [¶]Harvard Microchemistry Facility, Harvard University, Cambridge, Massachusetts 02138

Accessibility of the genome to DNA-binding transcription factors is regulated by proteins that control the acetylation of amino-terminal lysine residues on nucleosomal histones. Specifically, histone deacetylase (HDAC) proteins repress transcription by deacetylating histones. To date, the only known regulatory mechanism of HDAC1 function is via interaction with associated proteins. Although the control of HDAC1 function by protein interaction and recruitment is well predated, we were interested in exploring HDAC1 regulation by post-translational modification. Human HDAC1 protein was analyzed by ion trap mass spectrometry, and two phosphorylated serine residues, Ser⁴²¹ and Ser⁴²³, were unambiguously identified. Loss of phosphorylation at Ser⁴²¹ and Ser⁴²³ due to mutation to alanine or disruption of the casein kinase 2 consensus sequence directing phosphorylation reduced the enzymatic activity and complex formation of HDAC1. Deletion of the highly charged carboxyl-terminal region of HDAC1 also decreased its deacetylase activity and protein associations, revealing its requirement in maintaining HDAC1 function. Our results reinforce the importance of protein associations in modulating HDAC1 function and provide the first step toward characterizing the role of post-translational modifications in regulating HDAC activity *in vivo*.

Although 30,000–40,000 genes exist in a human cell, only a fraction of those genes are transcribed into mRNA and ultimately translated into the encoding protein in any given cell type (1, 2). Transcription is regulated at many levels to selectively express only those proteins necessary for proper cell function. Although the role of DNA-binding transcription factors in controlling gene expression is well established, an additional level of regulation has been recently elucidated, the accessibility of genomic DNA to transcription factors (3).

The genome is tightly packed into chromosomes through nucleosomal protein-DNA complexes. Each nucleosome contains ~200 base pairs of double-stranded DNA wrapped twice around a histone protein core (4). Although the carboxyl-terminal region of the histones are involved in forming the nucleo-

some core, the amino-terminal, lysine-rich tails are located outside of the nucleosome core (5). Various studies revealed a correlation between the acetylation of amino-terminal lysine residues on histones and transcriptional activity in chromatin (3, 6). Therefore, the current model of transcriptional control includes DNA accessibility due to acetylation of histones.

The acetylation state of histones is dependent on the activity of two proteins, histone acetyltransferase and histone deacetylase (HDAC),¹ which acetylate and deacetylate histones, respectively. Loss of HDAC activity using small molecule inhibitors correlates with the hyperacetylation of nucleosomal histones (7, 8). Importantly, HDAC proteins in yeast and humans are necessary to achieve the full transcriptional repression and induction of diverse genes (9, 10).

Not surprisingly, HDAC proteins are critical in fundamental cellular events, including cell cycle control, differentiation, and cancer formation (11). A small molecule inhibitor of HDAC function, trichostatin, causes cell cycle arrest in mammalian cells at both G₁ and G₂ phases, while overexpression of HDAC1 in mouse cells results in reduced growth rate due to lengthening of G₂ and M phases (12, 13). Trichostatin induces terminal differentiation of murine erythroleukemia cells (8, 14) and induces apoptosis in neural, lymphoid, and colorectal cancer cell lines (15, 16). Finally, HDAC proteins interact with cellular proteins implicated in cancer development, including the retinoblastoma tumor suppressor (Rb), metastasis-associated protein 2 (MTA2), and nuclear hormone receptors like the retinoic acid receptor (17). As a well studied example, acute promyelocytic leukemia is associated with a fusion protein of retinoic acid receptor α and a promyelocytic leukemia zinc finger gene (promyelocytic leukemia zinc finger-retinoic acid receptor α) that recruits HDAC repression activity to block normal differentiation and lead to leukemia (18, 19). Trichostatin treatment of cells expressing promyelocytic leukemia zinc finger-retinoic acid receptor α derepressed transcription and allowed cells to differentiate normally (18, 19). With this precedent, HDAC inhibitors are being explored as potential drugs for the treatment of certain forms of leukemia (20, 21).

The human HDAC proteins are organized into classes based on their similarity to yeast HDAC proteins. Class I HDACs include HDAC1, HDAC2, HDAC3, and HDAC8, which are homologous to the yeast Rpd3 protein (22–26). Class II HDACs are similar to yeast HDA1 and include HDAC4, HDAC5, HDAC6, HDAC7, and HDAC9 (27–29). Finally, the recently

* This work is supported by National Institutes of Health Grants GM19837 (to M. K. H. P.) and GM38627 (to S. L. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[§] Present address: Dept. of Chemistry, Wayne State University, 410 W. Warren Ave., Detroit, MI 48202.

^{||} Howard Hughes Investigator. To whom correspondence should be addressed: Dept. of Chemistry and Chemical Biology, 12 Oxford St., Harvard University, Cambridge, MA 02138. Tel.: 617-495-5318; Fax: 617-495-0751; E-mail: sls@slsiris.harvard.edu.

¹ The abbreviations used are: HDAC, histone deacetylase; Rb, retinoblastoma tumor suppressor; RbAp, Rb-associated protein; MTA, metastasis-associated protein; REST, RE1-silencing transcription factor/neural restrictive silencing factor; IP, immunoprecipitation; CK1 and CK2, casein kinase 1 and 2, respectively; NRD, nucleosome remodeling and deacetylating; MS, mass spectrometry; DRB, 5,6-dichloro-1- β -ribofuranosylbenzimidazole; RPLC, reverse phase liquid chromatography.

identified NAD-dependent sirtuin (SIRT) proteins are homologous to the yeast Sir2 protein (30, 31).

HDAC function is regulated by two known mechanisms. In the case of class II proteins, HDAC4 and HDAC5 shuttle between the nucleus and the cytoplasm via phosphorylation-dependent interaction with 14-3-3 protein (32–34). With class I proteins, HDAC1 and HDAC2 associate with proteins that modulate their deacetylase activity and recruitment to genomic regions (18, 35). Three characterized protein complexes contain HDAC1 and HDAC2. The Sin3 complex comprises HDAC1, HDAC2, RbAp48, RbAp46, mSin3, and Sin3-associated proteins 18 and 30 and interacts with DNA binding transcription factors, including Mad, Ikaros, REST, and nuclear hormone receptors (36–38). The nucleosome remodeling and deacetylating (NRD or NuRD) complex includes HDAC1, HDAC2, RbAp48, RbAp46, Mi-2 (or CHD), methyl-CpG-binding domain 3, and MTA2. The NRD complex combines deacetylation by HDAC proteins with ATP-dependent nucleosome remodeling to affect transcription (39–41). Recently, the CoREST complex, comprising HDAC1, HDAC2, CoREST, and p110, was identified (42). The identification of a group of repressor and corepressor proteins that bind HDAC proteins suggests a model where HDAC1 and HDAC2 are targeted to certain DNA promoters or chromosomal domains to affect transcription of cellular genes.

Although the control of HDAC1 and HDAC2 function by protein interaction and recruitment is well preceded, we were interested in exploring HDAC1 regulation by post-translational modification. We began our study by analyzing the sequence of HDAC1 by mass spectrometry. Two phosphorylated serines were unambiguously identified at Ser⁴²¹ and Ser⁴²³. Mutagenesis experiments revealed that phosphorylation at Ser⁴²¹ and Ser⁴²³ promoted the enzymatic activity and complex formation of HDAC1. The protein kinase casein kinase 2 (CK2) was shown to phosphorylate HDAC1 *in vitro*. Disruption of the CK2 consensus sequence directing phosphorylation of Ser⁴²¹ and Ser⁴²³ altered the enzymatic activity and complex formation of HDAC1. Glutamic acid and aspartic acid only partially substituted for the phosphoserines, indicating that the charge and size of phosphate are uniquely suited to maintaining HDAC1 function. Finally, our data support a model where the carboxyl-terminal region of HDAC1 is essential for maintaining the protein association and enzymatic activity of HDAC1.

EXPERIMENTAL PROCEDURES

Plasmids—All HDAC1 mammalian expression plasmids were constructed by inserting the HDAC1-FLAG sequence into the *NotI/EcoRI* sites of pBJ5 (22). Point mutants of HDAC1 were created by a two-step PCR amplification using the following primers (mutated bases are underlined): Not/HDAC1, GGGCTAGAGCGCGCGGATCCGCCATGGC-GCAGACGC; pBJ5–153rev, TATCATGTCTGGATCCGG; connecting primer, GAACCTCTCTCACAGGC; S421A, GCCTGTGAGGAAGAGTTCGCCGATTCTGAAGAGGAGGG; S423A, GCCTGTGAGGAAGAGTTCCTCCGATTCTGAAGAGGAGGG; S421A/S423A, GCCTGTGAGGAAGAGTTCGCCGATTCTGAAGAGGAGGG; S421E, GCCTGTGAGGAAGAGTTCGCCGATTCTGAAGAGGAGGG; S423E, GCCTGTGAGGAAGAGTTCGCCGATTCTGAAGAGGAGGG; S421E/S423E, GCCTGTGAGGAAGAGTTCGCCGATTCTGAAGAGGAGGG; S421D, GCCTGTGAGGAAGAGTTCGCCGATTCTGAAGAGGAGGG; S423D, GCCTGTGAGGAAGAGTTCGCCGATTCTGAAGAGGAGGG; S421D/S423D, GCCTGTGAGGAAGAGTTCGCCGATTCTGAAGAGGAGGG; S424A, GTGAGGAAGAGTTCGCCGATTCTGAGGAGGAGGAGGG; E424A, GTGAGGAAGAGTTCGCCGATTCTGAGGAGGAGGAGGG; E425A, CGAATTGCCTGTGAGGAGGAGTTCGCCGATTCTGAAGAGGAGGAGGGGGG; E426A, GGAAGAGTTCGCCGATTCTGAAGAGGAGGAGGAGGGGGGGGGC; AAGAAC; E424A/E426A, GTGAGGAAGAGTTCGCCGATTCTGAGGAGGAGGAGGGGGGGGGC; Sal-Δ390, CCGCGGTGCGACAGGGATGGCGTCTCAGGAATCGCCTGC. pSP/Gal-(1–147)-VP16 plasmids used in luciferase assays were constructed by inserting the PCR-

amplified HDAC1 sequence derived from pBJ5 into the *HindIII/EcoRI* sites using the following primers: *HindIII*/HDAC1, GGACCCAGCTT-GCCATGGCGCAGACGCGAGGGCACC CGGAGG; *EcoRI*/HDAC1, CCG-CCGGAATTCTTTATCATCATCATCTTTTATAATCCCC.

[³²P]Orthophosphate *In Vivo* Labeling—Simian virus 40 large T-antigen (T-Ag) Jurkat cells (40 × 10⁶) were washed and incubated in 4 ml of phosphate-free medium containing 1.2 mCi of [³²P]orthophosphate (PerkinElmer Life Sciences) for 4 h at 37 °C with 5% CO₂. Cells were washed three times with phosphate-buffered saline (10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 137 mM NaCl, 2.7 mM KCl, pH 7.4) and lysed in 1 ml of JLB (50 mM Tris, pH 8.0, 150 mM NaCl, 10% glycerol, and 0.5% Triton X-100) supplemented with 1 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml aprotinin, and 0.5 μg/ml leupeptin by rocking at 4 °C for 30 min. After pelleting cellular debris, the supernatant was incubated with 20 μl of α-HDAC1-conjugated agarose affinity gel in the presence and absence of HDAC1 peptide inhibitor (NH₂-CGEEK-PEAKGVKEEVKLA) for 1.5 h with rocking at 4 °C. The immunoprecipitates were washed three times with 1 ml of KA/LB (50 mM Tris, pH 8.0, 150 mM NaCl, 0.1% Nonidet P-40, 1 mM EDTA) and resuspended in 20 μl of 2× SDS to elute bound proteins (1× SDS buffer contains 100 mM Tris, pH 6.8, 10% glycerol, 4% SDS, and bromophenol blue). The eluted proteins were separated using SDS-PAGE.

Phosphorylation Site Determination by Ion Trap Mass Spectrometry—K-trap affinity resin was synthesized by deprotection of Alloc-protected K-trap and immobilization onto Affi-Gel 10 as described (22, 43). T-Ag Jurkat cells (1.25 × 10⁹) were lysed in JLB supplemented with 1 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml aprotinin, and 0.5 μg/ml leupeptin by rocking at 4 °C for 30 min. After cellular debris was pelleted by centrifugation, the protein concentration of the supernatant was determined by Bradford assay and diluted to 5 μg/μl with JLB. K-trap affinity resin (400 μl) was washed twice with JLB and incubated with the lysate at 4 °C for 14 h with rocking. The beads were washed with 10 ml of KA/LB twice quickly and three times more stringently by rocking for 10 min at room temperature. The washed beads were resuspended in 300 μl of 0.25× SDS buffer supplemented with freshly added 100 mM dithiothreitol and boiled for 5 min to elute bound protein. The supernatant was removed, and the elution was repeated with 100 μl of 0.25× SDS buffer. The 400 μl of eluted proteins were concentrated to 100 μl by evaporation, boiled, and separated using SDS-PAGE. After visualization with colloidal Coomassie staining (Novex), the band corresponding to HDAC1 was subjected to in gel reduction, carboxyamidomethylation, and tryptic digestion (Promega). Phosphorylated peptide sequences were determined in a two-stage microchromatographic run on a bimodal immobilized Fe³⁺ metal affinity/reverse phase liquid chromatography (RPLC) column. The bimodal column was packed in house with 5 cm of POROS MC support (PerkinElmer Life Sciences) followed by 5 cm of POROS R2 in a 75-μm inner diameter fused silica terminating in a 10-μm nanospray tip (New Objective). Phosphorylated peptides were retained by the immobilized Fe³⁺ metal affinity phase during RPLC of the nonphosphorylated peptides. Captured peptides were released by eluting with 100 mM KH₂PO₄, 1% acetic acid for subsequent RPLC concentration and separation. The chromatography was directly coupled to a LCQ DECA quadrupole ion trap mass spectrometer (ThermoFinnigan) through a custom nanoelectrospray source. The flow rate was 290 nL/min. The ion trap repetitively surveyed the range *m/z* 395–400, executing data-dependent tandem MS (MS/MS) for peptide sequence information on the four most abundant ions in each survey scan. MS/MS spectra were acquired with a relative collision energy of 30% and an isolation width of 2.5 daltons, and recurring ions were dynamically excluded. After data base correlation with the algorithm SEQUEST (44), phosphorylated peptides were confirmed by manual, *de novo* interpretation of the MS/MS spectra using Fuzzylons (45).

Cell Culture, Transfection, Immunoprecipitation, and Antibodies—T-Ag Jurkat cells (46) were grown in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin, and streptomycin. FLAG epitope-tagged HDAC1 was transiently transfected by electroporation into T-Ag Jurkat cells. Forty-eight hours post-transfection, cells were lysed at 4 °C by rotation in JLB with 1 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml aprotinin, and 0.5 μg/ml leupeptin. After cellular debris was pelleted by centrifugation, HDAC1-FLAG proteins were immunoprecipitated from the supernatant by incubation at 4 °C with α-FLAG M2 agarose affinity gel (Sigma) for 2 h with rocking. When immunoprecipitation was performed with T-Ag Jurkat lysates, 10 × 10⁶ cells were lysed as described and incubated with the protein-specific antibody for 30 min, followed by incubation with protein G-agarose (Life Technologies, Inc.) for 1 h. For Western blot analysis, the beads were washed with JLB three times, diluted with 1× SDS buffer, and separated by

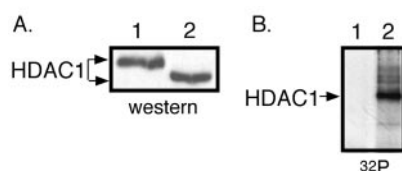


FIG. 1. **HDAC1 is phosphorylated *in vivo*.** A, T-Ag Jurkat lysates were incubated in the absence (lane 1) and presence (lane 2) of calf intestinal phosphatase and separated by SDS-PAGE. HDAC1 protein, indicated with arrows, was visualized using α -HDAC1 antibody (22). B, immunoprecipitated, ³²P-labeled HDAC1 is visualized by autoradiography (lane 2). As a control, the HDAC1 immunoprecipitation was also performed in the presence of a competitor HDAC1 peptide (lane 1). The HDAC1 protein is indicated with an arrow.

SDS-PAGE. Antibodies against HDAC1 (22) and MTA2 (42) and CoREST (42) have been described. Antibodies against FLAG (Sigma), RbAp48 (Upstate Biotechnology, Inc., Lake Placid, NY), mSin3A (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), I κ B α (Upstate Biotechnology), and CK2 (Upstate Biotechnology) were purchased.

Histone Deacetylation Assay—[³H]Acetate-incorporated histones were isolated from butyrate-treated HeLa cells by acid extraction as described (47). Immunoprecipitates were incubated with $\sim 0.4 \mu\text{g}$ (1,000 dpm) of histones for 2 h at 37 °C in 50 μl of HD buffer (20 mM Tris, pH 8.0, 150 mM NaCl, and 10% glycerol). The reaction was terminated by the addition of an equal volume of stop solution (0.5 M HCl and 0.2 M acetic acid), and deacetylase activity was determined by scintillation counting of the ethyl acetate-soluble [³H]acetic acid (22). Inhibition of enzymatic activity by trichostatin A was determined by incubating the immunoprecipitates with 500 nM trichostatin A (Sigma) for 30 min prior to the addition of histones.

Luciferase Assay—For each assay, 10⁷ T-Ag Jurkat cells were transfected by electroporation with 50 ng of luciferase reporter, 50 ng of cytomegalovirus- β -galactosidase reporter, and 50 ng of protein expression construct (36). Twenty-four hours post-transfection, luciferase assays were performed (Promega). Normalized luciferase values were calculated by dividing the luciferase activity by the β -galactosidase activity.

In Vitro and in Vivo Phosphorylation—T-Ag Jurkat cell lysates (50 μl) were incubated with 20 units of calf intestinal phosphatase for 1 h at 30 °C. The calf intestinal phosphatase was denatured for 5 min at 65 °C, and lysates were incubated with 500 units of casein kinase 2 or 1000 units of casein kinase 1 at 30 °C for 1 h. When treated with 10 μM 5,6-dichloro-1- β -ribofuranosylbenzimidazole (DRB) or 200 nM KT5720, T-Ag Jurkat cells were harvested after a 45-min incubation. DRB, KT5720, recombinant CK1, and recombinant CK2 were purchased from Calbiochem.

RESULTS

HDAC1 Is a Phosphoprotein—To determine whether HDAC1 is a phosphoprotein, simian virus 40 large T-Ag Jurkat cell lysates were treated with alkaline phosphatase, and HDAC1 was visualized using an HDAC1-specific antibody (Fig. 1A). In the presence of phosphatase, HDAC1 migrated more quickly in a polyacrylamide gel than untreated HDAC1, consistent with the possibility that HDAC1 is phosphorylated.

To verify that HDAC1 is phosphorylated, T-Ag Jurkat cells were labeled *in vivo* with [³²P]orthophosphate, and HDAC1 was immunoprecipitated with an HDAC1-specific antibody. Immunoprecipitated HDAC1, which migrated by SDS-PAGE identically to HDAC1 from lysates (data not shown), was labeled with ³²P, indicating that the protein is phosphorylated *in vivo* (Fig. 1B). These results are consistent with previous experiments indicating that HDAC1 is post-translationally modified by phosphorylation (48).

Determination of Phosphorylated Residues—To identify residues on HDAC1 that are phosphorylated, HDAC1 was purified from T-Ag Jurkat cells using K-trap, an affinity resin comprising modified trapoxin, an irreversible inhibitor of HDAC, attached to Affi-Gel 10 (Fig. 2A; Ref. 22). After separation by SDS-PAGE, the purified HDAC1 was excised from the gel (Fig. 2B) and analyzed by mass spectrometry. Two phosphorylated

serine residues were unambiguously identified, Ser⁴²¹ and Ser⁴²³ (Fig. 2C). Interestingly, every phosphorylated peptide observed was doubly phosphorylated at Ser⁴²¹ and Ser⁴²³; no peptide detected contained only one of the two identified phosphorylated serines.

The phosphorylated serines are located in the highly charged, carboxyl-terminal tail of HDAC1, removed from the catalytic site (Fig. 2C). A sequence comparison of the class I HDAC proteins, HDAC1, HDAC2, HDAC3, and HDAC8, indicates that only HDAC1 and HDAC2 contain serines in equivalent sequence positions. HDAC3 contains two potential CK2 consensus sites; however, a lack of sequence conservation in the C-terminal region of HDAC3 makes comparison with HDAC1 and HDAC2 difficult. HDAC8 does not contain the carboxyl-terminal charged tail, where the serine residues are located. For comparison, the yeast RPD3 protein contains a truncated carboxyl-terminal tail; however, no homologous serines exist in the equivalent HDAC1 Ser⁴²¹ and Ser⁴²³ positions (not shown). The fact that the phosphorylated serines identified are located in the carboxyl-terminal charge tail of HDAC1 is consistent with previous results indicating that HDAC1 is phosphorylated between residues 387 and 482 (48).

Phosphorylation Promotes Enzymatic Activity—To determine whether phosphorylation influences enzymatic activity, Ser⁴²¹ and Ser⁴²³ were independently and simultaneously mutated to alanine. Mutant HDAC1 proteins fused to a FLAG epitope tag were expressed in T-Ag Jurkat cells, which contain only small amounts of endogenous HDAC2 (49). The HDAC1 fusion protein was immunoprecipitated with α -FLAG affinity resin, and enzymatic activity was determined *in vitro*. Western blot experiments confirmed that only the FLAG-tagged HDAC1 protein bound to the α -FLAG affinity resin, and not detectable quantities of endogenous HDAC1 protein (data not shown). Under conditions where equal amounts of HDAC1 or mutant proteins were immunoprecipitated, mutant proteins displayed significantly diminished deacetylase activity compared with the wild-type protein (Fig. 3A). HDAC1 S421A, S423A, and S421A/S423A mutants demonstrated 33.0 ± 4.2 , 25.3 ± 2.9 , and $22.9 \pm 3.1\%$ activity compared with wild-type, respectively. The minimal activity displayed by the mutants was lost upon treatment with trichostatin A, an inhibitor of HDAC activity, indicating that the residual activity may be the result of partially active enzyme or a small amount of co-immunoprecipitated, endogenous HDAC activity (data not shown; Ref. 50).

To confirm that phosphorylation of HDAC1 promotes enzymatic activity *in vivo*, luciferase assays were performed. HDAC1 and mutants were expressed in T-Ag Jurkat cells as fusion proteins to Gal4 and VP16, which activate transcription of the luciferase gene under the control of four tandem Gal4 DNA binding sites. Where Gal4-VP16 activated the transcription of luciferase, the HDAC1-Gal4-VP16 fusion almost entirely repressed transcription due to deacetylase activity of HDAC1 (Fig. 3B), consistent with previous studies (49). The HDAC1 S421A, S423A, and S421A/S423A mutant fusion proteins activated transcription of luciferase under conditions where the wild-type HDAC1 fusion protein did not (Fig. 3B), consistent with the fact that the mutants display diminished deacetylase activities *in vitro*. Interestingly, the phosphorylation mutants activated the expression of luciferase to a greater extent than the HDAC1 H141A mutant (Fig. 3B), which is mutated at a conserved catalytic histidine residue (49).

Acidic Residues Partially Substitute for Phosphorylated Serine in HDAC1—Glutamic acid and aspartic acid have been previously used as mimics of phosphorylated serine residues (51). To test whether glutamic acid or aspartic acid substitutes for phosphoserine in HDAC1, Ser⁴²¹ and Ser⁴²³ were individ-

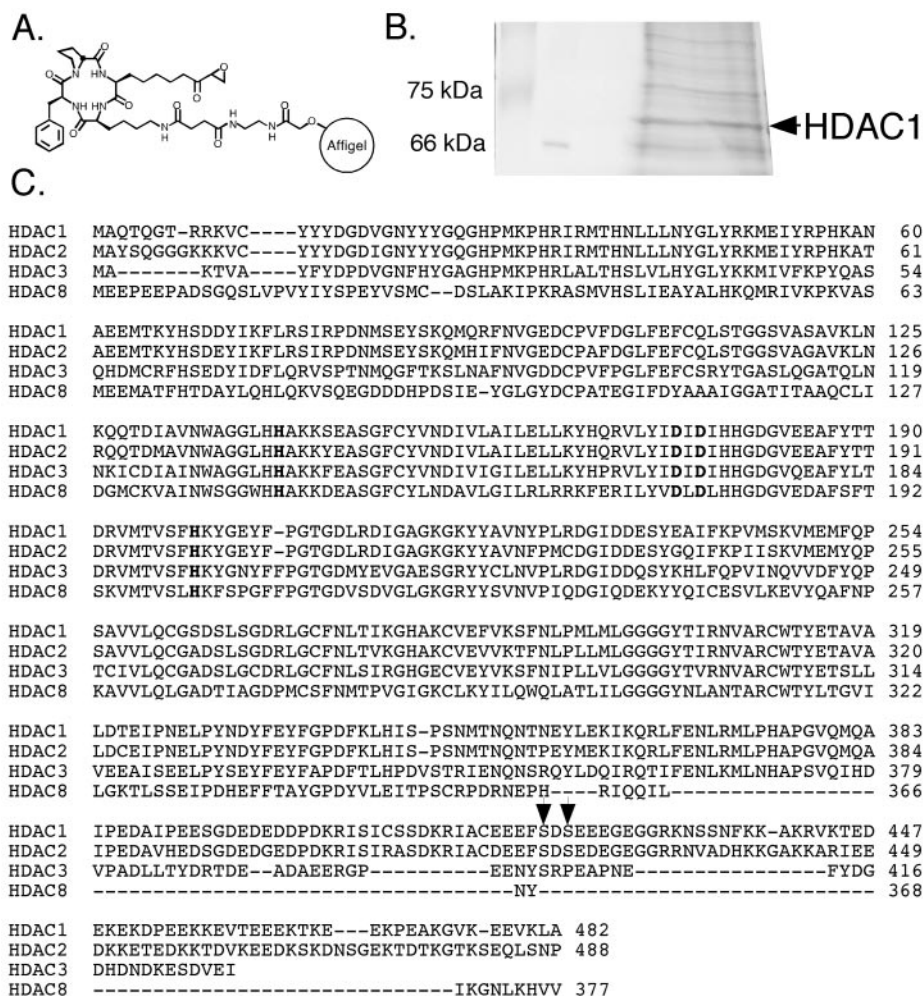


FIG. 2. HDAC1 is phosphorylated at Ser⁴²¹ and Ser⁴²³. A, shown is the K-trap affinity resin used to purify HDAC1 (22). B, K-trap-purified HDAC1 was separated by SDS-PAGE and Coomassie-stained to visualize protein. HDAC1, shown with an arrow, was excised from the gel and analyzed by mass spectrometry to identify phosphorylated residues. The size of molecular weight markers is shown. C, serines 421 and 423 in HDAC1, which are phosphorylated according to mass spectrometric analysis, are indicated with arrows. The conserved residues involved in catalysis are in *boldface type*. All other human class I HDAC proteins identified to date, HDAC2 (GenBankTM U31814), HDAC 3 (GenBankTM U75697), and HDAC 8 (GenBankTM AAF73076), are aligned below the HDAC1 sequence (GenBankTM U50079) using the Clustal method.

usually and simultaneously mutated and tested for enzymatic activity (Fig. 4). The S421D or S421E HDAC1 mutants efficiently deacetylated histones *in vitro* with 82.5 ± 2.9 and $81.8 \pm 2.9\%$ of wild-type activity, respectively. With the S423D and S423E mutants, acidic residues were less effective at mimicking phosphorylation, demonstrating 40.9 ± 3.0 and $48.7 \pm 2.6\%$ enzymatic activity of wild-type HDAC1. Finally, the double mutation of Ser⁴²¹ and Ser⁴²³ to aspartic acid or glutamic acid (S421D/S423D or S423E/S423E) only partially substituted for phosphorylation with 34.1 ± 2.6 and $38.5 \pm 0.2\%$ wild-type activity. Although the aspartic and glutamic acid mutants displayed diminished activity compared with wild-type, they maintained greater enzymatic activity than the corresponding alanine mutants (Fig. 3A). These results demonstrate that acidic residues only partially substitute for phosphoserine in deacetylase assays *in vitro*.

Carboxyl-terminal Region of HDAC1 Is Required for Activity—One possible mechanism explaining the requirement of serine phosphorylation for HDAC1 activity is that the highly charged carboxyl-terminal region, where Ser⁴²¹ and Ser⁴²³ are located, physically blocks the catalytic site of HDAC1. According to the model, the carboxyl-terminal region properly positions itself to allow substrate binding and deacetylation only when Ser⁴²¹ and Ser⁴²³ are phosphorylated.

To test whether the carboxyl-terminal region of HDAC1 physically inhibits enzymatic activity, a truncated mutant of HDAC1 lacking the charged carboxyl-terminal region was expressed and tested for enzymatic activity (Fig. 5). HDAC1-(1–390) demonstrated $22.1 \pm 2.3\%$ wild-type activity. These data do not support the model where the carboxyl-terminal region

physically inhibits enzymatic activity. Rather, the data suggest that the carboxyl-terminal region is critical for enzymatic activity, similar to previous results with mouse HDAC1, demonstrating that the amino-terminal region is required to maintain enzymatic activity (50).

HDAC1 Is a Substrate of CK2 *In Vitro* and *In Vivo*—Ser⁴²¹ and Ser⁴²³ of HDAC1 are located in a consensus site for the protein kinase CK2 (Figs. 2C and 6A). To determine whether CK2 is capable of phosphorylating Ser⁴²¹ and Ser⁴²³ *in vitro*, phosphatase-treated lysates were incubated with recombinant CK2 catalytic domain, and HDAC1 was separated and assessed for changes in migration through the polyacrylamide gel. As shown in Fig. 6B, HDAC1 incubated with CK2 migrated more slowly on a polyacrylamide gel than phosphatase-treated HDAC1 or HDAC1 incubated with casein kinase 1 (compare lane 3 with lanes 2 and 4), suggesting that HDAC1 is a substrate for CK2 but not CK1. These results are consistent with previous work demonstrating that human HDAC1 is phosphorylated *in vitro* by CK2 (48).

To determine whether HDAC1 is a substrate of CK2 *in vivo*, the CK2 consensus sites that potentially direct phosphorylation of Ser⁴²¹ and Ser⁴²³ in HDAC1 were disrupted individually and simultaneously by replacing the critical glutamic acids with alanine (Fig. 6A). If disruption of the CK2 sites results in reduced phosphorylation, we would expect the mutants to display reduced deacetylase activity compared with wild type. The enzymatic activities of the mutants were assessed and are shown in Fig. 6C. Consistent with the prediction, all CK2 consensus site mutants displayed reduced deacetylase activity compared with wild-type HDAC1. The double mutant (E424A/

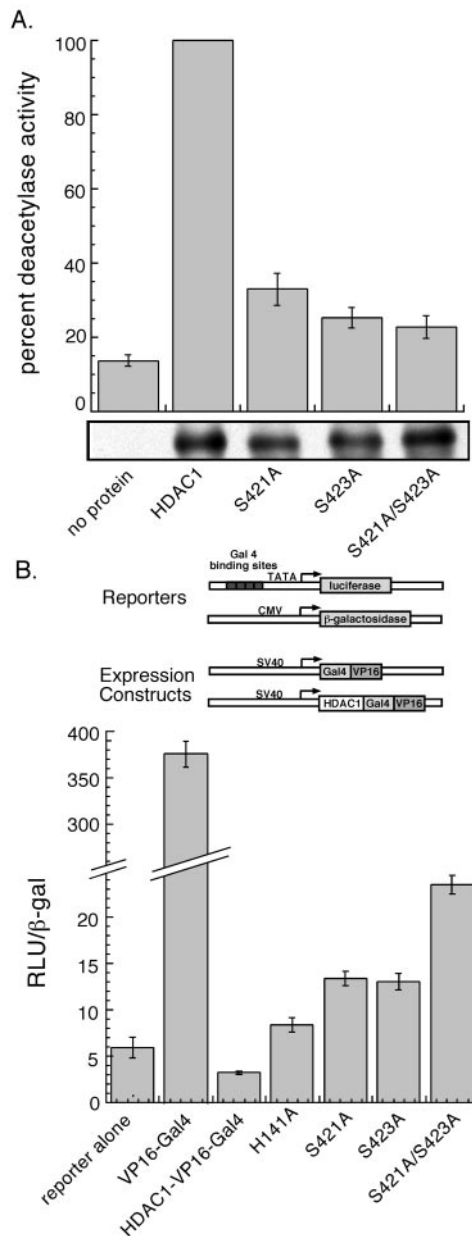


FIG. 3. Mutation of Ser⁴²¹ and Ser⁴²³ to alanine in HDAC1 reduces enzymatic activity. A, phosphorylation of Ser⁴²¹ and Ser⁴²³ was disrupted by alanine mutation. HDAC1 wild-type or mutants fused to a FLAG tag were expressed in T-Ag Jurkat cells and immunoprecipitated using α-FLAG-agarose resin. Equivalent amounts of immunoprecipitated protein were either incubated with [³H]labeled acetylated histones to determine enzymatic activity (*histogram*) or separated by SDS-PAGE and analyzed by Western blot to assess protein quantities (*gel image*). Enzymatic activity is shown as a percentage of HDAC1 wild-type activity (set at 100%). The S.E. of at least four independent experiments is shown. B, HDAC1 wild-type or mutants fused to the Gal4-(1–147) DNA binding domain and VP16 activation domain were expressed in T-Ag Jurkat cells in the presence of a luciferase gene reporter under the control of four tandem Gal4 binding sites. A β-galactosidase gene reporter under the control of the cytomegalovirus promoter was also included to normalize for transfection efficiencies. The amount of expressed luciferase and β-galactosidase in the cell lysates was assessed according to the manufacturer's procedure (Promega). Luciferase values (relative light units (RLU)) are calculated by dividing the luciferase activity by the β-galactosidase activity. The S.E. of three experiments is shown.

E426A), disrupting phosphorylation of Ser⁴²¹ and Ser⁴²³, displayed $30.9 \pm 1.6\%$ activity of wild-type HDAC1, while the E426A mutant, directing phosphorylation of Ser⁴²³ only, demonstrated $48.4 \pm 7.2\%$ of wild-type activity. Mutation of the

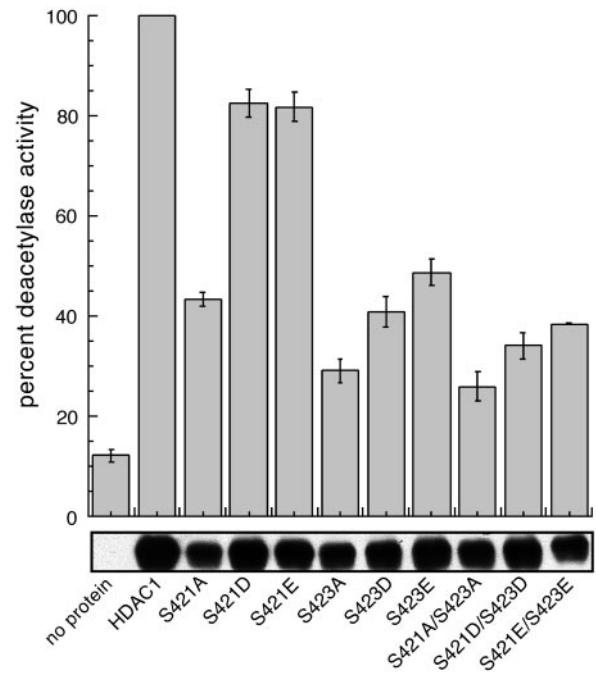


FIG. 4. Glutamic acid partially substitutes for phosphorylated Ser⁴²¹ and Ser⁴²³ in HDAC1. A, Ser⁴²¹ and Ser⁴²³ were mutated to glutamic acid and aspartic acid as described under "Experimental Procedures." HDAC1 wild-type or mutants fused to a FLAG tag were expressed in T-Ag Jurkat cells and immunoprecipitated using α-FLAG-agarose resin. Immunoprecipitates were treated as in Fig. 3A.

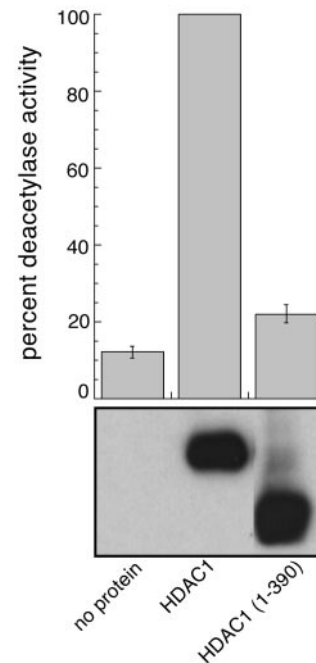


FIG. 5. The carboxyl-terminal tail of HDAC1 is necessary for full enzymatic activity. The carboxyl-terminal charged tail of HDAC1 was deleted to create the HDAC1-(1–390) truncation mutant. HDAC1 wild type or mutant fused to a FLAG tag was expressed in T-Ag Jurkat cells and immunoprecipitated using α-FLAG-agarose resin. Immunoprecipitates were treated as described in the legend to Fig. 3A.

glutamic acid directing Ser⁴²¹ phosphorylation (E424A) was not as effective in reducing the enzymatic activity of HDAC1, maintaining $78.5 \pm 5.4\%$ of wild-type HDAC1 activity. As a critical control, mutation of a nearby glutamic acid not directing CK2 phosphorylation (E425A) displayed $108 \pm 4.3\%$ of wild-type activity, indicating that the loss of a negatively

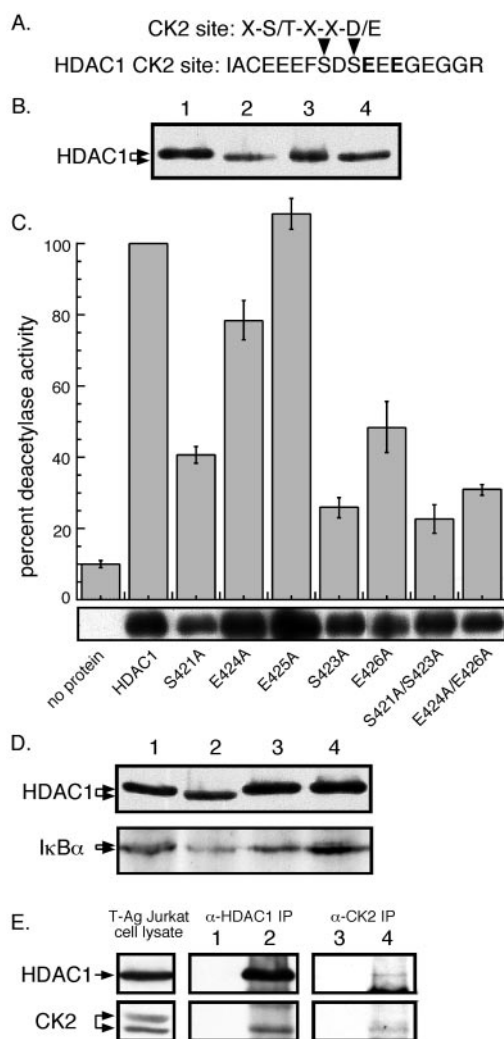


FIG. 6. Ser⁴²¹ and Ser⁴²³ on HDAC1 are phosphorylated by CK2. **A**, the consensus sequence for CK2 is shown, where an aspartic or glutamic acid directs phosphorylation of an amino-terminal serine located two residues away. The HDAC1 CK2 consensus sequence governing phosphorylation of Ser⁴²¹ and Ser⁴²³ is also shown, with the phosphorylated serines indicated with arrows and the directing glutamic acids (Glu⁴²⁴ and Glu⁴²⁶) in **boldface type**. **B**, cell lysates from T-Ag Jurkat cells containing endogenous HDAC1 were incubated in the absence (lane 1) or presence (lanes 2–4) of calf intestine phosphatase. Once dephosphorylated, CK2 (lane 3) or CK1 (lane 4) was added. After separation of protein in the lysates by SDS-PAGE, HDAC1 was visualized using an HDAC1-specific antibody (22). **C**, the glutamic acid residues directing phosphorylation of Ser⁴²¹ and Ser⁴²³ in HDAC1 were mutated individually (E424A and E426A) or simultaneously (E424A/E426A). In addition, a glutamic acid not involved in directing CK2 phosphorylation of HDAC1 was also mutated as a control (E425A). HDAC1 wild-type or mutants fused to a FLAG tag were expressed in T-Ag Jurkat cells and immunoprecipitated using α-FLAG-agarose resin. Immunoprecipitates were treated as in Fig. 3A. **D**, T-Ag Jurkat cells were incubated for 45 min in the absence (lane 1) and presence of small molecule inhibitors of CK2 (DRB; lane 3) or cAMP-dependent protein kinase (KT5720; lane 4), and cellular proteins were separated by SDS-PAGE. Phosphatase-treated lysates were also included for comparison (lane 2). HDAC1 and the positive control IκBα were visualized using a specific antibody. **E**, HDAC1 or CK2 was immunoprecipitated from T-Ag Jurkat cell lysates and separated using SDS-PAGE (lanes 2 and 4). As controls, cellular lysates and immunoprecipitates using protein G-agarose alone (without antibody) were also included (lanes 1 and 3). Proteins were visualized using specific antibodies.

charged residue is not responsible for the reduced deacetylase activity. In all cases, the CK2 consensus site mutation reduced activity to a lesser extent than the alanine mutation. However, the fact that all CK2 consensus site mutants demonstrated

reduced enzymatic activity compared with wild-type HDAC1 suggests that HDAC1 is a substrate for CK2 *in vivo*.

To provide further evidence that CK2 phosphorylates HDAC1 *in vivo*, T-Ag Jurkat cells were incubated in the presence of DRB, a specific small molecule inhibitor of CK2 enzymatic activity, and the migration of endogenous HDAC1 in a polyacrylamide gel was assessed. Interestingly, the migration of HDAC1 through a polyacrylamide gel was independent of whether CK2 activity was inhibited *in vivo* (Fig. 6D, compare lanes 1 and 3). In contrast, the migration of IκBα, a known CK2 substrate, was influenced by incubation with DRB (52). Because mutagenesis studies demonstrated that HDAC1 is a substrate of CK2 *in vivo*, these results raise the possibility that phosphorylation of HDAC1 is not dynamic during a 45-min incubation.

Finally, to probe the ability of CK2 to interact with HDAC1 *in vivo*, endogenous HDAC1 and CK2 were immunoprecipitated from T-Ag Jurkat lysates. As shown in Fig. 6E, HDAC1 and CK2 co-immunoprecipitated, indicating that the proteins interact *in vivo*. These combined data strongly argue that CK2 phosphorylates HDAC1 *in vivo*.

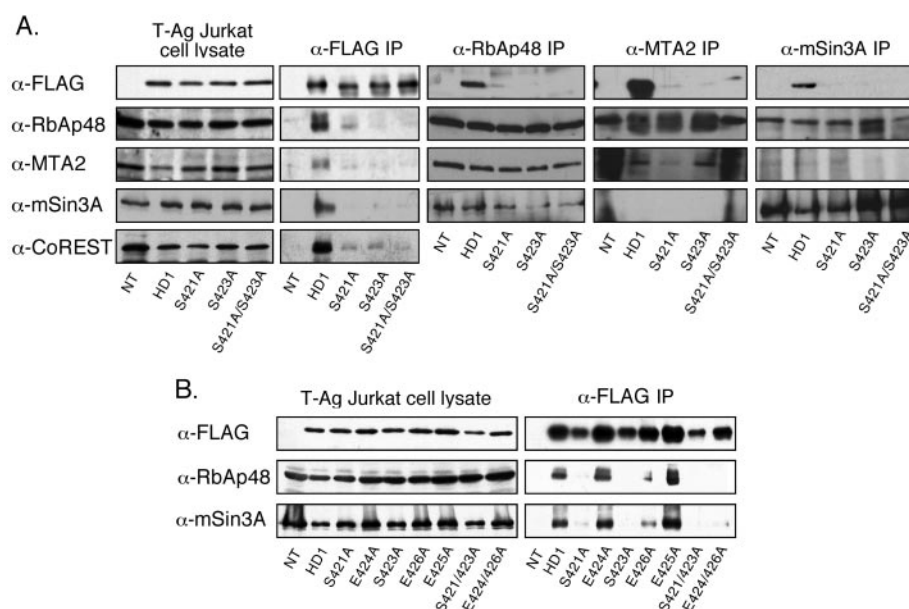
Phosphorylation Influences HDAC1 Complex Formation—HDAC1 function is regulated by association with cellular proteins. Not only are associated proteins able to recruit HDAC1 to specific DNA sequences for targeted deacetylation and transcriptional repression (35), but biochemically purified HDAC1 also displays higher deacetylase activity when in complex with associated proteins (40, 49). These previous results suggest that phosphorylation might affect deacetylase activity by disrupting complex formation.

To determine whether phosphorylation affects the protein interactions of HDAC1, immunoprecipitated FLAG-tagged HDAC1 and mutant proteins were probed for binding to HDAC1-associated proteins (Fig. 7). Phosphorylation site mutants did not interact with associated proteins as effectively as wild-type HDAC1. Where equivalent amounts of FLAG-tagged protein were immunoprecipitated, the S421A, S423A, and S421A/S423A mutants ineffectively co-immunoprecipitate proteins from HDAC1-containing complexes compared with wild-type HDAC1, including RbAp48 found in the Sin and NRD complexes, MTA-2 from the NRD complex, mSin3A from the Sin complex, and CoREST from the CoREST complex (Fig. 7A, compare lanes under α-FLAG IP). Co-immunoprecipitation of HDAC1 by RbAp48, MTA2, and mSin3A verified that HDAC1 binding was reduced with the S421A, S423A, and S421A/S423A mutants (Fig. 7A, compare lanes under α-RbAp48, α-MTA2 IP, and α-mSin3A IP). As expected, MTA-2 from the NRD complex did not co-immunoprecipitate with Sin3 from the Sin complex (Fig. 7A, compare α-MTA2 and α-mSin3A IP; Ref. 40). RbAp48 and mSin3A were also associated in the presence or absence of HDAC1, consistent with previous result demonstrating that they interact directly (Fig. 7A, compare α-RbAp48 and α-mSin3A IP; Ref. 38). Interestingly, MTA-2 and RbAp48 were associated in the presence or absence of the HDAC1-FLAG fusion protein, although they did not interact directly in glutathione S-transferase pull-down experiments (Fig. 3A, compare α-MTA2 and α-RbAp48; Ref. 40). Perhaps endogenous HDAC1 or another NRD complex member is involved in MTA-2/RbAp48 association.

To confirm that reduced phosphorylation at Ser⁴²¹ and Ser⁴²³ disrupts complex formation, HDAC1 mutants with CK2 consensus site alterations were probed for protein interactions. In this case, the ability of the mutants to bind associated proteins was roughly correlated with observed enzymatic activity (compare Fig. 6C with Fig. 7B). Where the E424A mutant, which disrupts phosphorylation of Ser⁴²¹, displayed

FIG. 7. HDAC1 phosphorylation site mutants disrupt interaction with associated proteins. A and B, HDAC1 wild-type or mutants fused to a FLAG tag

were expressed in T-Ag Jurkat cells and immunoprecipitated using α -FLAG agarose resin (Sigma) or protein G resin and α -RbAp48 (Upstate Biotechnology), α -MTA2 (42), or α -mSin3A (Santa Cruz Biotechnology). The immunoprecipitates were separated using SDS-PAGE, and proteins were visualized using specific antibodies.



$78.5 \pm 5.4\%$ of wild-type HDAC1 activity, it immunoprecipitated RbAp48 and mSin3A effectively compared with wild-type HDAC1. In the case of E426A or E424A/E426A, which displayed 48.4 ± 7.2 and $30.9 \pm 1.6\%$ activity of wild-type HDAC1, respectively, they poorly immunoprecipitated RbAp48 and Sin3A compared with HDAC1 wild type. In addition, deletion of the HDAC1 carboxyl-terminal tail that reduced enzymatic activity to $22.1 \pm 2.3\%$ disrupted interaction with associated proteins (data not shown). These results suggest that the enzymatic activity of HDAC1 phosphorylation site mutants may be related to their ability to interact with associated proteins.

DISCUSSION

Studies with human, mouse, and maize HDAC proteins demonstrated that histone deacetylases are phosphoproteins (32, 48, 53). To identify phosphorylation sites on HDAC1, K-trap-purified human HDAC1 protein was analyzed by mass spectrometry, and two phosphorylated serine residues, Ser⁴²¹ and Ser⁴²³, were unambiguously identified. Disruption of phosphorylation at Ser⁴²¹ and Ser⁴²³ by alanine mutation or mutation of the CK2 consensus sites reduced HDAC1 enzymatic activity and perturbed its binding to interacting proteins.

One possible result of mutagenesis is the nonspecific global unfolding of the HDAC1 mutants. Three experiments refute this possibility and argue that phosphorylation specifically results in altered HDAC1 activity. First, all HDAC1 mutants expressed were not degraded in our experiments, indicating that they are folded sufficiently to prevent protease digestion *in vivo*. Second, mutagenesis of the negatively charged Glu⁴²⁵, a residue in the vicinity of the CK2 consensus sites but not involved in directing phosphorylation, did not affect deacetylase activity or complex formation (Figs. 6C and 7B). The E425A control experiments indicate that loss of phosphoserine, and not the neutralization of any negatively charged residue in the region, affects deacetylase activity and complex formation of HDAC1. Finally, mutation of Ser⁴²¹ and Ser⁴²³ to glutamic acid only partially substituted for phosphorylation, suggesting that phosphoserine is uniquely suited to maintain HDAC1 activity. Our data suggest that HDAC1 specifically requires phosphorylation at Ser⁴²¹ and Ser⁴²³ to allow proper activity.

Although HDAC2 contains equivalent serines at HDAC1 Ser⁴²¹ and Ser⁴²³, the other class I HDACs do not (Fig. 2C). The lack of conservation of Ser⁴²¹ and Ser⁴²³ among the class I HDACs may reflect the differences in regulation of these pro-

teins. For example, HDAC2 is found in all characterized HDAC1-containing complexes, indicating that HDAC1 and HDAC2 are similarly regulated *in vivo* via a protein association mechanism (54). Consistent with the possibility that HDAC2 is phosphorylated, phosphatase treatment alters the migration of HDAC2 through a polyacrylamide gel (data not shown). The fact that HDAC2 contains Ser⁴²¹ and Ser⁴²³ and phosphorylation at Ser⁴²¹ and Ser⁴²³ promotes complex formation of HDAC1 suggests that HDAC2 may interact with associated proteins in the same manner as HDAC1. On the other hand, HDAC3 is known to bind HDAC4, HDAC5, HDAC7, and the nuclear hormone receptor corepressor; however, multiprotein complexes containing HDAC3 are not well characterized (32, 55, 56). Although HDAC3 contains two CK2 consensus sequences in its short C-terminal region, the lack of conservation of HDAC3 in the C-terminal region compared with HDAC1 and HDAC2 may indicate that HDAC3 does not function through the same complexes as HDAC1 and HDAC2 but rather through HDAC3-specific complexes. Regulation of HDAC8, the newest addition to the class 1 HDACs, has not yet been characterized. The fact that HDAC8 does not contain the carboxyl-terminal region suggests that it may be regulated through a distinct mechanism.

HDAC1 function is regulated by associated proteins, which govern enzymatic activity and specific DNA sequence recruitment (18, 40). Co-immunoprecipitation experiments demonstrated that the interaction of HDAC1 with associated proteins is phosphorylation-dependent. Interestingly, the ability of HDAC1 to interact with associated proteins was roughly correlated with HDAC1 enzymatic activity; when HDAC1 phosphorylation site mutants bound poorly to interacting proteins, including RbAp48, MTA-2, Sin3A, and CoREST, they also displayed reduced enzymatic activity (compare Fig. 6C with Fig. 7B). In addition, the HDAC1 H141A mutant, which has reduced enzymatic activity but still interacts with RbAp48 and mSin3A, was not as effective in activating luciferase gene expression as the phosphorylation site mutants (Fig. 3B; Ref. 49). Although more studies are needed to assign a causative role for associated proteins in modulating deacetylase activity, our results reinforce the importance of protein association in regulating HDAC1 function *in vivo*.

Glutathione S-transferase protein pull-down experiments with mouse HDAC1 deletion mutants identified the 51-amino

acid amino-terminal region of HDAC1 as mediating interaction with RbAp48 and Sin3A (50). Our results reveal that the carboxyl-terminal region of HDAC1 is also required to maintain enzymatic activity. The structure of HDLP, an HDAC homolog from hyperthermophilic bacterium *Aquifex aeolicus*, does not include the carboxyl-terminal region of HDAC1, but the 51-amino-terminal amino acids of HDLP are partially solvent exposed and available for protein interactions (57). Taken together, our results support a model where a large structural domain composed of the amino-terminal and carboxyl-terminal tails of HDAC1 cooperate to allow protein association.

Our results also suggest that Ser⁴²¹ and Ser⁴²³ are constitutively phosphorylated and buried when properly organized. Specifically, phosphatase-treated HDAC1 maintained the same enzymatic activity as wild-type HDAC1, despite the fact that unphosphorylated Ser⁴²¹ and Ser⁴²³ inactivated HDAC1 (data not shown; Ref. 48). We hypothesize that Ser⁴²¹ and Ser⁴²³ are inaccessible to the phosphatase enzyme because they are buried, either in the HDAC1 globular structure or at the interface with an associated protein. The marked change in HDAC1 migratory ability when phosphatase-treated, as shown in Fig. 1, may be caused by the removal of phosphate at solvent-accessible sites but not at Ser⁴²¹ and Ser⁴²³. Consistent with this hypothesis is the fact that CK2 phosphorylation of HDAC1 in Fig. 6B resulted in a decreased migratory shift compared with that in Fig. 1. In addition, incubation of cells with DRB, an inhibitor of CK2 activity, did not alter the migration of HDAC1 through a polyacrylamide gel, despite the fact that HDAC1 is a substrate for CK2 *in vitro* and *in vivo*. These data suggest that HDAC1 is not dynamically phosphorylated during a 45-min time scale. The observation by mass spectrometry that HDAC1 was always doubly phosphorylated and the fact that HDAC1 migrates as a single band by PAGE is consistent with the possibility that HDAC1 is constitutively phosphorylated at Ser⁴²¹ and Ser⁴²³. Although more studies are necessary to determine under what conditions HDAC1 becomes dephosphorylated *in vivo*, these results support a model where Ser⁴²¹ and Ser⁴²³ are constitutively phosphorylated and inaccessible to phosphatase enzymes. Perhaps Ser⁴²¹ and Ser⁴²³ phosphorylation is essential for organizing the large structural domain that mediates protein interactions. The fact that the mutants were stable in the presence of endogenous proteases suggests that the domain organization necessary to support protein association may be subtle.

Consistent with the proposed structural model is the fact that Ser⁴²¹ and Ser⁴²³ are substrates for CK2. CK2 is involved in diverse cellular events, such as transcription, proliferation, and development, and is essential for viability in numerous organisms, including *Saccharomyces cerevisiae* (58). CK2 is acidophilic, requiring one or more acidic residues positioned downstream from the target serine, including glutamic acid, aspartic acid, phosphoserine, and phosphotyrosine, for recognition and phosphorylation. For this reason, CK2 is thought to be involved in a mechanism of hierarchical phosphorylation, where one phosphorylation event leads to subsequent protein modifications (59). Importantly, CK2 is constitutively active due to the proper positioning of an activation arm, and CK2 activity is predominantly regulated by expression levels. The high basal activity of CK2 would ensure that HDAC1 is constitutively phosphorylated and able to bind associated proteins and deacetylate.

The physiological significance of phosphorylation at Ser⁴²¹ and Ser⁴²³ by CK2 remains unclear. As suggested, CK2 phosphorylation may serve predominantly a structural role in organizing the catalytic and protein interaction sites on HDAC1. Another possibility is that CK2 phosphorylation of HDAC1

leads to additional post-translational modifications that are critical for HDAC1 function. In fact, hierarchical phosphorylation events often occur in highly charged regions, analogous to the highly charged, carboxyl-terminal tail of HDAC1 and involve kinases with acidotropic or basotropic substrate specificities, such as CK2 (60). Perhaps phosphorylation of HDAC1 facilitates its further modification by phosphorylation or acetylation, leading to a fully active protein.

HDAC1 and CK2 are thought to be involved in cancer development. CK2 concentrations are increased in rapidly proliferating tumors, including those in breast, prostate, and lung (58). Although the relationship between heightened CK2 activity and proliferating tumors is still unclear, CK2 phosphorylates a number of proteins possibly involved in tumor proliferation, such as Myc and p53 (58). We speculate that the enhanced CK2 concentrations in proliferating tumors may result in enhanced HDAC1 activity or an increased fraction of functionally active HDAC1. In the same way that unnaturally increased HDAC1 recruitment by retinoic acid receptor 1-promyelocytic leukemia zinc finger protein to DNA target sites in acute promyelocytic leukemia is linked to leukemia development, in this case by inhibiting the differentiation of promyelocytes into later lineages, enhanced HDAC1 activity in tumors may be involved in maintaining a proliferating state, characteristic of an early lineage cell type. In fact, the MTA-2 protein, associated with metastatic tumors, is an essential component of the HDAC1-containing NRD complex (40). Elucidating how CK2 modulates HDAC1 function *in vivo* is the first step toward understanding the role of HDAC1 and CK2 in cancer development.

Acknowledgments—We thank J. Neveu and K. Pierce for high pressure liquid chromatography and mass spectrometry and Christina M. Grozinger and Brad E. Bernstein for comments.

REFERENCES

- Lander, E., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., and Howland, J., et al. (2001) *Nature* **409**, 860–921
- Venter, J., Adam, M. D., Meyers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt, R. A., Gocayne, J. D., Amanatides, P., Ballew, R. M., Huson, D. H., and Wortman, J. R., et al. (2001) *Science* **291**, 1304–1351
- Grunstein, M. (1992) *Sci. Am.* **267**, 68–74
- Kornberg, R. D., and Klug, A. (1981) *Sci. Am.* **244**, 52–64
- Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) *Nature* **389**, 251–260
- Grunstein, M. (1997) *Nature* **389**, 349–352
- Van Lint, C., Emiliani, S., and Verdin, E. (1996) *Gene Exp.* **5**, 245–253
- Richon, V. M., Emiliani, S., Verdin, E., Webb, Y., Breslow, R., Rifkind, R. A., and Marks, P. A. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 3003–3007
- Vidal, M., and Gaber, R. F. (1991) *Mol. Cell. Biol.* **11**, 6317–6327
- Bernstein, B. E., Tong, J. K., and Schreiber, S. L. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 13708–13713
- Kouzarides, T. (1999) *Curr. Opin. Genet. Dev.* **9**, 40–48
- Hoshikawa, Y., Kwon, H. J., Yoshida, M., Horinouchi, S., and Beppu, T. (1994) *Exp. Cell Res.* **214**, 189–197
- Bartl, S., Taplick, J., Lager, G., Khier, H., Kuchler, K., and Seiser, C. (1997) *Mol. Cell. Biol.* **17**, 5033–5043
- Jung, M., Brosch, G., Kolle, D., Scherf, H., Gerhauser, C., and Loidl, P. (1999) *J. Med. Chem.* **42**, 4669–4679
- Medina, V., Edmonds, B., Young, G. P., James, R., Appleton, S., and Zalewski, P. D. (1997) *Cancer Res.* **57**, 3697–3707
- Salminen, A., Tapiola, T., Korhonen, P., and Suuronen, T. (1998) *Mol. Brain Res.* **61**, 203–206
- Cress, W. D., and Seto, E. (2000) *J. Cell. Physiol.* **184**, 1–16
- Grignani, F., De Matteis, S., Nervi, C., Tomassoni, L., Gelmetti, V., Cioce, M., Fanelli, M., Ruthardt, M., Ferrara, F. F., Zamir, I., Seiser, C., Grignani, F., Lazar, M. A., Minucci, S., and Pelicci, P. G. (1998) *Nature* **391**, 815–818
- Lin, R. J., Nagy, L., Inoue, S., Shao, W., Miller, W. H., and Evans, R. M. (1998) *Nature* **391**, 811–814
- Warrell, R. P., Jr., He, L. Z., Richon, V., Calleja, E., and Pandolfi, P. P. (1998) *J. Natl. Cancer Inst.* **90**, 1621–1625
- Kramer, O. H., Gottlicher, M., and Heinzl, T. (2001) *Trends Endocrinol. Metab.* **12**, 294–300
- Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996) *Science* **272**, 408–411
- Yang, W. M., Inouye, C., Zeng, Y., Bearss, D., and Seto, E. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 12845–12850
- Yang, W. M., Yao, Y. L., Sun, J. M., Davie, J. R., and Seto, E. (1997) *J. Biol. Chem.* **272**, 28001–28007

25. Hu, E., Chen, Z., Fredrickson, T., Zhu, Y., Kirkpatrick, R., Zhang, G., Johanson, K., Sung, C., Liu, R., and Winkler, J. (2000) *J. Biol. Chem.* **275**, 15254–15264
26. Van den Wyngaert, I., de Vries, W., Kremer, A., Neefs, J., Verhasselt, P., Luyten, W. H. M. L., and Kass, S. U. (2000) *FEBS Lett.* **478**, 77–83
27. Grozinger, C. M., Hassig, C. A., and Schreiber, S. L. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 4868–4873
28. Kao, H. Y., Downes, M., Ordentlich, P., and Evans, R. M. (2000) *Genes Dev.* **14**, 55–66
29. Zhou, X., Marks, P. A., Rifkind, R. A., and Richon, V. M. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 10572–10577
30. Imai, S., Armstrong, C. M., Kaerberlein, M., and Guarente, L. (2000) *Nature* **403**, 795–800
31. Frye, R. A. (1999) *Biochem. Biophys. Res. Commun.* **260**, 273–279
32. Grozinger, C. M., and Schreiber, S. L. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 7835–7840
33. McKinsey, T. A., Zhang, C. L., Lu, J., and Olson, E. N. (2000) *Nature* **408**, 106–111
34. McKinsey, T. A., Zhang, C. L., and Olson, E. N. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 14400–14405
35. Knoepfler, P. S., and Eisenman, R. N. (1999) *Cell* **99**, 447–450
36. Hassig, C. A., Fleischer, T. C., Billin, A. N., Schreiber, S. L., and Ayer, D. E. (1997) *Cell* **89**, 341–347
37. Zhang, Y., Iratni, R., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (1997) *Cell* **89**, 357–364
38. Zhang, Y., Sun, Z., Iratni, R., Erdjument-Bromage, H., Tempst, P., Hampsey, M., and Reinberg, D. (1998) *Mol. Cell* **1**, 1021–1231
39. Zhang, Y., LeRoy, G., Seelig, H., Lane, W. S., and Reinberg, D. (1998) *Cell* **95**, 279–289
40. Zhang, Y., Ng, H., Erdjument-Bromage, H., Tempst, P., Bird, A., and Reinberg, D. (1999) *Genes Dev.* **13**, 1924–1935
41. Tong, J. K., Hassig, C. A., Schnitzler, G. R., Kingston, R. E., and Schreiber, S. L. (1998) *Nature* **395**, 917–921
42. You, A., Tong, J. K., Grozinger, C. M., and Schreiber, S. L. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 1454–1458
43. Taunton, J., Collins, J. L., and Schreiber, S. L. (1996) *J. Am. Chem. Soc.* **118**, 10412–10422
44. Eng, J. K., McCormick, A. L., and Yates, J. R. I. (1994) *J. Am. Soc. Mass Spectrom.* **5**, 976–989
45. Chittum, H., Lane, W., Carlson, B., Roller, P., Lung, F., Lee, B., and Hatfield, D. (1998) *Biochemistry* **37**, 10866–10870
46. Northrop, J. P., Ullman, K. S., and Crabtree, G. R. (1993) *J. Biol. Chem.* **268**, 2917–2923
47. Carmen, A. A., Rundlett, S. E., and Grunstein, M. (1996) *J. Biol. Chem.* **271**, 15837–15844
48. Cai, R., Kwon, P., Yan-Neale, Y., and Sambuccetti, L. (2001) *Biochem. Biophys. Res. Commun.* **283**, 445–453
49. Hassig, C. A., Tong, J. K., Fleischer, T. C., Owa, T., Grable, P. G., Ayer, D. E., and Schreiber, S. L. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 3519–3524
50. Taplick, J., Kurtev, V., Kroboth, K., Posch, M., Lechner, T., and Seiser, C. (2001) *J. Mol. Biol.* **308**, 27–38
51. Huang, W., and Erikson, R. L. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 8960–8963
52. Lin, R., Beauparlant, P., Makris, C., Meloche, S., and Hiscott, J. (1996) *Mol. Cell. Biol.* **16**, 1401–1409
53. Kolle, D., Brosch, G., Lechner, T., Pipal, A., Helliger, W., Taplick, J., and Loidl, P. (1999) *Biochemistry* **38**, 6769–6773
54. Ng, H. H., and Bird, A. (2000) *Trends Biochem. Sci.* **25**, 121–126
55. Wen, Y. D., Perissi, V., Staszewski, L. M., Yang, W. M., Kronen, A., Glass, C. K., Rosenfeld, M. G., and Seto, E. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 7202–7207
56. Li, J., Wang, J., Wang, J., Nawaz, Z., Liu, J. M., Qin, J., and Wong, J. (2000) *EMBO J.* **19**, 4342–4350
57. Finnin, M. S., Donigian, J. R., Cohen, A., Richon, V. M., Rifkind, R. A., Marks, P. A., and Pavletich, N. P. (1999) *Nature* **401**, 188–193
58. Guerra, B., and Issinger, O. (1999) *Electrophoresis* **20**, 391–408
59. Guerra, B., Boldyreff, B., Sarno, S., Cesaro, L., Issinger, O., and Pinna, L. A. (1999) *Pharmacol. Ther.* **82**, 303–313
60. Roach, P. J. (1991) *J. Biol. Chem.* **266**, 14139–14142

Histone Deacetylase 1 Phosphorylation Promotes Enzymatic Activity and Complex Formation

Mary Kay H. Pflum, Jeffrey K. Tong, William S. Lane and Stuart L. Schreiber

J. Biol. Chem. 2001, 276:47733-47741.

doi: 10.1074/jbc.M105590200 originally published online October 15, 2001

Access the most updated version of this article at doi: [10.1074/jbc.M105590200](https://doi.org/10.1074/jbc.M105590200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 60 references, 25 of which can be accessed free at <http://www.jbc.org/content/276/50/47733.full.html#ref-list-1>