Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 11295–11300, October 1997
Biochemistry

A histone deacetylase inhibitor potentiates retinoid receptor action in embryonal carcinoma cells

SAVERIO MINUCCI*, VALERIE HORN*†, NISAN BHATTACHARYYA*†, VALYA RUSSANOVA*, VASILY V. OGRYZKO*, LUCIA GABRIELE‡, BRUCE H. HOWARD*, AND KEIKO OZATO*§

*Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, and ‡Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Communicated by Igor B. Dawid, National Institutes of Health, Bethesda, MD, August 25, 1997 (received for review May 27, 1997)

ABSTRACT **Histone acetylation is thought to have a role in transcription. To gain insight into the role of histone acetylation in retinoid-dependent transcription, we studied the effects of trichostatin A (TSA), a specific inhibitor of histone deacetylase, on P19 embryonal carcinoma cells. We show that coaddition of TSA and retinoic acid (RA) markedly enhances neuronal differentiation in these cells, although TSA alone does not induce differentiation but causes extensive apoptosis. Consistent with the cooperative effect of TSA and RA, coaddition of the two agents synergistically enhanced transcription from stably integrated RA-responsive promoters. The transcriptional synergy by TSA and RA required the RA-responsive element and a functional retinoid X receptor (RXR)/retinoic acid receptor (RAR) heterodimer, both obligatory for RA-dependent transcription. Furthermore, TSA led to promoter activation by an RXR-selective ligand that was otherwise inactive in transcription. In addition, TSA enhanced transcription from a minimum basal promoter, independently of the RA-responsive element. Finally, we show that TSA alone or in combination with RA increases *in vivo* endonuclease sensitivity within the RA-responsive promoter, suggesting that TSA treatment might alter a local chromatin environment to enhance RXR/RAR heterodimer action. Thus, these results indicate that histone acetylation influences activity of the heterodimer, which is in line with the observed interaction between the RXR/RAR heterodimer and a histone acetylase presented elsewhere.**

Acetylation of the amino termini of core histones has been linked to formation of transcriptionally competent chromatin (for reviews, see refs. 1 and 2). At present the mechanism by which histone acetylation contributes to transcriptional activation of a specific gene is not fully understood. However, available evidence suggests that histone acetylation has a role in facilitating the activity of sequence-specific transcription factors, because histone acetylation is reported to alter nucleosomal templates and modulate binding of transcription factors *in vitro* (refs. 3–5; for reviews, see refs. 6 and 7). Histone deacetylase inhibitors such as sodium butyrate, trapoxin, and trichostatin A (TSA) increase acetylated histones in many cell types (for review, see ref. 8). Unlike sodium butyrate that elicits pleiotropic effects, TSA is thought to specifically inhibit histone deacetylase activity (9). For this reason, TSA has been used as a tool to study the consequences of histone acetylation *in vivo* (2, 8, 10).

Retinoid receptors, retinoic acid receptor (RAR) and retinoid X receptor (RXR), are members of the nuclear hormone receptor superfamily. These receptors bind to the retinoic acid-responsive element (RARE) as RXR/RAR heterodimer and regulate retinoic acid (RA)-dependent gene expression (for reviews, see refs. 11–13). Although heterodimer binding to the RARE does not require ligand *in vitro*, ligand is required for heterodimer occupancy of the RARE *in vivo* in some promoters (14, 15). These and our recent observations that RA increases *in vivo* endonuclease sensitivity in an RA-responsive promoter (41) suggest that transcription by liganded heterodimer occurs in conjunction with an alteration of chromatin. The recent findings that coactivators and corepressors of nuclear hormone receptors are complexed with histone acetylases and deacetylases (16–21) may suggest that ligand-induced chromatin alterations are in some way affected by histone acetylation. The activity of other nuclear hormone receptors may also be affected by histone acetylation, because sodium butyrate and TSA are reported to affect transcription mediated by steroid and thyroid hormones (22, 23).

This work was undertaken based on our initial observation that TSA potentiates RA-induced neuronal differentiation in P19 cells. We found that TSA markedly potentiates RA-dependent transcription from a stably integrated promoter in these cells. This transcriptional potentiation was in part attributed to the activity of RXR/RAR heterodimers. Results of endonuclease sensitivity assays indicate that TSA leads to an alteration of local chromatin structure that favors heterodimer binding to the RARE.

MATERIALS AND METHODS

TSA and Retinoids. TSA was obtained from Wako Biochemicals (Osaka) and dissolved in ethanol. All-*trans*-RA was obtained from Sigma. The RXR-selective ligand SR11237 (24, 25) synthesized at Hoffmann–La Roche was provided by A. Levin.

Transfections and Luciferase Assays. RA-responsive luciferase reporter genes have been described (25). Transfection of P19 cells was performed by lipofection using the Lipofectamine reagent (GIBCO/BRL) (25). To construct P19 cells with stably integrated luciferase reporter genes, parental cells were cotransfected with the reporter and the pSV-neo construct in a 10:1 molar ratio (typically using 4.5 μg of the reporter and 0.5 μg of the neo marker in a 60-mm dish) and selected with G418 (250 μg/ml; GIBCO/BRL) for 10–15 days (26). Clones were isolated and propagated as described (27). In some experiments 100–1,000 colonies of G418-resistant cells were pooled and used for analysis. To obtain P19 cells expressing the dominant negative RXRβ DNA binding do-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

0027-8424/97/9411295-6$0.00/0
PNAS is available online at http://www.pnas.org.

Abbreviations: TSA, trichostatin A; RA, retinoic acid; RARE, retinoic acid-responsive element; RXR, retinoid X receptor; RAR, retinoic acid receptor; DBD, DNA binding domain; H4, histone 4.

†V.H. and N.B. contributed equally to this work.
§To whom reprint requests should be addressed at: Building 6, Room 2A01, Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-2753. e-mail: ozatok@dir6.nichd.nih.gov.

main (DBD−) (26) and a luciferase reporter, cells were cotransfected with the luciferase reporter and pcxn2-RXRβ DBD− or the control pcxn2 vector and RARβ2s(GL3)-Luc in a 1:10 ratio (26) and pooled transfectants were obtained as above. Luciferase activities were normalized by protein concentrations as described (26).

Analysis of Acetylated Histones. This procedure was as described (18, 28). Briefly, nuclear preparations from P19 cells treated with TSA or RA were extracted with acid. The extracted proteins were resolved on a Triton/acid urea polyacrylamide gel and stained by Coomassie blue.

Endonuclease Sensitivity Assay. Nuclear preparations (20 μg of DNA equivalent) from P19 cells were digested with *Sma*I (240 units) or *Hin*cII (100 units, both from Promega) at 37°C for 30 min in digestion buffer (10 mM Tris·HCl, pH 7.4/15 mM NaCl/60 mM KCl/0.1 mM EDTA/5 mM $MgCl_2$/5% glycerol/1 mM DTT). DNA was purified, digested with appropriate enzymes, and resolved on a 2.2% agarose gel. Filters were hybridized to ^{32}P-labeled probes indicated in each experiment. The conditions for digestion and hybridization had been optimized before experiments, which are presented elsewhere (41).

Neuronal Differentiation and Cell Cycle Analysis. These assays were performed as described (27).

Apoptosis. DNA fragmentation was monitored by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay as described (29). Briefly, P19 cells treated with TSA or RA were fixed in 1% formaldehyde in PBS. Cells were incubated with biotinylated dUTP, dNTPs, and terminal deoxynucleotidyl transferase and then stained with fluorescein isothiocyanate-avidin (all from Boehringer Mannheim). Incorporation of dUTP by single cells was monitored on a FACScan flow cytometer with CELLQUEST data analysis software (Beckton Dickinson).

RESULTS

TSA Increases Acetylated Histones in P19 Cells. To ascertain whether TSA treatment leads to acetylation of core histones in P19 cells, acid-extractable proteins were analyzed on a Triton/acid urea gel (Fig. 1*C*). As noted before (18, 28), histone 4 (H4) exhibited the best resolution among other histones. Before TSA treatment, H4 were primarily in the unacetylated or mono-/diacetylated form (lanes 1 and 5). Four hours of TSA treatment at both 10 ng/ml (+) and 100 ng/ml (++) led to some increase in tri- and tetraacetylated forms. After 20 h, tri- and tetraacetylated H4 were greatly increased, with the unacetylated form of H4 almost undetectable. Addition of RA itself did not alter the acetylation pattern or affect

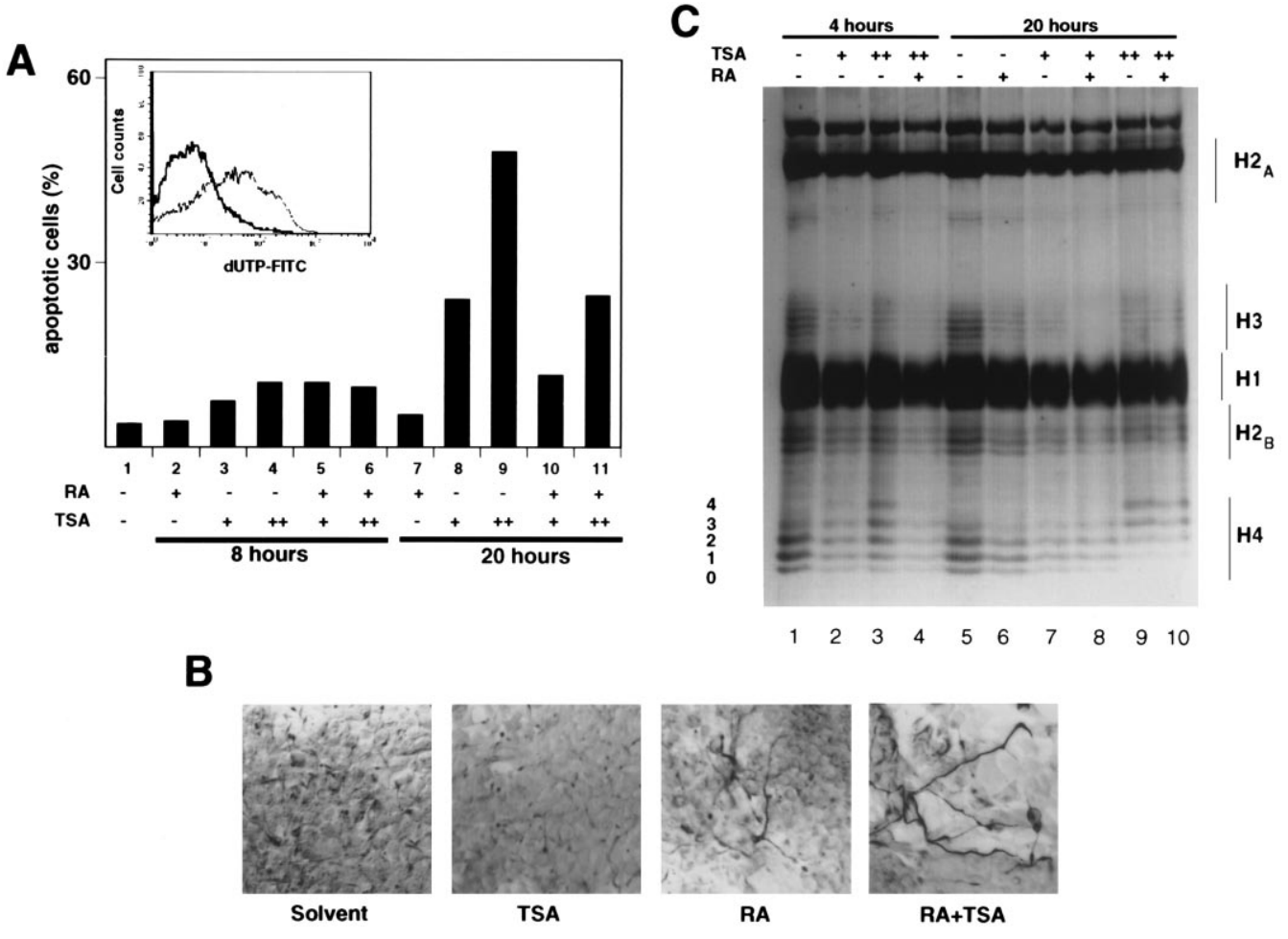

FIG. 1. Biological effect of TSA. (*A*) Apoptosis. P19 cells were treated with TSA at 10 ng/ml (+), 100 ng/ml (++), or RA (1 μM) for 8 or 20 h and the percent of apoptotic cells was determined by TUNEL assay. (*Inset*) A flow cytometry profile of DNA fragmentation after 20 h of TSA treatment (100 ng/ml) (thin line) compared with untreated cells (thick line). (*B*) Neuronal differentiation. Cells were treated with solvent alone, TSA at 5 ng/ml, RA (1 μM), or RA (1 μM) and TSA (5 ng/ml) for 48 h, followed by a further 48-h incubation without treatment. Neurofilaments were detected by immunohistochemical staining. (*C*) Histone acetylation patterns after TSA treatment. Cells were treated with TSA [10 ng/ml (+) or 100 ng/ml (++)] with or without RA (1 μM) for indicated time, and acid-extractable proteins were resolved in an acid urea gel. Positions of core histones are shown on the right. The number on the left indicates the degree of histone H4 acetylation.

Table 1. Cell Cycle Analysis

Treatment	Phase		
	G_1, %	S, %	G_2/M, %
Solvent	30.8	48.3	20.5
RA (1 μM)	38.2	40.6	20.6
TSA (2 ng/ml)	41.2	39.5	19.2
RA + TSA	44.5	38.6	16.4

P19 cells were treated with the indicated agents for 48 h and stained with propidium iodide, and the percentage of cells in each stage of cell cycle was analyzed on a FACScan as described (27). The data are the average of three experiments.

the changes caused by TSA. Thus, TSA treatment increases global levels of histone acetylation in P19 cells with complex kinetics.

Effects of TSA on RA-Induced Neuronal Differentiation and Apoptosis. Retinoids not only stimulate neuronal differentiation but also inhibit cell growth and cause apoptosis in EC cells (27). To assess biological role of histone acetylation, we first investigated whether TSA affects apoptosis and differentiation in P19 cells. Results of TUNEL assays in Fig. 1*A* show that TSA treatment causes DNA fragmentation in a large fraction of P19 cells. After 24 h of TSA treatment, DNA fragmentation occurred in more than 50% of P19 cells. DNA fragmentation was observed reproducibly with a wide range of TSA concentrations (10–500 ng/ml), peaking at 20–24 h after treatment. Although RA also caused DNA fragmentation, the extent was much less than that by TSA. These results suggest that increased histone acetylation leads to rapid and extensive apoptosis in P19 cells. Interestingly, coaddition of RA and TSA significantly reduced the percentage of apoptotic cells (Fig. 1*A*, compare lanes 10 and 11 to lanes 8 and 9), suggesting that the cell death pathway induced by TSA may be distinct from that by RA. Although TSA caused apoptosis in other cells such as NIH 3T3 cells and U2OS cells, it was much less extensive than in P19 cells (data not shown).

In Fig. 1*B*, the effect of TSA on neuronal differentiation was investigated by immunocytochemical detection of neurofilaments and by morphological inspection (27). Although a 48-h treatment with RA alone led to differentiation of P19 cells (Fig. 1*B*), as expected, TSA alone neither changed cell morphology nor induced neurofilament expression. In these experiments TSA was added at less than 10 ng/ml to circumvent extensive apoptosis. When cells were treated with both RA and TSA, neuronal differentiation was markedly enhanced, as evidenced by a large increase in neurofilament-positive cells, many of which had longer and thicker neuronal processes than those observed with RA treatment alone. We also examined alterations of cell cycle profiles following TSA treatment. Results of flow cytometry analysis are shown in Table 1. TSA treatment increased the fraction of cells in G_1 phase and decreased those in S and G_2 phases, similar to the changes noted by RA (27). Addition of the two agents further increased cells in G_1 and decreased those in S and G_2 phases. These results indicate that TSA inhibits cell cycle progression in P19 cells (1, 2, 8).

TSA Potentiates Retinoid-Dependent Promoter Activity. The enhanced differentiation of P19 cells observed by TSA suggests the possibility that histone acetylation influences retinoid-dependent transcription. We examined whether TSA affects promoter activity of the RARβ2 gene that had been stably integrated into P19 cells (27). The RARβ2 promoter contains a canonical RARE and is strongly activated by RA (14, 25, 30, 31). The promoter also contains a cAMP-like responsive element and an auxiliary RARE (CRE and *rare*, in Fig. 2). At an early stage (4 h after treatment), RA alone led to 35-fold enhancement in promoter activity, but treatment with TSA, alone or added with RA, caused no change in reporter activity. However, 8 and 20 h after treatment, coaddition of RA and TSA led to dramatic synergy in reporter activation, yielding a greater than 400-fold induction, but RA alone gave less than 75-fold induction in reporter activity. TSA alone also gave modest reporter activation (<35-fold) 8 and 20 h after treatment. Thus, TSA synergistically enhances RA-dependent transcription in P19 cells, although its effect is delayed relative to that of RA. The RA dose–response curve was not altered by addition of TSA (data not shown), suggesting that the synergy was not due to a change in ligand binding of the heterodimer. Although less prominent, synergistic enhancement of promoter activity was observed with NIH 3T3 and U20S cells (data not shown).

TSA Treatment Renders an Inactive RXR Ligand Active in Transcription. Ligands selective for RAR stimulate transcrip-

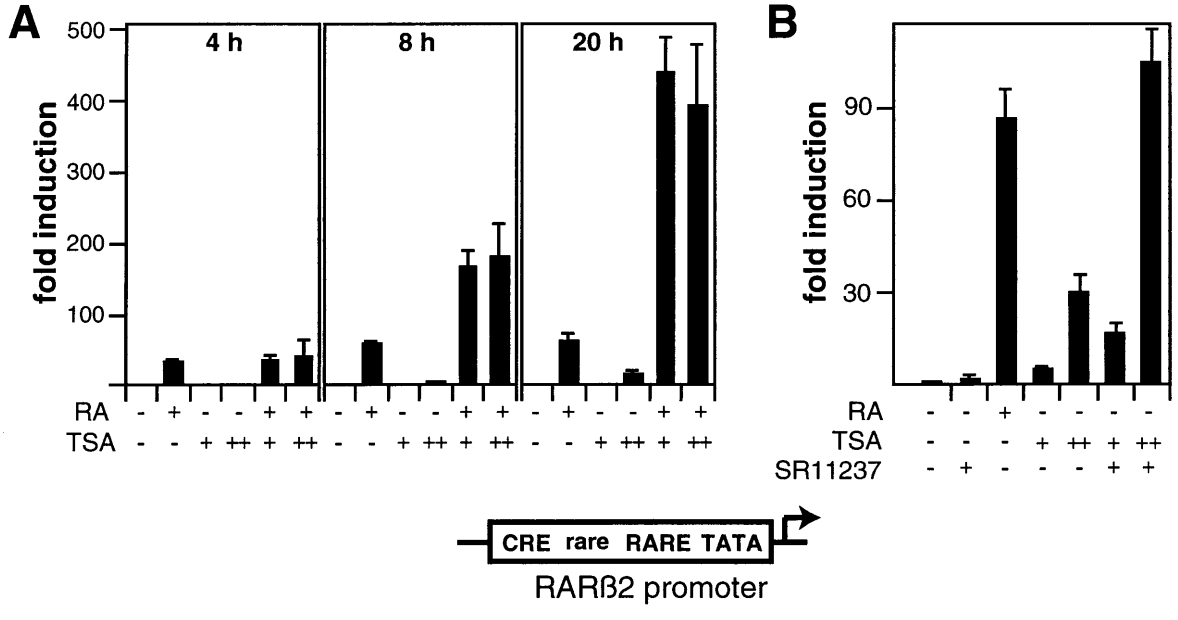

FIG. 2. Synergistic enhancement of RARβ2 promoter activity by TSA and RA. (*A*) P19 cells stably transfected with the RARβ2 promoter fused to the luciferase gene (14, 25, 27) were treated with RA (1 μM) or TSA [10 ng/ml (+) or 100 ng/ml (++)] for indicated period of time and luciferase activity was measured as described (25). (*B*) P19 cells were treated with RA (1 μM) or SR11237 (1 μM) and TSA as above for 20 h.

tion from promoters containing the RARE, but ligands for RXR do not, as liganded RXR and RAR have distinct roles (11–13, 15, 24, 25). RXR ligands, however, contribute to promoter activation, when added with RAR ligands (15, 25). We asked whether TSA treatment could change the transcriptional property of RXR by testing an RXR-selective ligand, SR11237, for reporter activation (Fig. 2*B*). Addition of this ligand alone did not stimulate the promoter activity, as expected. However, coaddition of TSA and SR11237 led to an almost 100-fold activation of promoter activity, much greater than activation by TSA alone (20- to 30-fold). Thus, TSA treatment enables a liganded RXR to activate transcription without requiring the partner RAR to bind to ligand. These results indicate that TSA treatment changes the way in which the liganded heterodimer functions *in vivo*.

TSA Potentiates RA-Dependent Promoter Activity Through the RARE. To investigate whether the transcriptional potentiation by TSA was mediated through the RARE, we next tested an artificial reporter in which the RARE was mutated and no longer bound the heterodimer (25). In this promoter, the RARE was connected to the basal promoter that had only a TATA box and initiator (Fig. 3). As shown in Fig. 3*A*, the control reporter with the intact RARE responded to both RA and TSA added alone, and coaddition of the two agents led to further enhancement in reporter activity. As expected, the reporter with the mutated RARE was not activated by RA. However, TSA alone enhanced activity of the mutated reporter, at levels higher than that by the control reporter, the basis of which has not been studied. Nevertheless, coaddition of the two agents failed to elicit synergistic transcription from the mutant reporter. These results indicate that TSA enhances transcription through the RARE as well as through the basal promoter. In support of these results, the RARα antagonist RO41–5253 (25) abrogated synergy by RA and TSA but did not affect promoter activation by TSA alone (data not shown).

Potentiation of Promoter Activation by TSA Requires the RXR/RAR Heterodimer. Results in Fig. 3*A* suggest a role for the RXR/RAR heterodimer in synergistic transcription. We tested whether TSA could enhance RA-dependent transcription when heterodimers are nonfunctional. P19 cells were stably transfected with a dominant negative RXRβ DBD− (26) along with the RA-responsive reporter used in Fig. 3*A*. This dominant negative RXR lacks the DBD and RXR/RAR heterodimers formed with this RXR are unable bind to the RARE (26). We and others showed that such a dominant negative receptor represses RA-responsive promoter activity (26, 32). As shown in Fig. 3*B*, cells transfected with RXRβ DBD− did not elicit synergistic transcription in response to TSA and RA, although they elicited enhanced promoter activation by TSA alone. In contrast, the expected synergy was observed in control transfectants (pcxn-2 in Fig. 3*B*). As previously noted (26), RXRβ DBD− proteins were expressed in these cells as confirmed by immunoblot analysis, and addition of RA did not enhance promoter activity in these cells (data not shown). These results indicate that a functional RXR/RAR heterodimer is required for synergistic activation of the promoter by TSA and RA but not for transcription through the basal promoter by TSA.

TSA Treatment Enhances Endonuclease Sensitivity in the RARβ Promoter *in Vivo*. In some steroid-responsive promoters, addition of hormones leads to an alteration of chromatin structure, which can be assessed by altered endonuclease sensitivity in and around the promoter (32–34). We recently showed that RA treatment of P19 cells causes an increase in endonuclease sensitivity within the RARβ2 promoter, which closely correlated with the RA-induced *in vivo* footprint and transcriptional activation of the promoter (41). Herein we examined whether TSA treatment alters sensitivity to nuclease digestion in the RARβ2 promoter. *Sma*I was chosen, because it cuts a site near the RARE (shaded in Fig. 4*A*) in the

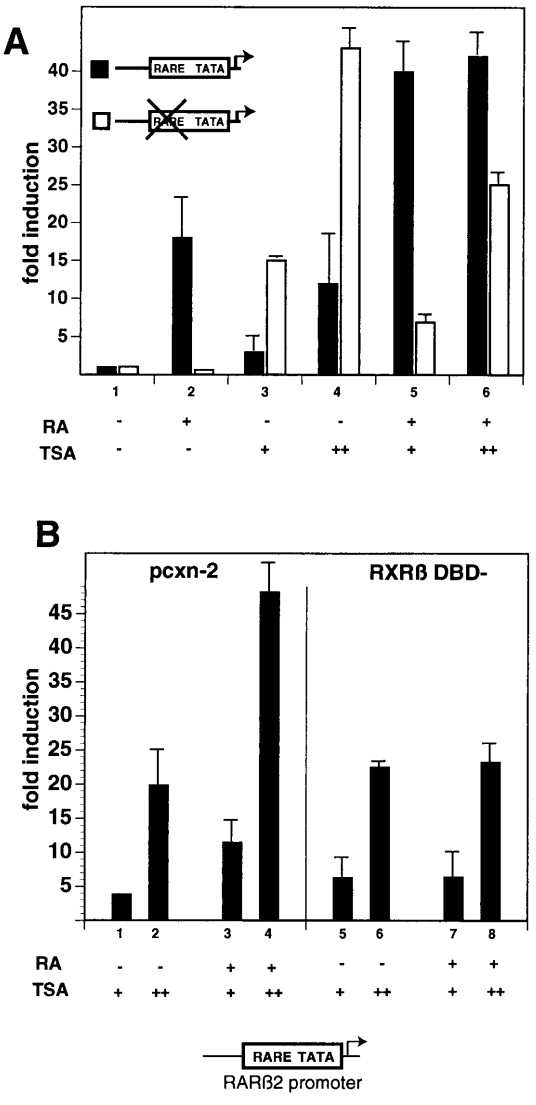

FIG. 3. Requirement of the RARE and RXR/RAR heterodimer for synergistic promoter activation by TSA and RA. (*A*) Promoter activity with a mutated RARE. P19 cells stably transfected with the indicated reporters were treated with RA or TSA (same concentrations as in Fig. 2) for 20 h and luciferase activity as measured as above. (*B*) Analysis of a dominant negative RXRβ. P19 cells stably transfected with the promoter and RXRβ DBD− or control vector (pcxn-2) were treated with RA or TSA as above.

promoter (boxed, positions −142 to +14). Nuclei from P19 cells treated with TSA or RA were digested with *Sma*I *in vivo*, DNA was purified, and digestion products were detected by Southern blot hybridization using a ^{32}P-labeled probe corresponding to the RARβ2 promoter. As shown in Fig. 4*D*, P19 cells treated with RA alone showed increased *Sma*I digestion relative to untreated cells, as noted before (41). Furthermore, cells treated with TSA alone (100 ng/ml) also showed increased *Sma*I digestion relative to untreated cells (compare the ratio of the undigested, upper most band to two smaller digested fragments). Quantitation of *Sma*I digestion (Fig. 4*D Lower*) shows that *Sma*I cleavage in TSA- or RA-treated cells was 33% and 39%, respectively, both significantly higher than that by control cells (20%). In a separate experiment shown in Fig. 4*E*, TSA was added at a lower concentration (10 ng/ml) with RA. Although TSA alone at this low concentration did not affect *Sma*I digestion (16%), coaddition of TSA and RA led to greater *Sma*I digestion (33% by TSA plus RA vs. 22% by RA alone). Similar increased *Sma*I sensitivity was seen 8 h

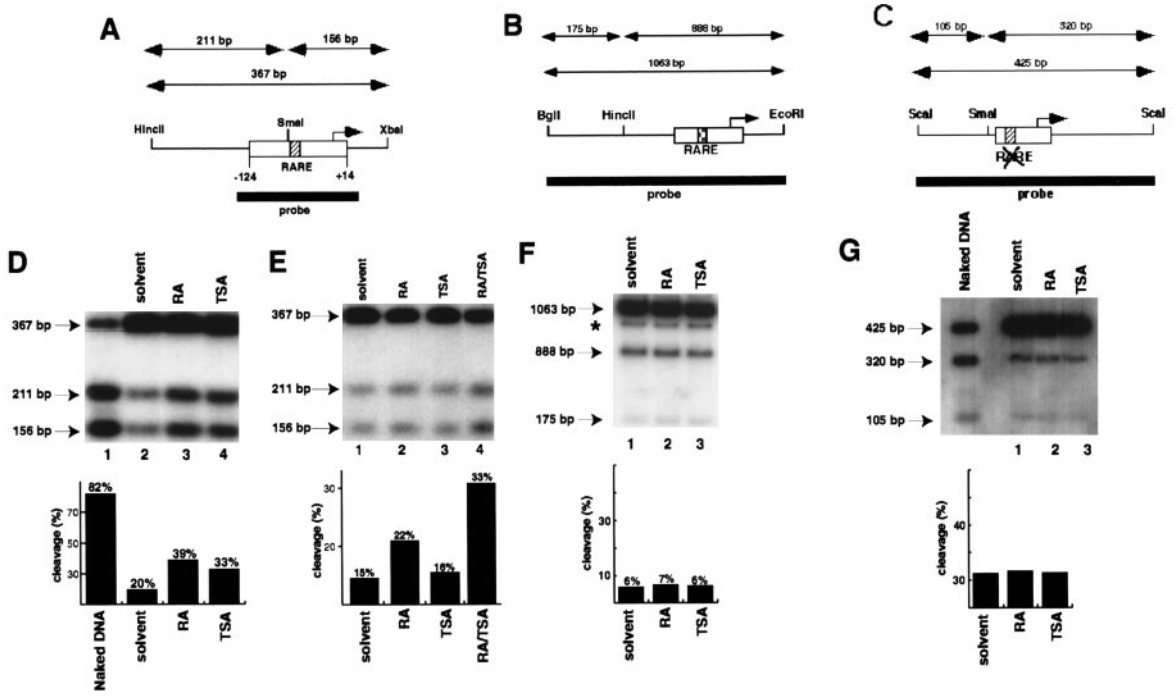

FIG. 4. Endonuclease sensitivity in the RARβ by TSA. (*A–C*) Diagrams of promoters and endonuclease digestion. The promoters are boxed. Upper and lower arrows indicate digested and undigested fragments. Solid bars indicate ^{32}P-labeled probes used in each digestion. P19 cells stably transfected with the RARβ-luciferase promoter (*A* and *B*) or the mutant RARE-TATA (*C*) were tested. (*D*, *F*, and *G*) Cells were treated with TSA (100 ng/ml) or RA (1 μM) for 20 h. (*E*) Cells were treated with RA (1 μM) plus TSA (10 ng/ml) for 20 h. Nuclei were digested with *Sma*I (*D*, *E*, and *G*) or *Hin*cII (*F*) and hybridized with indicated probes. The upper most band and lower bands represent the undigested or *Sma*I- or *Hin*cII-digested bands. The length of undigested and digested fragments are shown on the left. Genomic DNA was digested with the enzymes *in vitro* (naked DNA) and used as a control. The lower-most panels indicate percent digestion quantified on a PhosphoImager.

of TSA treatment (data not shown). To examine whether the increased sensitivity to *Sma*I was specific for a sequence in the RARβ2 promoter, we tested sensitivity to *Hin*cII, another restriction enzyme that cuts a site outside the promoter (see the *Hin*cII site upstream from the promoter in Fig. 4*B*). Results in Fig. 4*F* show that *Hin*cII digestion produced two digested fragments (888 bp and 175 bp) whose levels were not affected either by TSA or RA. Thus, TSA by itself and in combination with RA increases *Sma*I sensitivity in a promoter-specific manner, suggesting that histone acetylation contributes to an alteration of chromatin structure in the RARβ promoter. Interestingly, the promoter containing the mutated RARE tested in the preceding section (Fig. 3*A*) did not elicit increased *Sma*I sensitivity in response to either TSA or RA (Fig. 4*G*). These results suggest that increased *Sma*I sensitivity seen by the two agents is dependent on the intact RARE, implications of which are discussed below.

DISCUSSION

Histone deacetylase inhibitors have been shown to inhibit cell growth and differentiation in some cell types (2, 8). In the present work we found that TSA potentiates RA-induced neuronal differentiation in P19 cells, although alone it did not induce differentiation in these cells. In addition, TSA led to extensive apoptosis in P19 cells. This and the recent report that TSA causes rapid apoptosis in rat thymocytes (35) suggest an intriguing possibility that histone acetylation has a role in programmed cell death. TSA-induced cell death in P19 cells, however, was offset by addition of RA, although RA itself caused moderate apoptosis (Fig. 1). These results raised the possibility that histone acetylation might modulate RA action.

Synergy Between RA and TSA. The main thrust of this work is the observation that the combined treatment with RA and TSA synergistically activated transcription from RA-responsive promoters (Fig. 2). The synergy required the intact RARE and the ligand-bound functional RXR/RAR receptors (Fig. 3). These results raises the possibility that increased histone acetylation caused by TSA treatment alters a local chromatin structure leading to enhanced recruitment of the heterodimer to the RARE. Such chromatin alterations might enable even those heterodimers bound only to an RXR ligand to be recruited to the promoter, which does not occur under normal circumstances (Fig. 2*B*). Compatible with the role of histone acetylation in factor recruitment, Lee *et al.* (3) showed that acetylation of core histones stimulates binding of TFIIIA to the 5S RNA gene *in vitro*, which may occur through destabilization of nucleosomes. Histone-acetylation-regulated recruitment of heterodimers may involve corepressor and coactivators that are known to dissociate from or associate with the heterodimer after ligand binding (36–39).

Results in Fig. 3 suggest that increased histone acetylation caused by TSA leads to stimulation of transcription from a basal promoter without an activator. Because TBP binding to the TATA box is shown to be inhibited when the template is chromatinized (40), recruitment of basal factors (polymerase II holoenzyme and TFIID, and possibly additional factors) may be precluded under "normal" conditions where nucleosomal histones in and near the promoter are not excessively acetylated. It is possible that increased histone acetylation results in increased recruitment of basal factors leading to transcription without an upstream activator.

TSA-Induced Restriction Site Accessibility and Chromatin Opening. Data in Fig. 4 demonstrate that TSA increases *Sma*I sensitivity in the promoter in a site-specific manner. It is remarkable that the same site shows increased sensitivity to RA (Fig. 4 and ref. 41). Our results suggest that TSA, both alone and in combination with RA, alters a chromatin structure within the RARβ2 promoter. However, we do not know the precise mechanism by which this alteration occurs. A simple possibility would be that *Sma*I accessibility reflects recruitment of heterodimers to the RARE, which is stimulated

by histone acetylation. When histones are hyperacetylated by TSA treatment, some unliganded heterodimers may be recruited to the RARE causing increased *Sma*I accessibility. Such recruitment, if it had occurred, appears transcriptionally abortive, because no promoter activation through the RARE ensued in the absence of ligand (Fig. 3). Nevertheless, this scenario may prove correct because induction of *Sma*I sensitivity was not observed with the reporter that contained a defective RARE after TSA or RA treatment (Fig. 4*F*). Alternatively, however, it is possible that the *Sma*I site in the promoter is inherently sensitive to histone acetylation and can be opened without heterodimer binding. In this scenario, the increased *Sma*I sensitivity seen by RA treatment alone (Fig. 4) may simply be a result of histone acetylation that occurred concomitant with heterodimer binding. In support of such a possibility, Van Lindt *et al.* (4) reported that TSA induces chromatin modifications in the stably integrated HIV promoter that closely resembles modifications associated with factor binding and promoter activation. These authors noted that TSA caused endonuclease sensitivity changes without apparent factor binding and even under the conditions where active transcription was inhibited.

Finally, our observations may support the idea that the histone acetylation contributes to the enhanced function of RXR/RAR heterodimer in the normal conditions. Consistent with this idea, we have recently observed that a histone acetyltransferase, PCAF interacts with liganded heterodimers that are bound to the RARE and stimulate RA dependent transcription (J. Blanco, personal communication). Recently, a reciprocal situation has been reported in which nuclear hormone receptors and other specific transcription factors associate with histone deacetylases (20, 21).

We thank Drs. A. Levin, A. Dey, J. Blanco, J. Wong, and A. P. Wolffe for reagents and discussions.

1. Jeppesen, P. (1997) *BioEssays* **19,** 67–74.
2. Turner, B. M. & O'Neil, L. P. (1995) *Semin. Cell. Biol.* **6,** 229–236.
3. Lee, D. Y., Hayes, J. J. & Wolffe, A. P. (1993) *Cell* **72,** 73–84.
4. Van Lindt, C., Emiliani, S., Ott, M. & Verdin, E. (1996) *EMBO J.* **15,** 1112–1120.
5. Vettese, D. M., Grant, P. A., Hebbes, T. R., Crane, R. C., Allis, C. D. & Workman, J. L. (1996) *EMBO J.* **15,** 2508–2518.
6. Wolffe, A. P. & Pruss, D. (1996) *Cell* **84,** 817–819.
7. Brownell, J. E. & Allis, C. D. (1996) *Curr. Opin. Genet. Dev.* **6,** 176–184.
8. Yoshida, M., Horinouchi, S. & Beppu, T. (1995) *BioEssays* **17,** 423–430.
9. Kijima, M., Yoshida, M, Sugita, K. Horinouchi, S. & Beppu, T. (1993) *J. Biol. Chem.* **268,** 22429–22431.
10. Ogryzko, V. V., Hirai, T. H., Russanova, V. R., Barbie, D. A. & Howard, B. H. (1996) *Mol. Cell. Biol.* **16,** 5210–5218.
11. Chambon, P. (1996) *FASEB J.* **10,** 940–954.
12. Mangersdorf, D. J. & Evans, R. M. (1995) *Cell* **83,** 841–850.
13. Minucci, S. & Ozato, K. (1996) *Curr. Opin. Genet. Dev.* **6,** 567–574.
14. Dey, A., Minucci, S. & Ozato, K. (1994) *Mol. Cell. Biol.* **12,** 3590–3599.
15. Chen, J. Y., Clifford, J., Zusi, C., Starrett, J. E., Tortolani, D., Ostrowski, J., Reczek, P., Chambon, P. & Gronemeyer, H. (1996) *Nature (London)* **382,** 819–822.
16. Chakravarti, D., LaMorte, V. J., Nelson, M. C., Nakajima, T., Schulman, I. G., Juguilon, H., Montminy, M. & Evans, R. M. (1996) *Nature (London)* **383,** 99–103.
17. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.-C., Heyman, R. A., Rose, D. W., Glass, C. K. & Rosenfeld, M. G. (1996) *Nature (London)* **85,** 403–414.
18. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H. & Nakatani, Y. (1996) *Cell* **87,** 953–959.
19. Beato, M., Herrlich, P. & Schutz, G., (1995) *Cell* **83,** 851–857.
20. Nagy, L., Kao, H.-Y., Chakravarti, D., Lin, R. J., Hassig, C. A., Ayer, D. E., Schreiber, S. L. & Evans, R. M. (1997) *Cell* **89,** 373–380.
21. Laherty, C. D., Yang, W.-M., Sun, J.-M., Davie, J. R., Seto, E. & Eisenman, R. N. (1997) *Cell* **89,** 349–356.
22. Bresnick, E. H., John, S., Berad, D. S., Lefebvre, P. & Hager, G. L. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 3977–3981.
23. Bartsch, J., Truss, M., Bode, J. & Beato, M. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 10741–10746.
24. Lehmann, J. M., Jong, L., Fanjul, A., Cameron, J. F., Lu, X.-P., Haefner, P., Dawson, M. I. & Pfahl, M. (1992) *Science* **258,** 1944–1946.
25. Minucci, S., Leid, M., Toyama, R., Saint-Jeannet, J.-P., Peterson, V. J., Horn, V., Ishmael, J. E., Bhattacharyya, N., Dey A., Dawid, I. B., & Ozato, K. (1997) *Mol. Cell. Biol.* **17,** 644–655.
26. Minucci, S., Zand, D. J., Dey, A., Marks, M. S., Nagata, T., Grippo, J. F. & Ozato, K. (1994) *Mol. Cell. Biol.* **14,** 360–372.
27. Horn, V., Minucci, S., Ogryzko, V., Adamson, E., Howard, B., Levin, A. & Ozato, K. (1996) *FASEB J.* **10,** 1071–1077.
28. West, M. H. P. & Bonner, W. M. (1980) *Biochemistry* **19,** 3238–3245.
29. Gorczyca, W., Gong, J. & Darynkiewicz, Z. (1993) *Cancer Res.* **53,** 1945–1951.
30. Valcarcel, R., Holtz, H., Jemenez. C. G., Barretino, D. & Stunnenberg, H. G. (1994) *Genes Dev.* **8,** 3068–3079.
31. Folkers, G. E. & van der Saag, P. T. (1995) *Mol. Cell. Biol.* **15,** 5868–5878.
32. Archer, T. K, Lee, H.-L., Cordingley, M. G., Mymryk, J. S., Fragoso, G., Berard, D. S. & Hager, G. L. (1994) *Mol. Endocrinol.* **8,** 568–576.
33. Truss, M., Bartsch, J., Schelbert, A., Hache, R. J. G. & Beato, M. (1995) *EMBO J.* **14,** 1737–1751.
34. Wade, P., Pruss, D. & Wolffe, A. P. (1997) *Trends Biochem. Sci.* **22,** 128–132.
35. Lee, E., Furukubo, T., Miyabe, T, Yamaguchi, A. & Kariya, K. (1996) *FEBS Lett.* **395,** 183–187.
36. Horlein, A. J., Naar, A. M., Heinzel, T., Torchia, J., Glass, B., Kurokawa, R., Ryan, A., Kamei, Y., Soderstrom, M., Glass, C. K. & Rosenfeld, M. G., (1995) *Nature (London)* **377,** 397–403.
37. Chen, D. & Evans, R. M. (1995) *Nature (London)* **377,** 454–457.
38. Onate, S. A., Tsai, S. Y., Tsai, M.-J. & O'Malley, N. W. (1995) *Science* **270,** 1354–1357.
39. Von Bauer, E., Zechel, C. Heery, D., Heine, M. J. S., Garnier, J.-M., Vivat, V., Le Douarin, B., Gronemeyer, H., Chambon, P. & Losson, R. (1996) *EMBO J.* **15,** 110–224.
40. Imbalzano, A. N., Kwon, H., Green, M. R. & Kingston, R. E. (1994) *Nature (London)* **370,** 481–485.
41. Bhattacharyya, N., Dey, A., Minucci, S., Zimmer, A., John, S., Hager, G. & Ozato, K. (1997) *Mol. Cell Biol.*, in press.