Journal of Cellular Biochemistry 104:2143–2154 (2008)

Histone Deacetylase 5 Represses the Transcription of Cyclin D3

Sangita Roy, Audrey C. Shor, Tapan K. Bagui, Edward Seto, and W. Jackson Pledger*

Molecular Oncology Program, Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, Florida 33612

Abstract Histone deacetylases (HDACs) modulate the transcription of a subset of genes by various means. HDAC5 is a class II HDAC whose subcellular location is signal-dependent. At present, its known gene targets are few in number. Here we identify a new HDAC5 target: the gene encoding the cell cycle-regulatory protein cyclin D3. When overexpressed in Balb/c-3T3 cells or mouse embryo fibroblasts, HDAC5 substantially reduced the activity of the cyclin D3 promoter and the abundance of endogenous cyclin D3 protein. Conversely, conditions that blocked HDAC5 function increased cyclin D3 expression: treatment of cells with the class I/II HDAC inhibitor trichostatin A (TSA), depletion of HDAC5 from cells by RNA interference, and cytoplasmic sequestration of HDAC5 by co-expression of catalytically active calcium/calmodulin-dependent protein kinase. HDAC5 interacted with the cyclin D3 promoter in vivo, and the HDAC5-responsive element was within 118 base pairs upstream of the transcription start site. Mutation of the Sp1 site and the cyclic AMP response element within this region did not affect the responsiveness of the cyclin D3 promoter to HDAC5 or TSA. J. Cell. Biochem. 104: 2143–2154, 2008.

Key words: cyclin D3; HDAC5; trichostatin A; fibroblasts

Histone deacetylases (HDACs) indirectly interact with gene promoters and repress or in some instances enhance transcription [Nusinzon and Horvath, 2005; Clayton et al., 2006]. The canonical HDACs are divided into two phylogenetic groups: class I (HDACs 1, 2, 3 and 8, related to the yeast deacetylase Rpd3) and class II (HDACs 4, 5, 6, 7, 9, and 10, related to the yeast deacetylase Hda1) [Thiagalingam et al., 2003]. Another HDAC, HDAC11, shares homology with both class I and class II HDACs [Gao et al., 2002]. Determinants of HDAC activity include post-translational modifications and interactions with co-repressors or other proteins [Thiagalingam et al., 2003]. As an additional regulatory mechanism, HDACs 4 and 5 shuttle between the nucleus and the cytoplasm in a signal-dependent manner [Grozinger and Schreiber, 2000; McKinsey et al., 2000a].

Grant sponsor: NIH; Grant numbers: CA93544, CA67360.

*Correspondence to: W. Jackson Pledger, Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, FL 33612.
E-mail: jack.pledger@moffitt.org

Received 14 January 2008; Accepted 28 February 2008
DOI 10.1002/jcb.21771

Sequence-specific transcription factors on gene promoters recruit HDACs to promoters, where they deacetylate surrounding histones [Luo and Dean, 1999]. Such factors include Sp1 and CREB (cyclic AMP response element binding protein) [Doetzlhofer et al., 1999; Canettieri et al., 2003]. Consequences of histone deacetylation in the context of transcriptional repression are: *one*, localized chromatin condensation and reduced access of basal transcription factors to promoters, and *two*, removal of docking sites (i.e., acetyl groups) for co-activators or basal transcription factors [Yang, 2004; Clayton et al., 2006]. How HDACs activate transcription is unclear; this process may involve the concerted actions of HDACs and histone acetyltransferases and perhaps other histone-modifying enzymes [Nusinzon and Horvath, 2005]. HDACs also modulate transcription by deacetylating non-histone proteins; HDAC substrates include the transcription factors p53 and E2F, which are inactive when deacetylated [Gu and Roeder, 1997; Juan et al., 2000; Martinez-Balbas et al., 2000; Marzio et al., 2000].

HDACs do not repress (or activate) transcription globally: expression analyses of cells treated with inhibitors of class I/II HDACs estimate the percentage of HDAC-responsive

genes as 2% to 7% [Van Lint et al., 1996; Mariadason et al., 2000; Della Ragione et al., 2001]. Less is known about the gene targets of class II HDACs than of class I HDACs. Most notably, HDACs 4, 5, 7 and 9 inactivate genes involved in myogenesis by interacting with the MEF2 transcription factors [Miska et al., 1999; Lemercier et al., 2000; Lu et al., 2000a,b; McKinsey et al., 2002].

Here we identify a novel target of HDAC5: the gene encoding the cell cycle-regulatory protein cyclin D3. We show that HDAC5 interacts with the proximal region of the cyclin D3 promoter in vivo and represses promoter activity in mouse fibroblasts. Trichostatin A (TSA), an inhibitor of class I/II HDACs [Yoshida et al., 1990], increases promoter activity, and responsiveness to TSA and HDAC5 does not require intact Sp1 binding sites or the cyclic AMP response element (CRE) in the proximal region of the promoter.

MATERIALS AND METHODS

Cell Culture

Balb/c-3T3 mouse fibroblasts were cultured in Dulbecco's modified Eagle's medium supplemented with 4 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, and 10% calf serum. Mouse embryo fibroblasts (MEFs) were prepared from 15- or 16-day-old embryos. After removal of the head and internal organs, embryos were minced and plated individually in 100 mm tissue culture dishes containing medium supplemented with 10% fetal calf serum.

Cyclin D3 Reporter Assays

The full-length cyclin D3 promoter was generated from mouse genomic DNA by PCR (forward primer: 5′ TAGGCAGGCCTTGAATTGTCTGTA 3′; reverse primer: 5′ ACTCTT GCTCTCACCCGTGATAG 3′) and was cloned into the luciferase reporter plasmid pGL3-Basic at KpnI and SmaI sites. The promoter sequence contains 993 base pairs upstream and 96 base pairs downstream of the transcription start site defined by Wang et al. [1996]. Exponentially growing cells in medium containing 10% serum were co-transfected with 3 μg reporter plasmid and 25 ng pCMV-β-galactosidase using lipofectamine according to the instructions of the manufacturer (Invitrogen).

Mutated sites for primers are underlined. Primers used for mutation of the distal Sp1 site (−129) [Wang et al., 1996] of the cyclin D3 promoter are: forward, 5′ GCCCTTTCCGACTGCGGCCAATCACCTTAGAACGTTGTGACGTAGG 3′; reverse, 5′ CCTACGTCACAACGTTCTAAGGTGATTGGCCGCAGTCGGAAAGGGC 3′.

Primers used for mutation of the proximal Sp1 site (−72) are: forward, 5′ GCTACATCGTCGCGAGGGTTTAGTCGCCTGTCAGGGAAGCGG 3′; reverse, 5′ CCGCTTCCCTGACAGGCGACTAAACCCTCGCGACGATGTAGC 3′.

Primers used for mutation of the GC-rich site (−155) are: forward, 5′ GGAGGGAGGGTATAGCGTTTGCCAGCCCTTTCCGACTGCGG 3′; reverse, 5′ CCGCAGTCGGAAAGGGCTGGCAAACGCTATACCCTCCCTCC 3′.

Primers used for mutation of the CRE (−109) are: forward, 5′ CCCCTTAGAACGTTGCAGCGTAGGAGCATTCCA 3′; reverse, 5′ TGGAATGCTCCTACGCTGCAACGTTCTAAGGGGCGGG 3′.

Ribonuclease Protection Assay (RPA)

Total RNA was isolated from cells in Trizol reagent (Gibco BRL). RPAs were performed using the RiboQuant MultiProbe RNase Protection Assay System and a custom-made probe set (PharMingen).

Western Blotting

Cells were rinsed with phosphate-buffered saline (PBS), harvested by scraping, and collected by centrifugation. Cell pellets were resuspended in lysis buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.1% Tween-20, 10% glycerol, 1 mM NaF, 0.1 mM vanadate, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml leupeptin, and 1 mM dithiothreitol) and incubated on ice for 15 min. Insoluble material was removed by centrifugation. Cell extracts normalized for amount protein were resolved by SDS–PAGE and transferred to nitrocellulose membranes. Membranes were blocked in PBS containing 0.1% Tween-20 and 5% instant milk and incubated with primary antibody in PBS containing 0.1% Tween for 2 h at room temperature or overnight at 4°C. Proteins recognized by the primary antibody were detected by enhanced chemiluminescence using a horseradish peroxidase-coupled secondary antibody according to the instructions of the manufac-

turer (Pierce). Cyclin D3, HDAC5, and Flag antibodies were obtained from Sigma.

Chromatin Immunoprecipitation Assay (ChIP)

Formaldehyde was added to the culture medium to a final concentration of 1%, and cells were incubated for 10 min at room temperature with gentle shaking to crosslink DNA and associated proteins. Glycine was added to a final concentration of 125 mM, and cells were rocked for 5 min. Cells were lysed in a buffer containing 5 mM Pipes (pH 8.0), 85 mM KCL, 0.5% NP-40, 1 mM Na_3VO_4, 20 mM NaF, 1 mM $Na_4P_2O_7$, 0.5 mM PMSF, 0.1 mM dithiothreitol, and EDTA-free protease inhibitor cocktail (Roche Diagnostics). Pellets were washed with lysis buffer without NP-40 and incubated for 10 min on ice in a buffer containing 50 mM Tris (pH 8.1), 1% SDS, 10 mM EDTA, 0.5 mM PMSF, and protease inhibitors. Pellets were sonicated, and debris was removed by centrifugation at 13,000 rpm for 10 min at 4°C.

Supernatants were diluted 10-fold in ChIP dilution buffer (16.7 mM Tris, pH 8.1, 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, and 167 mM NaCl) and precleared with protein A/G-plus agarose. Samples were incubated overnight with antibody and subsequently with protein A/G-plus agarose for 2 h at 4°C. Immunocomplexes were washed sequentially with TSE-500 buffer (20 mM Tris, pH 8.1, 0.1% SDS, 1% Triton X-100, and 2 mM EDTA), LiCl wash buffer (100 mM Tris, pH 8.1, 500 mM LiCl, 1% NP-40, and 1% sodium deoxycholate), and TE buffer (10 mM Tris, pH 8.0, and 1 mM EDTA). Immunocomplexes were removed from the agarose by two elutions, each with 150 μl 1% SDS and 50 mM $NaHCO_3$.

After addition of 30 μl of 5 M NaCl (final concentration, 500 mM), eluates were heated overnight at 65°C to reverse protein-DNA crosslinks. Samples were incubated for 1 h at 37°C with proteinase K and RNase ONE to remove protein and any contaminating RNA. DNA was recovered by phenol-chloroform extraction and ethanol precipitation.

Primers for PCR amplification were: 5′ CCCTCCAGAAAGTTCTCTCTACCC 3′ (forward) and 5′ CCCTCGCGACGATGTAGCAACCGTG 3′ (reverse). These primers amplify a 201 bp fragment of the cyclin D3 promoter beginning 269 bp upstream of the transcription start site. PCR was carried out for 30 cycles (95°C for 1 min, 55°C for 30 s, and 72°C for 30 s). PCR products were resolved on 2% agarose gels.

Immunostaining

Cells were rinsed with PBS and fixed with 4% paraformaldehyde for 5 min at room temperature. Fixed cells were rinsed with PBS and incubated in blocking buffer (10% goat serum, 3% bovine serum albumin, and 0.1% Triton X-100 in PBS) for 1 h at room temperature. Cells were incubated with antibody to Flag in blocking buffer at 4°C overnight and washed three times, 30 min/wash, with wash buffer (1% goat serum, 3% bovine serum albumin, 0.1% Triton X-100 in PBS). Cells were incubated with anti-mouse horse radish peroxidase-linked antibody in blocking buffer for 1 h at room temperature and washed as above. Cells were stained with DAPI to indicate nuclei.

Electrophoretic Mobility Shift Assay (EMSA)

Cells were lysed in a buffer containing 20 mM Hepes, pH 7.9, 1 mM EDTA, 1 mM EGTA, and 1% NP-40. After centrifugation, cell pellets were resuspended in a buffer containing 20 mM Hepes, pH 7.9, 1 mM EDTA, 1 mM EGTA, 420 mM NaCl, and 20% glycerol and were incubated for 30 min on ice to extract nuclear proteins. For binding reactions, nuclear extracts (7 μg) were incubated for 30 min at room temperature in a buffer containing 10 mM Hepes, pH 7.9, 50 mM KCl, 1 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 0.5 μg/ml bovine serum albumin, 1.8 μg salmon sperm DNA, and double-stranded, end-labeled oligonucleotide probe. Samples were electrophoresed on 5% polyacrylamide gels containing 0.25× TBE and 2.5% glycerol.

RESULTS

TSA Increases the Activity of the Cyclin D3 Promoter

The D cyclins (D1, D2, D3) and their cyclin-dependent kinase (CDK) partners (CDK4 and CDK6) elicit events required for the entry of cells into S phase [Olashaw and Pledger, 2002]. Microinjection of cells with neutralizing antibodies to the D cyclins inhibits G1 progression, whereas overexpression of the D cyclins reduces G1 transit time [Baldin et al., 1993; Quelle et al., 1993; Bartkova et al., 1998; Herzinger and Reed, 1998]. Transcriptional activation and

transcriptional repression are key determinants of D cyclin abundance; thus knowledge of the mechanisms controlling these processes is important. Our studies focus on the contribution of HDACs to cyclin D3 expression.

Effects of TSA on cyclin D3 promoter activity were determined for cycling Balb/c-3T3 cells and MEFs. Cells were transfected with a luciferase reporter plasmid (pGL3-Basic) containing the full-length mouse cyclin D3 promoter (−993 to +96 relative to the transcription start site). Forty-eight hours after transfection, cells received 10 ng/ml TSA for 8 h. As shown in Figure 1A (left and middle panels), TSA increased cyclin D3 promoter activity more than fourfold in both Balb/c-3T3 cells and MEFs.

TSA also activated the cyclin D3 promoter when added to quiescent cells (Fig. 1A, right panel). To induce quiescence, Balb/c-3T3 cells were serum-starved for 24 h before exposure to TSA. G1 arrest was confirmed by flow cytometry of cells stained with propidium iodide (data not shown). Fold stimulation by TSA was similar in cycling and quiescent cells.

Effects of TSA on the abundance of cyclin D3 mRNA and protein were determined by RPA and Western blotting, respectively. Growing Balb/c-3T3 cells and MEFs received TSA for the indicated times (Fig. 1B). Consistent with its stimulatory actions on cyclin D3 promoter activity, TSA increased amounts of cyclin D3 mRNA (top panels) and protein (bottom panels) in both populations. Increases in mRNA abundance were observed 3–6 h after addition of TSA to cells; increases in protein abundance were observed within 8 h. Collectively, the data in Figure 1A,B link HDAC inactivation to cyclin D3 expression.

Three additional inhibitors were tested: AG490, which inhibits Janus kinase activity, and PD98059 and U0126, which inhibit MEK activity; MEK phosphorylates and activates ERK1 and ERK2. None of these inhibitors affected the activity of the cyclin D3 promoter in Balb/c-3T3 cells or MEFs (Fig. 1C).

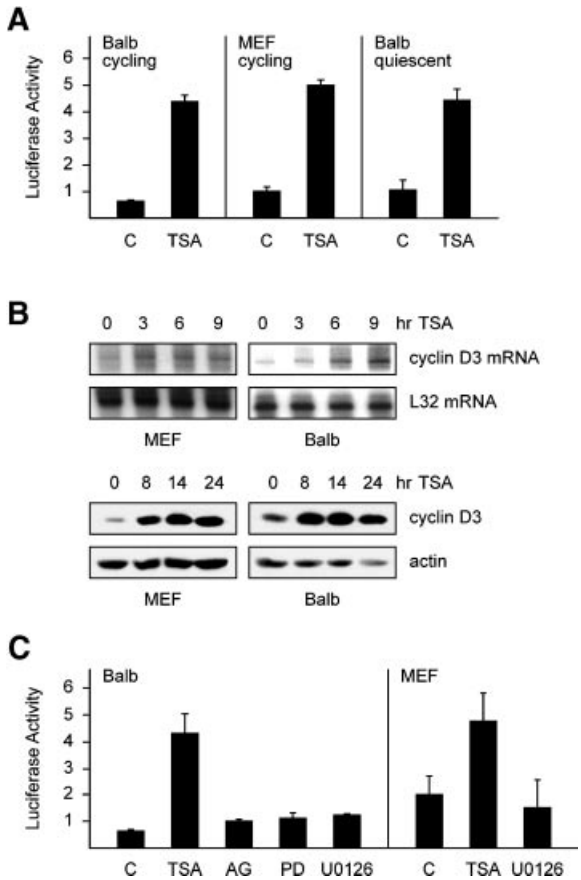

Fig. 1. TSA increases cyclin D3 expression. **A**: Exponentially proliferating Balb/c-3T3 cells and MEFs were transfected with pGL3-Basic containing the full-length mouse cyclin D3 promoter. Cycling cells: 48 h after transfection, cells received 10 ng/ml TSA for 8 h. Quiescent cells: cells prestarved in medium containing 0.1% serum for 24 h received TSA for 8 h. Luciferase activity is normalized to β-galactosidase activity, and error bars show standard deviation. C, control. **B**: Cycling Balb/c-3T3 cells and MEFs received 10 ng/ml TSA for the indicated times. **Top panels**: Amounts of cyclin D3 mRNA and L32 mRNA (loading control) were determined by RPA. **Bottom panels**: amounts of cyclin D3 and actin (loading control) were determined by Western blotting. **C**: Cycling Balb/c-3T3 cells and MEFs received 10 ng/ml TSA (positive control), 25 μM AG490 (AG), 76 μM PD98059 (PD), or 10 μM U0126 for 8 h. Luciferase activity is normalized to β-galactosidase activity, and error bars show standard deviation. C, control.

HDAC5 Represses the Activity of the Cyclin D3 Promoter

To determine which HDACs repress the activity of the cyclin D3 promoter, we coexpressed the full-length cyclin D3 promoter and Flag-tagged HDACs 1–11 in MEFs. Western blots with anti-Flag antibody verified expression of all HDACs (Fig. 2A). Of the HDACs, HDAC5 was the most potent repressor of cyclin D3 promoter activity. Activity in cells expressing Flag-HDAC5 was approximately 15% of that of cells expressing vector alone. Although less effective, three additional class II HDACs (HDACs 4, 6, and 7) also reduced promoter activity, as did the class I HDAC, HDAC8. Interestingly, and for reasons

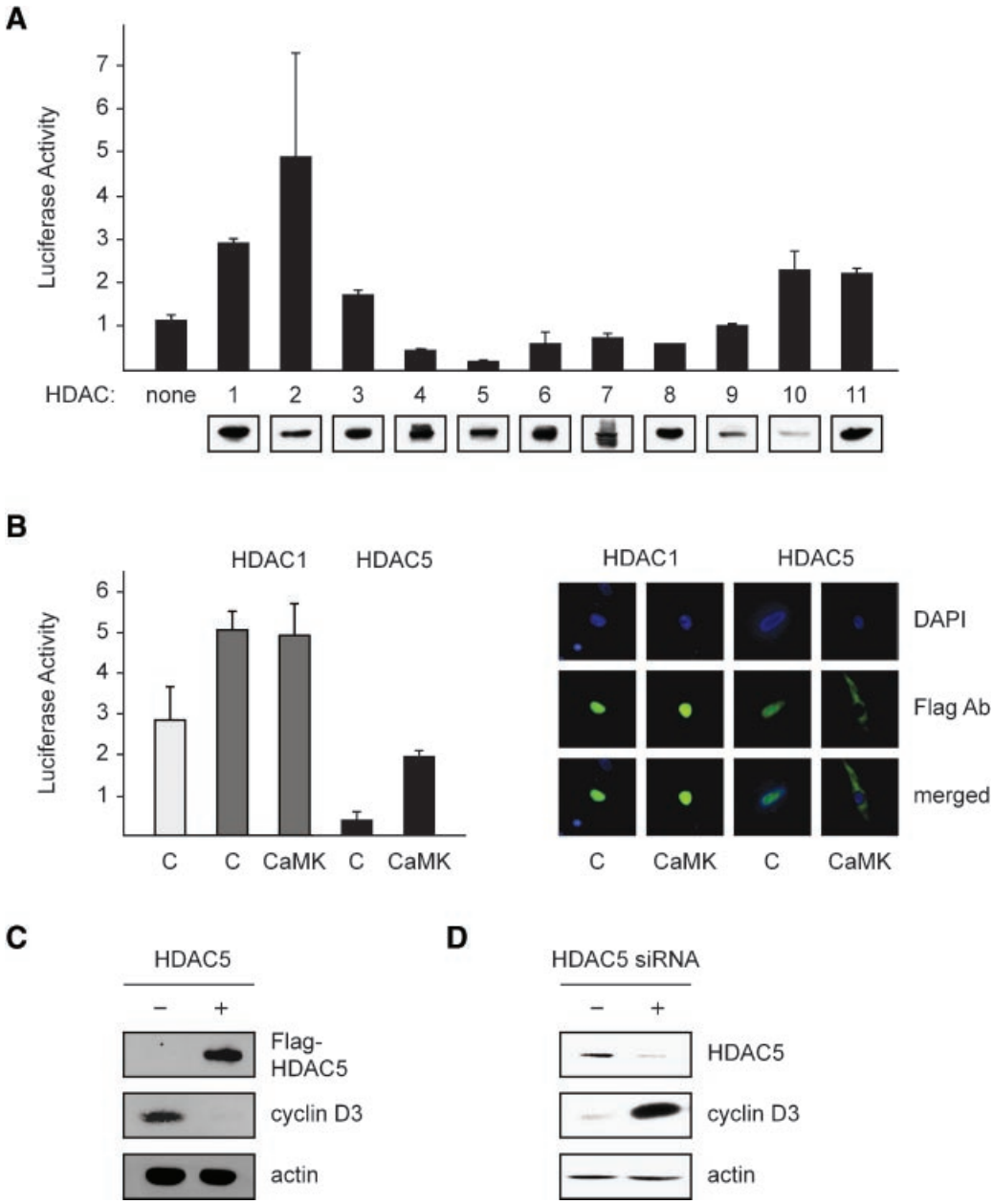

Fig. 2. HDAC5 represses cyclin D3 expression. **A**: MEFs were co-transfected with pGL3-Basic containing the full length cyclin D3 promoter and pcDNA3.1 encoding Flag-tagged HDACs 1–11. Cells were harvested 48 h after transfection. Bar graph: luciferase activity is normalized to β-galactosidase activity, and error bars show standard deviation. Western blots: cell extracts were immunoblotted with antibody to Flag to monitor amounts of ectopic proteins. **B**: Balb/c-3T3 cells were transfected with pGL3-Basic containing the full length cyclin D3 promoter and with plasmids encoding Flag-HDAC1 (HDAC1), Flag-HDAC5 (HDAC5), and CaMK as indicated. **Left panel**: luciferase activity was determined 48 h after transfection and is normalized to β-galactosidase activity. Error bars show standard deviation. **Right panel**: cells were incubated with antibody to Flag and secondary antibody and co-stained with DAPI 48 h after transfection. C, control. **C**: MEFs were infected with adenovirus alone (−) or adenovirus expressing Flag-HDAC5 (+). Cells were harvested 48 h after infection. Amounts of Flag-HDAC5, cyclin D3 and actin were determined by Western blotting. **D**: MEFs were incubated with lipofectamine and HDAC5 siRNA (+) or control oligonucleotides (−) (Dharmacon) for 72 h. Amounts of HDAC5, cyclin D3, and actin were determined by Western blotting.

unknown, HDACs 1 and 2 increased promoter activity somewhat.

HDAC5 translocates from the nucleus to the cytoplasm when phosphorylated by calcium/calmodulin-dependent protein kinase (CaMK) [McKinsey et al., 2000a]. As shown here, co-expression of active CaMK in Balb/c-3T3 cells partially reversed the repression of cyclin D3

promoter activity by Flag-HDAC5 (Fig. 2B, left panel). Flag-HDAC1, which is not targeted by CaMK, had similar (and slightly stimulatory) effects on cyclin D3 promoter activity in both the presence and absence of ectopic CaMK. Immunofluorescence confirmed the nuclear location of HDAC5, HDAC1, and HDAC1 plus CaMK and the cytoplasmic location of HDAC5 plus CaMK (right panel).

Consistent with transcriptional repression of the cyclin D3 gene, amounts of cyclin D3 protein were subnormal in cells overexpressing HDAC5 (Fig. 2C). In these experiments, MEFs were infected with adenovirus alone or adenovirus expressing Flag-HDAC5; 60% of cells were infected as monitored by immunofluorescence with green fluorescent protein (data not shown). Conversely, cyclin D3 accumulated in cells depleted of endogenous HDAC5 by RNA interference. As shown in Figure 2D, MEFs transfected with HDAC5 siRNA oligonucleotides expressed less HDAC5 (thus confirming its depletion) and more cyclin D3 than did cells expressing control oligonucleotides.

Localization of the TSA- and HDAC5-Responsive Region of the Cyclin D3 Promoter

To map the region(s) of the cyclin D3 promoter targeted by TSA and HDAC5, the cyclin D3 promoter was serially deleted from its 5′ end. Deletion mutants in pGL3-Basic were expressed in Balb/c-3T3 cells and MEFs, and cells received TSA for 8 h. Deletion to −168 did not affect promoter responsiveness to TSA in either cell line (Fig. 3A,B). Further deletion to −68 substantially reduced both basal and TSA-stimulated activity in both cell lines. An internal deletion of base pairs −268 to −68 also reduced basal and stimulated activity (Fig. 3B). These findings place the TSA-responsive element downstream of −168. Interestingly, deletion of the region between −368 and −268

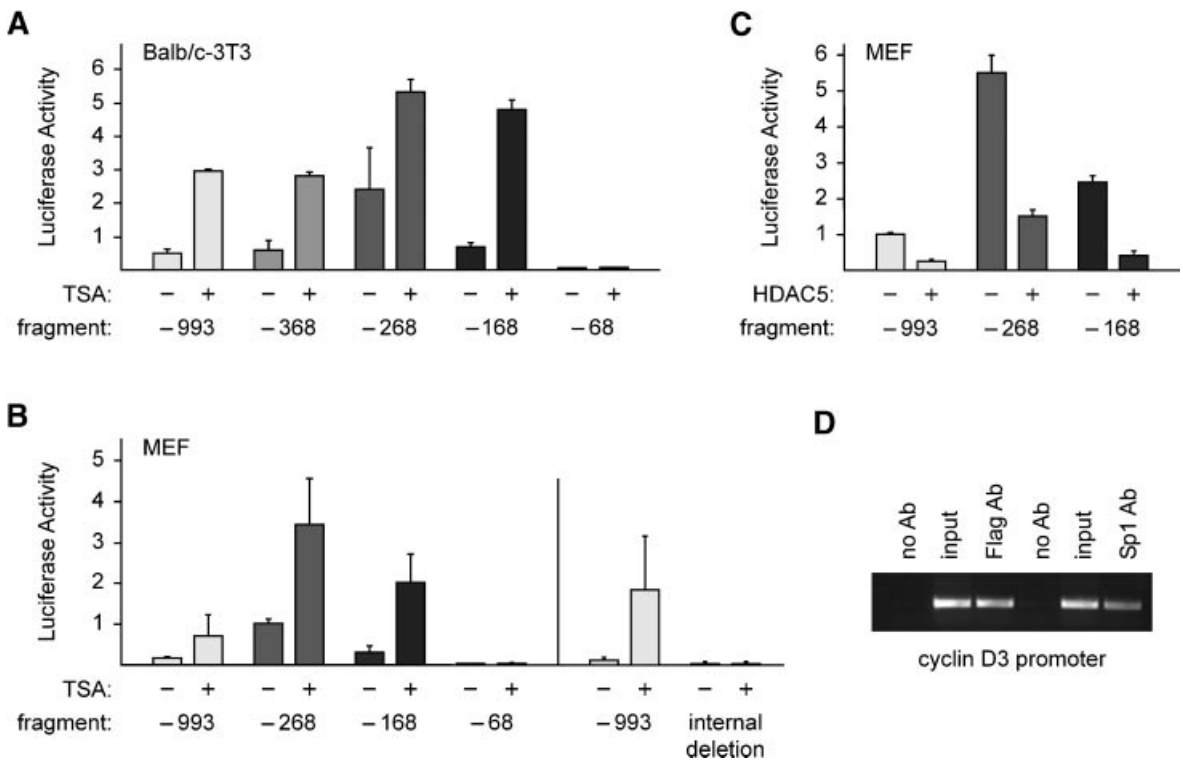

Fig. 3. Localization of the TSA- and HDAC5-responsive region of the cyclin D3 promoter. **A**,**B**: Balb/c-3T3 cells (A) and MEFs (B) were transfected with pGL3-Basic containing the full length cyclin D3 promoter (−993) or promoters serially deleted from the 5′ end. MEFs were also transfected with the full length promoter containing an internal deletion (−268 to −68). Cells received 10 ng/ml TSA 48 h after transfection and were harvested 8 h thereafter. Luciferase activity is normalized to β-galactosidase activity, and error bars show standard deviation. **C**: MEFs were co-transfected with pGL3-Basic containing the indicated cyclin D3 promoter constructs and pcDNA3.1 encoding HDAC5. Luciferase activity is normalized to β-galactosidase activity, and error bars show standard deviation. **D**: Flag-tagged HDAC5 was expressed in MEFs by transient transfection. ChIP assays were performed as described in Materials and Methods Section. Cross-linked chromatin was immunoprecipitated with antibody to Flag or Sp1, and PCR was performed using primers that generate the −269 to −68 region of the cyclin D3 promoter.

markedly increased promoter activity in both the presence and absence of TSA, thus indicating the presence of a repressor element in this region.

When overexpressed in MEFs, HDAC5 repressed the activity of two 5′ truncated cyclin D3 promoters, −268 and −168 (Fig. 3C). Thus, HDAC5 and TSA target a similar region of the promoter. Moreover, chromatin immunoprecipitation (ChIP) assays show that HDAC5 interacts with the cyclin D3 promoter in vivo (Fig. 3D). In this experiment, Flag-tagged HDAC5 was expressed in MEFs, crosslinked chromatin was immunoprepitated with antibody to Flag, and PCR was performed using primers that generate the −269 to −68 region of the cyclin D3 promoter. Antibody to Flag coprecipitated this region of the promoter, as did antibody to Sp1.

Mutation of Sp1 Sites

To more precisely map the TSA-responsive region of the cyclin D3 promoter, we deleted the promoter in 25 bp increments beginning at −168. Deletion mutants in pGL3-Basic were expressed in Balb/c-3T3 cells, and cells received TSA for 8 h before harvest. The −168, −143, and −118 promoter fragments were responsive to TSA (Fig. 4A). The basal activity of the −93 promoter fragment was near baseline; thus, whether TSA affects its activity is unclear. Similar results were obtained for MEFs (data not shown). These data further delineate the location of the TSA response element of the cyclin D3 promoter: it is downstream of −118.

The TSA-responsive region of the cyclin D3 promoter contains an Sp1 binding site at −72 [Wang et al., 1996], and our studies show interaction of Sp1 with the proximal region of the cyclin D3 promoter in vivo (see Fig. 3D). Sp1 sites are GC or GT boxes; they are present in numerous promoters including those involved in growth regulation [Black et al., 2001; Kaczynski et al., 2003]. Members of the Sp/Krüppel-like factor family (e.g., Sp1) bind Sp1 sites to activate or repress transcription. All family members have highly conserved C terminal DNA-binding domains containing

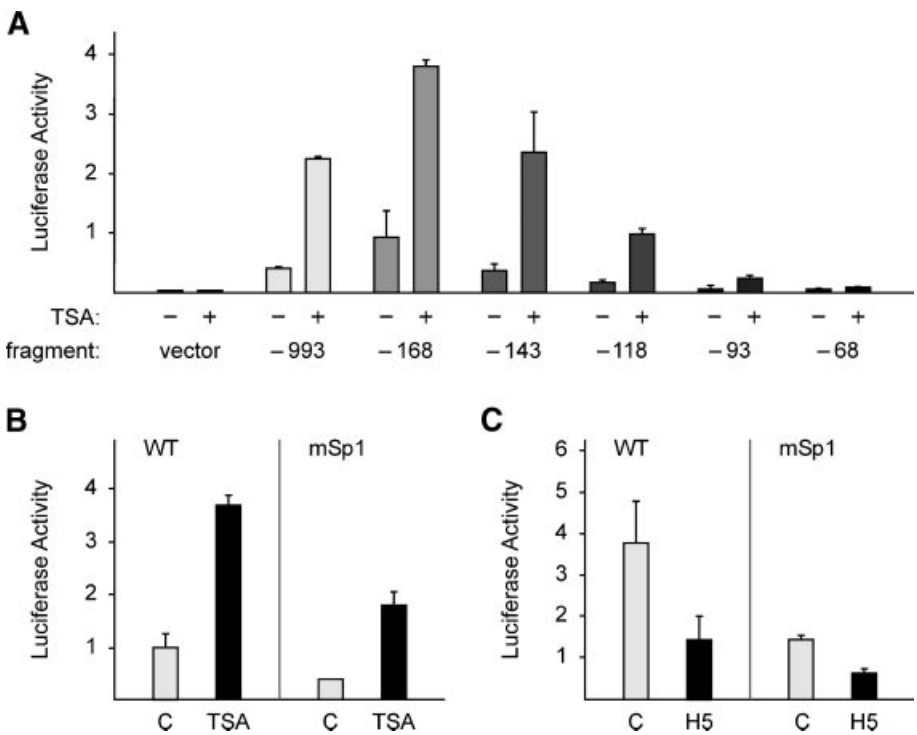

Fig. 4. Lack of effect of Sp1 mutations on the responsiveness of the cyclin D3 promoter to TSA and HDAC5. **A**: Balb/c-3T3 cells were transfected with vector alone, the full length cyclin D3 promoter (−993), or the indicated truncated promoters. Cells received 10 ng/ml TSA for 8 h before harvest. **B**,**C**: MEFs were transfected with pGL3-Basic containing the full-length cyclin D3 promoter, either wild-type (WT) or containing mutations of two Sp1 sites (−129 and −72) and the GC-rich region (−155) (mSp1). Cells received 10 ng/ml TSA for 8 h before harvest (B) or were co-transfected with pcDNA3.1 encoding HDAC5 (H5) (C). C, control. A–C: Luciferase activity is normalized to β-galactosidase activity, and error bars show standard deviation.

three zinc fingers. Previous studies show binding of Sp1 to oligonucleotides containing the −72 site [Wang et al., 1999]. Sp1 associates with class I and (at least in vitro) class II HDACs including HDAC5 [Margueron et al., 2003].

We mutated the −72 site within the context of the full-length cyclin D3 promoter. This construct also contained mutations in the Sp1 site at −129 and in the GC-rich region at −155 (whether this region is a bona fide Sp1 site is unclear). The wild-type and the mutant promoters were expressed in MEFs by transient transfection. Cells were either co-transfected with HDAC5 or treated with TSA for 8 h before harvest. Although less active than the wild-type promoter, the mutant promoter responded to TSA and HDAC5 in the same way as did the wild-type promoter: TSA increased its activity (Fig. 4B), whereas HDAC5 reduced its activity (Fig. 4C). This finding indicates that Sp1 sites do not mediate the effects of TSA and HDAC5 on the activity of the cyclin D3 promoter.

Mutation of the CRE

As another possibility, we examined the CRE at −109. This site is within the TSA-responsive region (see Fig. 4A) and is protected in footprinting analyses [Wang et al., 1999]. Its sequence (TGACGTAG) diverges somewhat from the canonical palindromic CRE sequence (TGACGTCA); however, half-sites (TGACG and CGTAG) are also functional [Mayr and Montminy, 2001; Smith et al., 2007]. We show that a oligonucleotide probe corresponding to the −118 to −93 region of the cyclin D3 promoter associates with a protein(s) in MEF nuclear extracts (EMSA, Fig. 5A). Excess cold wild-type probe abolished the shift, whereas an excess of an unrelated probe (the serum-inducible element) did not. Mutations within the CRE prevented (Fig. 5B, lanes 4, 5) or substantially reduced (#6) complex formation, whereas those outside the CRE did not (lanes 1, 2, 3, 7, 8). These findings show that MEF nuclear extracts

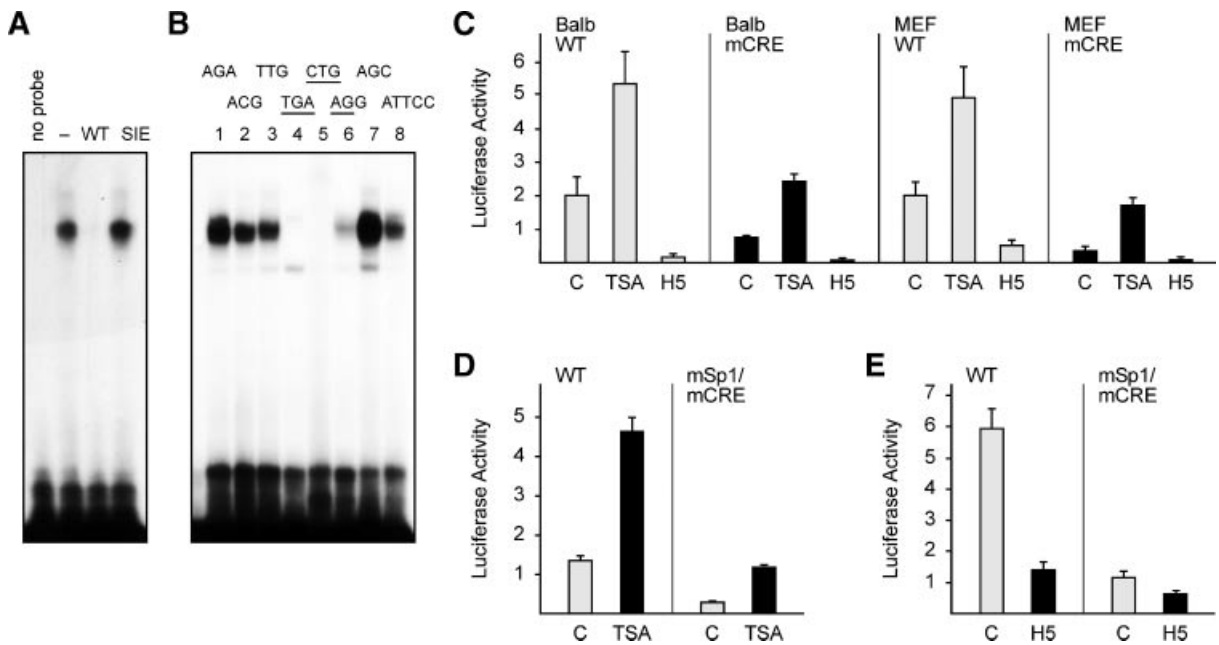

Fig. 5. Lack of effect of CRE mutations on the responsiveness of the cyclin D3 promoter to TSA and HDAC5. **A**: EMSAs were performed as described in Materials and Methods Section. Nuclear extracts of MEFs were incubated with a radiolabeled double-stranded oligonucleotide probe corresponding to the −118 to −93 region of the cyclin D3 promoter. For competitions, reaction mixtures contained a 100-fold excess of unlabeled probe corresponding to the radiolabeled probe (WT) or a 100-fold excess of an unrelated probe (SIE). **B**: Sequential triple mutations were made in the −118 to −93 sequence (#1 to 7). For #8, the final five bases were all mutated. Mutated probes were radiolabeled and EMSAs were performed. The sequence of the −118 to −93 region is shown; the CRE is underlined. **C**: Balb/c-3T3 cells and MEFs were transfected with wild-type cyclin D3 promoter (WT) or mutant promoter containing a CRE mutation (the same mutation as in the #5 probe in B) (mCRE). Cells received 10 ng/ml TSA for 8 h before harvest or were co-transfected with HDAC5 (H5). C, control. **D**,**E**: MEFs were transfected with wild-type cyclin D3 promoter (WT) or mutant promoter containing mutations in the two Sp1 sites, the GC-rich site, and the CRE (mSp1/mCRE). Cells received 10 ng/ml TSA 8 h before harvest (D) or were co-transfected with HDAC5 (E). C–E: Luciferase activity is normalized to β-galactosidase activity, and error bars show standard deviation.

contain a protein that specifically interacts with the CRE. Proteins that bind the CRE include CREB and CREM (CRE modulator); both proteins associate with HDACs [Mayr and Montminy, 2001; Guan et al., 2002; Canettieri et al., 2003; Tenbrock et al., 2006].

To assess the need for the CRE for HDAC responsiveness, we mutated the CGT site of the CRE in the context of the full-length cyclin D3 promoter. As shown in Figure 5B (lane 5), this mutation abolishes the protein-binding activity of the CRE. Balb/c-3T3 cells and MEFs were transfected with mutant or wild-type promoter and either co-transfected with HDAC5 or treated with TSA. Results were similar for both cell lines: reduced basal activity of the mutant promoter, and increases and decreases in activity in response to TSA and HDAC5, respectively (Fig. 5C). We also mutated the cyclin D3 promoter at all four tested sites: both Sp1 sites, the GC-rich site, and the CRE. The quadruple mutation did not negate the effects of TSA (Fig. 5D) or HDAC5 on promoter activity (Fig. 5E). Collectively, our studies show that these agents regulate the activity of the cyclin D3 promoter via elements other than Sp1 and CRE.

DISCUSSION

Our data show that TSA increases the activity of the cyclin D3 promoter and the abundance of cyclin D3 mRNA and protein in Balb/c-3T3 cells and MEFs. We suggest that promoter activation contributes to protein accumulation but do not exclude post-translational events. Others demonstrated stabilization of cyclin D3 by HDAC inhibitors in other systems [Blottiere et al., 2003; Florenes et al., 2004]. Stabilization may result from the interaction of cyclin D3 with the cell cycle inhibitor $p21^{Cip1}$. HDAC inhibitors increase expression of $p21^{Cip1}$ [Nakano et al., 1997; Richon et al., 2000; Sambucetti et al., 1999; Sowa et al., 1999], and as shown by us and others, D cyclins are more stable when complexed to $p21^{Cip1}$ [Cheng et al., 1999; Bagui et al., 2003].

Our studies implicate HDAC5 in the repression of cyclin D3 expression. First, HDAC5 interacted with the proximal region of the cyclin D3 promoter in vivo. Second, depletion of HDAC5 by RNA interference increased cyclin D3 abundance. Third, overexpression of HDAC5 reduced cyclin D3 abundance and the activity of the cyclin D3 promoter. Co-expression of CaMK, which results in cytosolic sequestration of HDAC5, partially reversed the inhibitory effects of HDAC5 on promoter activity. In addition to HDAC5, HDACs 4, 6, 7, and 8 also inhibited the activity of the cyclin D3 promoter although to a lesser extent than did HDAC5.

At present, investigators have identified only a handful of HDAC5-responsive genes. Most notable are those whose products signal myoblast differentiation [Lemercier et al., 2000; Lu et al., 2000a; McKinsey et al., 2000b]. Other HDAC5-repressed genes encode osteocalcin [Kang et al., 2005] and major histocompatibility complex II [McKinsey et al., 2006], which are involved in osteoblast differentiation and antigen presentation, respectively. Our studies add the gene encoding cyclin D3 to the list of HDAC5-modulated genes.

Deletion analysis of the cyclin D3 promoter localized the TSA-activated, HDAC5-repressed element to the proximal region of the cyclin D3 promoter (i.e., downstream of −118). As reported by others, this region contains an Sp1-binding site and a CRE [Wang et al., 1999]. Both sites are protected from nuclease digestion in footprinting experiments [Wang et al., 1999]. Sp1 interacts with both class I and class II HDACs and recruits HDACs to several promoters (e.g., the telomerase, tyrosine hydroxylase, and HMG-CoA promoters) [Takakura et al., 2001; Choi et al., 2002; Camarero et al., 2003; Kim et al., 2003; Margueron et al., 2003]. Our data show interaction of Sp1 with the proximal region of the endogenous cyclin D3 promoter. Two CRE-binding proteins interact with HDACs: CREB and CREM. CREB associates with HDAC1 and HDAC2 (but not HDAC5) in HEK293 cells [Canettieri et al., 2003]; CREB2 associates with HDAC5 in neurons [Guan et al., 2002]; and CREMα associates with HDAC1 in T cells [Tenbrock et al., 2006]. HDAC inhibitors up-regulate the promoters for γ-globin, tyrosine hydroxylase, and interleukin-2 receptor β via the CRE [Shao et al., 2002; Kim et al., 2003; Sangerman et al., 2006].

Despite precedent for HDAC recruitment to Sp1 sites and CREs, mutation of these sites did not abrogate the responsiveness of the cyclin D3 promoter to TSA or HDAC5. Thus, they do not mediate the effects of TSA and HDAC5 on promoter activity in fibroblasts. In contrast, the HDAC inhibitor apicidin activated the cyclin D3 promoter in colon carcinoma cells in

an Sp1-dependent manner; in this system, promoter activation also required protein kinase C activity [Kim et al., 2007]. Whether differences in cell types account for the difference between these data and ours is not known at present.

In addition to the Sp1 site and the CRE, the TSA-responsive region of the cyclin D3 promoter also contains binding sites for NF-κB and GATA-1 and GATA-2 (identified on TFSEARCH with a cut-off of 80). Previous studies show that HDACs associate with NF-κB (HDACs 1, 2) and GATA-1 and GATA-2 (HDAC5) and repress expression of their target genes [Ashburner et al., 2001; Ozawa et al., 2001; Zhong et al., 2002; Watamoto et al., 2003]. Thus, it is possible that these proteins recruit HDAC5 to the cyclin D3 promoter. Although expressed primarily in hematopoietic cells, GATA proteins have been detected in fibroblasts [Wang et al., 2005]. Of interest are data showing HDAC-mediated NF-κB inhibition in resting cells [Zhong et al., 2002]; as shown here, TSA increases the activity of the cyclin D3 promoter in quiescent fibroblasts. We also note that NF-κB and GATA-1 are acetylated and that acetylation corresponds with enhanced DNA binding activity [Boyes et al., 1998; Deng et al., 2003].

HDACs repress the expression of both cell cycle inhibitors (e.g., $p21^{Cip1}$) and cell cycle activators (e.g., cyclins E and A and, as shown here, cyclin D3) and thus have both positive and negative effects on cell cycle progression [Nakano et al., 1997; Brehm et al., 1998; Stiegler et al., 1998; Sambucetti et al., 1999; Sowa et al., 1999; Richon et al., 2000; Kim et al., 2006]. HDAC inhibitors typically suppress cell growth [Dokmanovic and Marks, 2005]. Paradoxically, overexpression of HDAC5 also inhibits proliferation, as demonstrated for several tumor cell lines [Huang et al., 2002]. Whether HDAC5-mediated growth arrest results in part from repression of cyclin D3 expression is not known but is certainly possible. Amounts of HDAC5 are subnormal in colon cancers and in acute myeloid leukemia blasts and cell lines, and thus may account in part for their dysregulated proliferation [Scanlan et al., 2002; Bradbury et al., 2005].

ACKNOWLEDGMENTS

This work was supported by the Cortner-Couch endowed chair for research and NIH grants CA93544 and CA67360 to W.J.P. The authors thank Nancy Olashaw for manuscript preparation.

REFERENCES

Ashburner BP, Westerheide SD, Baldwin AS. 2001. The p65 (RelA) subunit of NF-κB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. Mol Cell Biol 21: 7065–7077.

Bagui TK, Mohapatra S, Haura E, Pledger WJ. 2003. $p27^{Kip1}$ and $p21^{Cip1}$ are not required for the formation of active D cyclin-cdk4 complexes. Mol Cell Biol 23:7285–7290.

Baldin V, Lukas J, Marcote MJ, Pagano M, Draetta G. 1993. Cyclin D1 is a nuclear protein required for cell cycle progression in G1. Genes Dev 7:812–821.

Bartkova J, Lukas J, Strauss M, Bartek J. 1998. Cyclin D3: Requirement for G1/S transition and high abundance in quiescent tissues suggest a dual role in proliferation and differentiation. Oncogene 17:1027–1037.

Black AR, Black JD, Azizkhan-Clifford J. 2001. Sp1 and Krüppel-like factor family of transcription factors in cell growth regulation and cancer. J Cell Physiol 188:143–160.

Blottiere HM, Buecher B, Galmiche JP, Cherbut C. 2003. Molecular analysis of the effect of short-chain fatty acids on intestinal cell proliferation. Proc Nutr Soc 62:101–106.

Boyes J, Byfield P, Nakatani Y, Ogryzko V. 1998. Regulation of activity of the transcription factor GATA-1 by acetylation. Nature 396:594–598.

Bradbury CA, Khanim FL, Hayden R, Bunce CM, White DA, Drayson MT, Craddock C, Turner BM. 2005. Histone deacetylases in acute myeloid leukaemia show a distinctive pattern of expression that changes selectively in response to deacetylase inhibitors. Leukemia 19:1751–1759.

Brehm A, Miska EA, McCance DJ, Reid JL, Bannister AJ, Kouzarides T. 1998. Retinoblastoma protein recruits histone deacetylase to repress transcription. Nature 391:597–601.

Camarero N, Nadal A, Barrero MJ, Haro D, Marrero PF. 2003. Histone deacetylase inhibitors stimulate mitochondrial HMG-CoA synthase gene expression via a promoter proximal Sp1 site. Nucleic Acids Res 31:1693–1703.

Canettieri G, Morantte I, Guzman E, Asahara H, Herzig S, Anderson SD, Yates JR, Montminy M. 2003. Attenuation of a phosphorylation-dependent activator by an HDAC-PP1 complex. Nat Struct Biol 10:175–181.

Cheng M, Olivier P, Diehl JA, Fero M, Roussel MF, Roberts JM, Sherr CJ. 1999. The $p21^{Cip1}$ and $p27^{Kip1}$ CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. EMBO J 18:1571–1583.

Choi HS, Lee JH, Park JG, Lee YI. 2002. Trichostatin A, a histone deacetylase inhibitor, activates the IGFBP-3 promoter by upregulating Sp1 activity in hepatoma cells: Alteration of the Sp1/Sp3/HDAC1 multiprotein complex. Biochem Biophys Res Commun 296:1005–1012.

Clayton AL, Hazzalin CA, Mahadevan LC. 2006. Enhanced histone acetylation and transcription: A dynamic perspective. Mol Cell 23:289–296.

Della Ragione F, Criniti V, Della Pietra V, Borriello A, Oliva A, Indaco S, Yamamoto T, Zappia V. 2001. Genes

modulated by histone acetylation as new effectors of butyrate activity. FEBS Lett 499:199–204.

Deng WG, Zhu Y, Wu KK. 2003. Up-regulation of p300 binding and p50 acetylation in tumor necrosis factor-α-induced cyclooxygenase-2 promoter activation. J Biol Chem 278:4770–4777.

Doetzlhofer A, Rotheneder H, Lagger G, Koranda M, Kurtev V, Brosch G, Wintersberger E, Seiser C. 1999. Histone deacetylase 1 can repress transcription by binding to Sp1. Mol Cell Biol 19:5504–5511.

Dokmanovic M, Marks PA. 2005. Prospects: Histone deacetylase inhibitors. J Cell Biochem 96:293–304.

Florenes VA, Skrede M, Jorgensen K, Nesland JM. 2004. Deacetylase inhibition in malignant melanomas: Impact on cell cycle regulation and survival. Melanoma Res 14: 173–181.

Gao L, Cueto MA, Asselbergs F, Atadja P. 2002. Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. J Biol Chem 277:25748–25755.

Grozinger CM, Schreiber SL. 2000. Regulation of histone deacetylase 4 and 5 and transcriptional activity by 14-3-3-dependent cellular localization. Proc Natl Acad Sci USA 97:7835–7840.

Gu W, Roeder RG. 1997. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell 90:595–606.

Guan Z, Giustetto M, Lomvardas S, Kim JH, Miniaci MC, Schwartz JH, Thanos D, Kandel ER. 2002. Integration of long-term-memory-related synaptic plasticity involves bidirectional regulation of gene expression and chromatin structure. Cell 111:483–493.

Herzinger T, Reed SI. 1998. Cyclin D3 is rate-limiting for the G1/S phase transition in fibroblasts. J Biol Chem 273: 14958–14961.

Huang Y, Tan M, Gosink M, Wang KK, Sun Y. 2002. Histone deacetylase 5 is not a p53 target gene, but its overexpression inhibits tumor cell growth and induces apoptosis. Cancer Res 62:2913–2922.

Juan LJ, Shia WJ, Chen MH, Yang WM, Seto E, Lin YS, Wu CW. 2000. Histone deacetylases specifically down-regulate p53-dependent gene activation. J Biol Chem 275:20436–20443.

Kaczynski J, Cook T, Urrutia R. 2003. Sp1- and Krüppel-like transcription factors. Genome Biol 4:206.

Kang JS, Alliston T, Delston R, Derynck R. 2005. Repression of Runx2 function by TGFβ through recruitment of class II histone deacetylases by Smad3. EMBO J 24:2543–2555.

Kim HS, Park JS, Hong SJ, Woo MS, Kim SY, Kim KS. 2003. Regulation of the tyrosine hydroxylase gene promoter by histone deacetylase inhibitors. Biochem Biophys Res Commun 312:950–957.

Kim S, Kang JK, Kim YK, Seo DW, Ahn SH, Lee JC, Lee CH, You JS, Cho EJ, Lee HW, Han JW. 2006. Histone deacetylase inhibitor apicidin induces cyclin E expression through Sp1 sites. Biochem Biophys Res Commun 342:1168–1173.

Kim YH, Lim JH, Lee TJ, Park JW, Kwon TK. 2007. Expression of cyclin D3 through Sp1 sites by histone deacetylase inhibitors is mediated with protein kinase C-δ (PKC-δ) signal pathway. J Cell Biochem 101:987–995.

Lemercier C, Verdel A, Galloo B, Curtet S, Brocard MP, Khochbin S. 2000. mHDA1/HDAC5 histone deacetylase interacts with and represses MEF2A transcriptional activity. J Biol Chem 275:15594–15599.

Lu J, McKinsey TA, Nicol RL, Olson EN. 2000a. Signal-dependent activation of the MEF2 transcription factor by dissociation from histone deacetylases. Proc Natl Acad Sci USA 97:4070–4075.

Lu J, McKinsey TA, Zhang CL, Olson EN. 2000b. Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. Mol Cell 6:233–244.

Luo RX, Dean DC. 1999. Chromatin remodeling and transcriptional regulation. J Natl Cancer Inst 91:1288–1294.

Margueron R, Licznar A, Lazennec G, Vignon F, Cavailles V. 2003. Oestrogen receptor α increases p21/WAF1/CIP1 gene expression and the antiproliferative activity of histone deacetylase inhibitors in human breast cancer cells. J Endocrinol 179:41–53.

Mariadason JM, Corner GA, Augenlicht LH. 2000. Genetic reprogramming in pathways of colonic cell maturation induced by short chain fatty acids: Comparison with trichostatin A, sulindac, and curcumin and implications for chemoprevention of colon cancer. Cancer Res 60: 4561–4572.

Martinez-Balbas MA, Bauer UM, Nielsen SJ, Brehm A, Kouzarides T. 2000. Regulation of E2F1 activity by acetylation. EMBO J 19:662–671.

Marzio G, Wagener C, Gutierrez MI, Cartwright P, Helin K, Giacca M. 2000. E2F family members are differentially regulated by reversible acetylation. J Biol Chem 275:10887–10892.

Mayr B, Montminy M. 2001. Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat Rev Mol Cell Biol 2:599–609.

McKinsey TA, Zhang CL, Lu J, Olson EN. 2000a. Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. Nature 408:106–111.

McKinsey TA, Zhang CL, Olson EN. 2000b. Activation of the myocyte enhancer factor-2 transcription factor by calcium/calmodulin-dependent protein kinase-stimulated binding of 14-3-3 to histone deacetylase 5. Proc Natl Acad Sci USA 97:14400–14405.

McKinsey TA, Zhang CL, Olson EN. 2002. MEF2: A calcium-dependent regulator of cell division, differentiation and death. Trends Biochem Sci 27:40–47.

McKinsey TA, Kuwahara K, Bezprozvannaya S, Olson EN. 2006. Class II histone deacetylases confer signal responsiveness to the ankyrin-repeat proteins ANKRA2 and RFXANK. Mol Biol Cell 17:438–447.

Miska EA, Karlsson C, Langley E, Nielsen SJ, Pines J, Kouzarides T. 1999. HDAC4 deacetylase associates with and represses the MEF2 transcription factor. EMBO J 18:5099–5107.

Nakano K, Mizuno T, Sowa Y, Orita T, Yoshino T, Okuyama Y, Fujita T, Ohtani-Fujita N, Matsukawa Y, Tokino T, Yamagishi H, Oka T, Nomura H, Sakai T. 1997. Butyrate activates the WAF1/Cip1 gene promoter through Sp1 sites in a p53-negative human colon cancer cell line. J Biol Chem 272:22199–22206.

Nusinzon I, Horvath CM. 2005. Histone deacetylases as transcriptional activators? Role reversal in inducible gene regulation. Sci STKE 2005:re11.

Olashaw N, Pledger WJ. 2002. Paradigms of growth control: Relation to CDK activation. Sci STKE 2002:RE7.

Ozawa Y, Towatari M, Tsuzuki S, Hayakawa F, Maeda T, Miyata Y, Tanimoto M, Saito H. 2001. Histone deacetylase 3 associates with and represses the transcription factor GATA-2. Blood 98:2116–2123.

Quelle DE, Ashmun RA, Shurtleff SA, Kato JY, Bar-Sagi D, Roussel MF, Sherr CJ. 1993. Overexpression of mouse D-type cyclins accelerates G1 phase in rodent fibroblasts. Genes Dev 7:1559–1571.

Richon VM, Sandhoff TW, Rifkind RA, Marks PA. 2000. Histone deacetylase inhibitor selectively induces $p21^{WAF1}$ expression and gene-associated histone acetylation. Proc Natl Acad Sci USA 97:10014–10019.

Sambucetti LC, Fischer DD, Zabludoff S, Kwon PO, Chamberlin H, Trogani N, Xu H, Cohen D. 1999. Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific chromatin acetylation and antiproliferative effects. J Biol Chem 274:34940–34947.

Sangerman J, Lee MS, Yao X, Oteng E, Hsiao CH, Li W, Zein S, Ofori-Acquah SF, Pace BS. 2006. Mechanism for fetal hemoglobin induction by histone deacetylase inhibitors involves γ-globin activation by CREB1 and ATF-2. Blood 108:3590–3599.

Scanlan MJ, Welt S, Gordon CM, Chen YT, Gure AO, Stockert E, Jungbluth AA, Ritter G, Jager D, Jager E, Knuth A, Old LJ. 2002. Cancer-related serological recognition of human colon cancer: Identification of potential diagnostic and immunotherapeutic targets. Cancer Res 62:4041–4047.

Shao RH, Tian X, Gorgun G, Urbano AG, Foss FM. 2002. Arginine butyrate increases the cytotoxicity of DAB(389)IL-2 in leukemia and lymphoma cells by upregulation of IL-2Rβ gene. Leuk Res 26:1077–1083.

Smith B, Fang H, Pan Y, Walker PR, Famili AF, Sikorska M. 2007. Evolution of motif variants and positional bias of the cyclic-AMP response element. BMC Evol Biol 7 (Suppl 1):S15.

Sowa Y, Orita T, Hiranabe-Minamikawa S, Nakano K, Mizuno T, Nomura H, Sakai T. 1999. Histone deacetylase inhibitor activates the p21/WAF1/Cip1 gene promoter through the Sp1 sites. Ann NY Acad Sci 886:195–199.

Stiegler P, De Luca A, Bagella L, Giordano A. 1998. The COOH-terminal region of pRb2/p130 binds to histone deacetylase 1 (HDAC1), enhancing transcriptional repression of the E2F-dependent cyclin A promoter. Cancer Res 58:5049–5052.

Takakura M, Kyo S, Sowa Y, Wang Z, Yatabe N, Maida Y, Tanaka M, Inoue M. 2001. Telomerase activation by histone deacetylase inhibitor in normal cells. Nucleic Acids Res 29:3006–3011.

Tenbrock K, Juang YT, Leukert N, Roth J, Tsokos GC. 2006. The transcriptional repressor cAMP response element modulator α interacts with histone deacetylase 1 to repress promoter activity. J Immunol 177:6159–6164.

Thiagalingam S, Cheng KH, Lee HJ, Mineva N, Thiagalingam A, Ponte JF. 2003. Histone deacetylases: Unique players in shaping the epigenetic histone code. Ann NY Acad Sci 983:84–100.

Van Lint C, Emiliani S, Verdin E. 1996. The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation. Gene Expr 5:245–253.

Wang Z, Sicinski P, Weinberg RA, Zhang Y, Ravid K. 1996. Characterization of the mouse cyclin D3 gene: Exon/intron organization and promoter activity. Genomics 35: 156–163.

Wang Z, Zhang Y, Lu J, Sun S, Ravid K. 1999. Mpl ligand enhances the transcription of the cyclin D3 gene: A potential role for Sp1 transcription factor. Blood 93: 4208–4221.

Wang L, Tanaka S, Ramirez F. 2005. GATA-4 binds to an upstream element of the human α(I) collagen gene (COL1A2) and inhibits transcription in fibroblasts. Matrix Biol 24:333–340.

Watamoto K, Towatari M, Ozawa Y, Miyata Y, Okamoto M, Abe A, Naoe T, Saito H. 2003. Altered interaction of HDAC5 with GATA-1 during MEL cell differentiation. Oncogene 22:9176–9184.

Yang XJ. 2004. Lysine acetylation and the bromodomain: A new partnership for signaling. Bioessays 26:1076–1087.

Yoshida M, Kijima M, Akita M, Beppu T. 1990. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J Biol Chem 265:17174–17179.

Zhong H, May MJ, Jimi E, Ghosh S. 2002. The phosphorylation status of nuclear NF-κB determines its association with CBP/p300 or HDAC-1. Mol Cell 9:625–636.