Deacetylase Activity Is Required for cAMP Activation of a Subset of CREB Target Genes*

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Many hormones activate transcription by raising the level of cAMP within cells. In one well studied pathway, cAMP induces protein kinase A to phosphorylate the transcription factor CREB, which binds to a consensus sequence, the cAMP-regulated enhancer, found in many target genes. A generally accepted model suggests that phosphorylated CREB recruits the histone acetyltransferase CBP to activate transcription. In contrast, histone deacetylases have been linked to the cessation of CREB-dependent transcription. Here we tested this model in the regulation of endogenous CREB target genes. We used a constitutively active CREB mutant and microarray analysis to identify target genes in PC12 cells. We then tested the role of histone deacetylase activity in cAMP activation of four of these genes (c-FOS, ICER, NOR-1, and NUR77) by treating cells with the histone deacetylase inhibitor trichostatin A. Consistent with the generally accepted model, trichostatin A enhanced activation of c-FOS and NUR77 by cAMP. Surprisingly, trichostatin A blocked activation of ICER and NOR-1. The block of ICER and NOR-1 activation persisted in the presence of cycloheximide, indicating that the trichostatin A effect did not depend on new protein synthesis. This unexpected role of histone deacetylases in transcriptional activation of certain endogenous CREB target genes was not apparent in transfected reporter genes. Chromatin immunoprecipitation analysis indicated that the differential roles of histone deacetylases in activating or repressing CREB target genes was manifested at the level of preinitiation complex recruitment. These data indicate that histone deacetylases differentially regulate CREB target genes by contributing to either activation or cessation of transcription.

Hormones regulate gene transcription by initiating signal transduction cascades that ultimately influence the activity of transcription factors at promoters (1). These factors in turn recruit coregulators that either activate or repress transcription (2, 3). Coregulators fall into several groups based on the functions they perform, including physically linking transcription factors and RNA polymerase II (4), remodeling chromatin structure around promoters in an ATP-dependent manner (5), and catalyzing several types of covalent modifications of histone tails in promoter nucleosomes. Included in the last group are enzymes that can catalyze acetylation, methylation, phosphorylation, or ubiquitination of specific histone residues (6). These modifications are thought to form a code, indicating either activation or repression, that can be read by other cofactors (7). Perhaps the best studied of the histone-modifying proteins are the numerous enzymes that either add (histone acetyltransferases (HATs)) or remove (histone deacetylases (HDACs)) acetyl groups from histone tail lysines. Activating transcription factors have been found to recruit HATs, whereas repressors recruit HDACs (for a review, see 8). Hence, a general model has emerged in which HATs mediate activation and HDACs mediate repression.

Many hormones and neurotransmitters regulate transcription via the transcription factor CREB. CREB is expressed ubiquitously in all tissues, and CREB-regulated transcription has been implicated in a large number of physiological processes, including learning and memory (9), neuronal survival (10), and T-cell development (11). A consideration of known CREB target genes suggests functions in many other tissues as well (for a review, see Ref. 12). Thus, elucidating the mechanism of transcriptional activation by CREB is important for understanding many aspects of physiology.

Hormones can regulate transcription via CREB by causing an elevation of the second messenger cAMP, which activates protein kinase A. Protein kinase A phosphorylates CREB at serine 133 (13). CREB binds to cAMP-response elements (CREs) in promoters of many cAMP-regulated genes, and its phosphorylation recruits the HAT CBP to activate transcription (12). One recent study suggested that HDAC1 simultaneously associates with CREB and protein phosphatase 1, thereby causing dephosphorylation of CREB and the attenuation of transcription (14). Indeed, the HDAC inhibitor trichostatin A (TSA) has been shown to enhance activation of CRE reporter genes by cAMP (14, 15). Thus, the regulation of transcription by CREB, at least in the context of transfected reporter genes, appears to conform to the general model of HATs mediating activation and HDACs mediating repression.

In the present study, we tested this model by examining the role of HDACs in the regulation of transcription of endogenous CREB target genes by cAMP. First, we used a DNA microarray screen to identify genes up-regulated by a constitutively active CREB mutant. Then we measured activation of four of these genes (c-FOS, ICER, NOR-1, and NUR77) by cAMP in the absence and presence of the HDAC inhibitor TSA. CREB target genes could be grouped into two subsets based on the effects of TSA as follows: one in which TSA augments transcription activated by cAMP (c-FOS and NUR77) and another in which

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1 The abbreviations used are: HATs, histone acetyltransferases; HDACs, histone deacetylases; CREB, cAMP-response element-binding protein; CREs, cAMP-response elements; TSA, trichostatin A; RT, reverse transcriptase.
TSA blocks transcription (ICER and NOR-1). The augmentation of c-FOS and NUR77 transcription by TSA indicates that HDAC activity inhibits activation of these genes. In contrast, the inhibition of ICER and NOR-1 transcription by TSA shows that HDAC activity is required for cAMP activation of a subset of endogenous CREB target genes. This requirement for HDAC activity was not recapitulated in a stably incorporated reporter gene driven by the ICER promoter; activation of this reporter gene was actually slightly augmented by TSA. Thus, transfecting reporter genes may be subject to different regulatory mechanisms than genes in their native chromatin environment.

**EXPERIMENTAL PROCEDURES**

**Cell Culture, Drug Treatments, and Reporter Gene Assays—**For all experiments, PC12 cells were grown on poly-lysine-coated plates in Dulbecco's modified Eagle's medium with 10% neonatal calf serum and 5% fetal bovine serum plus 100 units/ml penicillin and 100 units/ml streptomycin. Medium for PC12 stable cell lines was supplemented with 700 μg/ml G418 (Invitrogen). Reporter genes stably incorporated into cell lines were 6× CRE-laCZ (16) and ICER-Luc, which consisted of −189 to +1 of the rat ICER promoter (numbering according to Ref. 17) cloned into the pβL3 luciferase vector (Promega). Cells were serum-starved (using media containing 0.5% neonatal calf serum and 0.25% fetal bovine serum) for 16 h prior to drug treatments. Drugs (all from Calbiochem) were used at the following concentrations: forskolin, 10 μM; trichostatin A, 100 μg/ml; amitriptyline, 1 μM, cycloheximide, 50 μg/ml. β-Galactosidase and luciferase activities were determined with a luminometer (E&G Berthold AutoLumat) and Tropix reagents (PerkinElmer Life Sciences) according to the manufacturer's instructions. Statistical analyses of reporter gene assay data (one-way analysis of variance and post tests using the Bonferroni correction) were performed with PRISM software (GraphPad). 

**Affymetrix Microarray Screen—**PC12 cells in 6-well plates were transfected with 1 μg of VP16-CREB expression vector (VP16-CREB in pcDNA3 from Invitrogen) or empty expression vector (pcDNA3) for 12 h with LipofectAMINE 2000 (Invitrogen). This procedure typically resulted in transfection of 60–90% of cells, as determined by separate transfections of a green fluorescent protein expression vector (data not shown). Total RNA was extracted 12 h after the end of the transfection with the RNeasy kit (Qiagen). Microarray assays were performed in the Affymetrix Microarray Core of the Oregon Health and Sciences University Gene Microarray Shared Resource, which uses Affymetrix Expression Analysis software (www.ohsu.edu/gmsr/amc). The screen was performed in duplicate on Affymetrix Rat Genome U34A microarray chips, and the genes listed in Table 1 were up-regulated in both screens.

**RT-PCR Assay—**Total RNA was collected with the RNeasy kit (Qiagen). Contaminating genomic DNA was removed with the DNA-free kit (Ambion). First strand cDNA synthesis was performed with Superscript II (Invitrogen). PCR primers were as described for ICER (19), c-FOS (20), NOR-1 (21), and NUR77 (22). cDNAs were amplified with Qiagen Taq for 22–25 cycles (ICER), 25–30 cycles (NOR-1), 20–22 cycles (NUR77), and 25–27 cycles (c-FOS). PCR products were verified by DNA sequencing.

**Chromatin Immunoprecipitation Assay—**Chromatin immunoprecipitation assays were performed according to the Upstate Biotechnology, Inc., protocol (www.upstate.com), with the following exceptions. 5–10× 10^6 cells were used for each sample. Formaldehyde fixation was performed at room temperature. Cells were lysed in 200 μl of RIPA lysis buffer (1% SDS, 10 mg/ml NaDodSO4, 2 mM EDTA, 0.1% sodium deoxycholate, 1% Triton X-100, pH 7.8). Sonicated lysate was brought up to 1 ml with RIPA buffer (same as RIPA lysis buffer except with 0.1% SDS). Antibodies (both from Santa Cruz Biotechnology) used for chromatin immunoprecipitations were H-224 (pol II) and C-18 (TFII.B). Low salt wash buffer was RIPA buffer. High salt wash buffer was RIPA buffer with 500 mM NaCl. DNA was purified from eluates from protein A/G beads (Santa Cruz Biotechnology) with the QIAquick PCR purification kit (Qiagen). The following primers were used to amplify the ICER, c-FOS, and NUR77 promoters in 35-cycle PCRs with Qiagen Taq: CATTCCGGGTTGTTTATACAGGAAGAG (ICER forward prim
CREB protein identified highly inducible CREB target genes in PC12 cells.

To confirm the CREB dependence of cAMP activation of ICER, c-FOS, NOR-1, and NUR77, we used the dominant negative CREB inhibitor A-CREB. A-CREB consists of the CREB leucine zipper domain with an acidic amphipathic extension on its N terminus (18). We transfected A-CREB into PC12 cells and selected transfected cells with a MACS selection kit (see “Experimental Procedures”). Fig. 1B shows that A-CREB inhibited induction of ICER, c-FOS, NOR-1, and NUR-77 by forskolin. These data further confirm that these four genes are CREB target genes.

HDAC Activity Is Required for Activation of a Subset of CREB Target Genes—To test the role of HDACs in transcriptional regulation of CREB target genes, we treated PC12 cells with the HDAC inhibitor TSA for 30 min prior to forskolin stimulation. Fig. 2A shows that TSA had gene-specific effects. TSA slightly enhanced activation of c-FOS and NUR77, as has been reported previously (39). Indeed, TSA treatment alone produced modest activation of c-FOS and NUR77 (Fig. 2C) as has been observed previously (39, 41), and this effect was not blocked by A-CREB, indicating that it is CREB-independent (Fig. 2D). Surprisingly, TSA inhibited the forskolin-induced activation of ICER and NOR-1. Fig. 2B shows that apicidin, an HDAC inhibitor that is structurally distinct from TSA (for a review, see Ref. 40), also inhibited ICER transcription. Thus, HDAC activity is required for activation of a subset of CREB target genes.

In principle, the inhibitory effect of HDAC inhibitors on ICER and NOR-1 transcription could be due to either a direct action (i.e. inhibition of an HDAC or HDACs required for activation of transcription) or an indirect action (e.g. de novo synthesis of a transcription factor that represses ICER and NOR-1). To rule out an indirect action, we tested the ability of TSA to inhibit forskolin-induced ICER and NOR-1 transcription in the presence of the protein synthesis inhibitor cycloheximide. Fig. 3 shows that TSA inhibition of ICER and NOR-1 transcription does not depend on de novo protein synthesis. PC12 cells were treated with 10 μM forskolin for 1 h. TSA (100 ng/ml) was added 30 min prior to forskolin stimulation. Cycloheximide (50 μg/ml), a protein synthesis inhibitor, was added 30 min prior to TSA. ICER and NOR-1 mRNAs were detected by RT-PCR.
tion was intact in the presence of cycloheximide. Thus, TSA does not block transcription of ICER and NOR-1 through an indirect action that requires protein synthesis.

Inhibition by TSA Does Not Occur in Stably Transfected CRE-driven Reporter Genes—The cAMP inducibility of the ICER and NOR-1 promoters is mediated by regions that contain four and three tandem CREs, respectively (17, 25). We tested whether inhibition of CREB target gene activation by TSA could be recapitulated in reporter genes driven by promoters containing multiple CREs. First, we used PC12 cells containing a stably incorporated reporter gene driven by six tandem CREs (16). Fig. 4A shows that TSA did not block but rather augmented activation of the 6×CRE reporter gene by forskolin. Next, we tested whether the region of the ICER promoter that contains four tandem CREs was sensitive to inhibition by TSA. A reporter gene containing this region of the ICER promoter was stably incorporated into PC12 cells. Fig. 4B shows that TSA produced a small but statistically significant augmentation of activation of the stably incorporated ICER reporter gene by forskolin. Thus, the CRE-containing portion of the ICER promoter is not sufficient to confer sensitivity to inhibition by TSA in the context of a stably incorporated reporter gene. Inhibition of CREB target gene activation by TSA is only observed in endogenous genes.

HDAC Activity Differentially Regulates Pol II and TFIIB Recruitment to the ICER, c-Fos, and NUR77 Promoters—Inhibition of ICER and NOR-1 expression by TSA could be due to a block of transcription or rapid degradation of ICER and NOR-1 mRNA. To test these two possibilities, we performed chromatin immunoprecipitation assays to examine recruitment of pol II and TFIIB to the ICER promoter. Due to the high content of AC repeats and GC-rich regions, we were unable to find PCR primers that could efficiently amplify the NOR-1 promoter. Fig. 5 shows that forskolin induced a robust recruitment of both pol II and TFIIB to the ICER promoter. TSA blocked recruitment of both proteins. These data indicate that TSA blocks formation of the preinitiation complex at the ICER promoter. Thus, inhibition of ICER expression by TSA is due to a block of transcription. In addition, these data show that HDAC activity is required for recruitment of pol II and TFIIB to the ICER promoter.

We also examined the role of HDAC activity in the recruitment of pol II and TFIIB to the c-Fos and NUR77 promoters (Fig. 5). These two proteins were present at the c-FOS promoter both basally and during forskolin stimulation. In contrast to the blocked recruitment observed at the ICER promoter, pol II and TFIIB remained bound to the c-FOS promoter in the presence of TSA. Similarly, pol II and TFIIB remained bound to the NUR77 promoter in the presence of TSA. Thus, HDAC activity is not required for pol II and TFIIB recruitment to the c-FOS and NUR77 promoters. Rather, HDAC activity antagonized recruitment of pol II and TFIIB at these two promoters; TSA treatment alone induced binding of pol II and TFIIB to the NUR77 promoter and enhanced binding of pol II to the c-FOS promoter. These data indicate that HDAC activity interferes with preinitiation complex formation under basal conditions at the c-FOS and NUR77 promoters, which may explain the previous observations that TSA stimulates c-FOS and NUR77 transcription (Fig. 2C) (39, 41).

**DISCUSSION**

In this study, we used a chimeric protein containing the activation domain of VP16 fused to the CRE-binding portion of CREB to identify several CREB target genes in PC12 cells. These target genes were all highly inducible and most contained multiple CREs in their promoters. The dominant negative A-CREB blocked cAMP activation of the four genes that we chose for further study (ICER, c-FOS, NOR-1, and NUR77), confirming that they are CREB targets. HDAC inhibitors blocked activation of a subset of these genes by cAMP. The block by HDAC inhibitors was rapid and independent of new protein synthesis. At the ICER promoter, HDAC inhibitor pre-
ventilated formation of the preinitiation complex. These data indicate that the general model of HATs mediating activation and HDACs mediating repression cannot fully describe transcriptional regulation by cAMP. In particular, HDAC activity is required for activation of transcription at a subset of CREB target gene promoters.

The idea that HDACs can play a role in activation is consistent with an increasing body of evidence in both yeast and mammalian systems. Bernstein et al. (42) reported that TSA treatment rapidly down-regulates a subset of yeast genes. Deletion of the HDAC Rpd3 down-regulates many of the same genes. Rpd3 may contribute to gene activation by deacetylating histone H4 lysine 12, which prevents recruitment of the silencer Sir3p (42). The yeast HDAC Hos2 is also involved in gene activation. Hos2 associates with the coding regions of actively transcribed genes and is required for efficient transcription (43). Set3, which forms a complex with Hos2 and another HDAC, Hat1 (44), was also required for efficient transcription of Hos2-activated genes (43). Thus, in yeast, it appears that multiple HDACs contribute to the activation of transcription. While this manuscript was in preparation, two studies were published indicating that HDACs can similarly contribute to transcriptional activation by STAT5 in mammalian cells. Rasche et al. (45) showed that deacetylase inhibitors block cytokine induction of STAT5 target genes and, as we observed at the ICER promoter, TSA blocked preinitiation complex formation at the promoters of STAT5 target genes. Xu et al. (46) reported that STAT5 recruits HDAC1 to mediate target gene activation. These observations, along with our data, support the idea that HDACs, typically associated with repression, may actually contribute to activation of many genes.

How might HDAC activity contribute to activation of ICER and NOR-1 transcription by cAMP in mammalian cells? Kudrnastani and Grunstein (47) have proposed that there is a deacetylated state of histones that is “permissive” for transcription initiation. We imagine that there are several potential ways in which the acetylation of histone lysines might block steps required for transcriptional activation at a particular promoter. First, an acetylated histone lysine might recruit a repressor that contains a bromodomain motif. Indeed, bromodomain motifs, which specifically bind acetylated lysines (48), are found in some repressors (49, 50). Second, a histone lysine that is acetylated prematurely might cause the recruitment of bromodomain-containing activators in an improper order. The precisely timed ordered recruitment of activators is thought to be critical for transcriptional activation (51). Finally, the acetylation of a histone lysine might prevent another modification required for activation, such as methylation (52). For example, methylation of histone H3 lysines 4 and 79 has been associated with transcriptional activation (53, 54), and methylation of histone H3 lysine 4 blocks binding of the transcriptional repressor complex NuRD (55). Thus, it is possible that some histone tail lysines in nucleosomes at the ICER and NOR-1 promoters must be deacetylated to permit the promoters to respond to cAMP.

Alternatively, the HDAC target might be a transcription factor or cofactor involved in ICER and NOR-1 transcription, and acetylation of this factor might block transcription at these promoters. Indeed, HATs can also be factor acetyltransferases, and factor acetylation can inhibit transcription, as has been shown to occur at the β-INTERFERON promoter. Acetylation of the architectural protein HMG I(Y) by CBP disrupts the enhanceosome and turns off β-INTERFERON transcription (56). In addition, acetylation of the acetylase ACTR by CBP disrupts the association of HAT coactivator complexes with the estrogen receptor at the pS2 promoter, which attenuates transcription (57). In this regard, it should be noted that CREB can be acetylated at three lysines (15), but acetylation of CREB appears to enhance transcriptional activation. In addition, we used chromatin immunoprecipitation assays to show that TSA has no effect on the level of CREB bound to the ICER promoter during forskolin stimulation (data not shown).

Fig. 4 showed that regions of promoters that contain CREs are not sufficient to confer sensitivity to inhibition by TSA in stably incorporated reporter genes. It is possible that the portions of the promoters used in these reporter genes do not contain sequences responsible for TSA inhibition. Alternatively, the chromatin structure of the reporter genes might be different from the native promoters. For example, the position of nucleosomes on the native ICER promoter might not be recapitulated on the ICER reporter gene. Also, the pattern of covalent modifications of the histone tails might not be the same in nucleosomes in native promoters versus stably incorporated reporter genes. In particular, a histone lysine that must be deacetylated in response to cAMP for activation of the native ICER promoter might be constitutively deacetylated in nucleosomes on the reporter gene. These considerations call into question the suitability of reporter genes, even those that are stably incorporated, for the study of HDACs in transcriptional regulation.

Finally, our data indicate that HDACs play multiple roles in the regulation of CREB target gene transcription. Fig. 5 illustrates two roles of HDACs that are promoter-specific. First, at the c-FOS promoter, TSA alone enhances recruitment of pol II. At the NUR77 promoter, TSA induces recruitment of both TFIIB and pol II. In addition, TSA activates both c-FOS and NUR77 transcription (Fig. 2C) (39, 41). Thus, at these two promoters, HDACs act to prevent formation of the preinitiation complex in order to suppress transcription under basal conditions. HDACs play a completely different role in regulation of ICER and NOR-1 transcription. For these genes, TSA blocks activation of transcription by cAMP (Fig. 1). Fig. 5 shows that TSA also blocks forskolin-induced recruitment of TFIIB and pol II at the ICER promoter. Thus, at the ICER promoter, HDAC activity is required for formation of the preinitiation complex. In addition to these promoter-specific functions, Canetti et al. (14) showed that HDACs can attenuate CREB-dependent transcription by promoting CREB dephosphorylation. It is possible that different HDACs may mediate each of these distinct
roles. Indeed, there are 11 known HDACs expressed in most cell types in mammals (58). The activity of each of the five HDACs in yeast affects different classes of genes (59). The presence of multiple HDACs within cells, and their ability to perform distinct roles in transcriptional regulation, may increase the diversity of mechanisms for activation of expression of CREB target genes.

What determines the nature of the role that HDACs play in activation of specific CREB target genes? Our data suggest two critical determinants: activation kinetics and preinitiation complex assembly. CREB target genes inhibited by TSA (ICER and NOR-1) had significantly slower activation kinetics (Fig. 1). This observation suggests that ICER and NOR-1 transcription involves an HDAC-dependent step that is not necessary for c-FOS and NUR77 transcription, allowing faster activation kinetics for the latter two genes. The difference in activation kinetics correlates with preinitiation complex assembly. In the case of ICER, TFIIB and pol II are not bound to the promoter under basal conditions, and TSA treatment alone induces recruitment of TFIIB and pol II, and deacetylase inhibition stimulates transcription of this gene (Fig. 2C) (39). This suggests that, under basal conditions, the NUR77 promoter exists in a state of readiness to bind the transcription complex under basal conditions may not require HDAC activity for activation and thus can be transcribed rapidly. In contrast, CREB target genes with slower activation kinetics may require an additional HDAC-dependent step for preinitiation complex assembly.

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REFERENCES

1. Brivanlou, A. H. & Darnell, J. E., Jr. (2002) Science 295, 813–818
2. Pazin, M. J. & Kadonaga, J. T. (1997) Cell 88, 325–328
3. Meinkoth, J., Alberts, A. S. & Feramisco, J. R. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5481–5485
4. Rachez, C. & Freedman, L. P. (2001) Curr. Opin. Cell Biol. 13, 272–278
5. Nusser, A., Blinder, D. & Schreiber, S. L. (2000) J. Biol. Chem. 275, 14313–14316
6. Pumplin, R., Malarkey, K., Aidulis, D., McLees, A. & Gould, G. W. (1997) Cell. Signal. 9, 323–328
7. Toscano, S., Bezouzdraia, O. & Tsingotjadou, A. (2001) Endocrinology 142, 663–670
8. Narlikar, G. J., Fan, H.-Y. & Kingston, R. E. (2002) Mol. Cell. Biol. 20, 999–1010
9. Kato, S., Shimojo, T., Kato, K., Watanabe, M. & Ohba, T. (2000) J. Biol. Chem. 275, 2475–2480
10. Zhang, Y. & Reinberg, D. (2002) Genes Dev. 16, 2321–2326
11. Taipale, J., Arumugam, S., Zhang, W., Zhang, J., Komorowski, R. & Anderson, B. D. (2000) J. Biol. Chem. 275, 19040–19045
12. Zhang, Y. & Reinberg, D. (2000) Genes Dev. 14, 2321–2326
13. Santos-Rosa, H., Schneider, R., Bannister, A. J., Sherriff, J., Bernstein, B. E., Tugdual, L., Schreiber, S. L., Mellor, J. & Kouzarides, T. (2002) Mol. Cell 9, 497–511
14. Im, H., Park, C., Feng, Q., Johnson, K. D., Keikhafer, C. M., Choi, K., Zhang, Y. & Brennick, E. H. (2003) J. Biol. Chem. 278, 18346–18352
15. Zegerman, P., Canas, B., Pappin, D. & Kouzarides, T. (2002) J. Biol. Chem. 277, 11621–11624
16. Munshi, N., Merika, M., Yie, J., Senger, K., Chen, G. & Thanos, D. (1998) Mol. Cell 2, 457–467
17. Chen, H., Lin, R. J., Xie, W., Wiltzius, D. & Evans, R. M. (1999) Cell 98, 675–686
18. de Ruijter, A. J. M., van Gennip, A. H., Caron, H. N., Kemp, S. & Van Kuiiken, A. B. P. (1998) Biochem. J. 370, 737–740
19. Roby, D., Suka, Y., Xenarios, I., Kurdistani, S. K., Wang, A., Suka, N. & Grunstein, M. (2002) Cell 109, 437–446
20. Pinaud, S. & Mirvishitch, J. (1988) J. Mol. Biol. 280, 785–798