Moderate increase in histone acetylation activates the mouse mammary tumor virus promoter and remolds its nucleosome structure

(chromatin structure/MMTV/progestins/glucocorticoids/butyrate)

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ABSTRACT The mouse mammary tumor virus (MMTV) promoter is regulated by steroid hormones through a hormone-responsive region that is organized in a positioned nucleosome. Hormone induction leads to a structural change of this nucleosome which makes its DNA more sensitive to cleavage by DNase I and enables simultaneous binding of all relevant transcription factors. In cells carrying either episomal or chromosomally integrated MMTV promoters, moderate acetylation of core histones, generated by treatment with low concentrations of the histone deacetylase inhibitors sodium butyrate or trichostatin A, enhances transcription from the MMTV promoter in the absence of hormone and potentiates transactivation by either glucocorticoids or progestins. At higher concentrations, histone deacetylase inhibitors reduce basal and hormone induced MMTV transcription. Inducing inhibitor concentrations lead to the same type of nucleosomal DNase I hypersensitivity as hormone treatment, suggesting that moderate acetylation of core histone activates the MMTV promoter by mechanisms involving chromatin remodeling similar to that generated by the inducing hormones.

The compaction of the eukaryotic genome in nuclear chromatin is likely to influence the accessibility of DNA sequences for recognition by DNA binding proteins. The basic unit of chromatin is the nucleosome, a flattened disk formed by core histones with a central (H3–H4) tetramer flanked by two H2A/H2B dimers, around which are wound 145 bp of DNA forming 1.8 negative superhelical turns (1). Wrapping of the DNA around the octamer of histones could hinder access to those sequences that are oriented with the relevant sites pointing toward the core histones (2). To study the contribution of chromatin organization to DNA binding and gene expression, inducible systems, such as the mouse mammary tumor virus (MMTV) promoter, are particularly well suited as they permit one to correlate the influence of the inducer on gene expression and on chromatin structure. In most of these systems, induction is accompanied by changes in chromatin structure often manifested in a localized hypersensitivity to cleavage by DNase I (3). In the MMTV promoter, which is organized into a positioned nucleosome, hormone induction leads to the appearance of a DNase I hypersensitive region within a cluster of hormone-responsive elements (4, 5). The mechanism responsible for this change in nucleosome sensitivity is unknown; while it requires binding of the hormone receptors and remodeling of the positioned nucleosome, it is not dependent on ongoing transcription (6).

One biochemical modification that is accompanied by structural changes in chromatin is acetylation of the N-terminal tails of the core histone, a reaction catalyzed by histone acetyltransferases and involving addition of acetyl to the ε-amino group of lysines (7). Apart from its role during chromatin replication, histone acetylation has been claimed to correlate with active regions of chromatin (8–10). Histone hyperacetylation has been reported to open up the nucleosome enhancing its DNase I sensitivity at the dyad axis (11–13) and to reduce the capacity of histone H1 to compact chromatin (14, 15). Genetic evidence for a role of histone acetylation in the control of gene expression derives from experiments in yeast. Mutation of those lysine residues in the amino-terminal tails of the core histones that are known to be substrates for acetylation influences the transcription of several inducible genes (reviewed in ref. 16). The influence of histone hyperacetylation on steroid hormone induced gene expression has been reported to be inhibitory (17) as well as stimulatory (18), depending on the experimental system. Early results were based on the use of sodium butyrate as an inhibitor of histone deacetylase (19–23). At 5–10 mM, this compound has been reported to inhibit induction of the MMTV promoter by glucocorticoids when the promoter was stably integrated into chromatin (24). Because butyrate is known to exert concentration dependent pleiotropic effects (21), we decided to investigate in more detail the influence of several concentrations of butyrate on basal and hormonally induced transcription of MMTV-driven reporters. In addition we have used a more selective inhibitor of histone deacetylases, (R)-trichostatin A (TSA), which is active at much lower concentrations than butyrate (25–27). Here we report an inhibitory effect of high doses of butyrate and TSA on basal and hormone-induced transcription from stably integrated MMTV promoters. Lower doses of both drugs, leading to moderate histone acetylation, rapidly enhance basal and induced transcription from the MMTV promoter. A previously identified alteration in nucleosome structure of the MMTV promoter, reflected in an hormone-dependent enhancement of cleavage by DNase I at the nucleosome dyad (6), was also induced by stimulatory concentrations of TSA in the absence of hormone. These results suggest that moderately increased histone acetylation induces changes in chromatin organization of the MMTV promoter similar to those accompanying hormone induction and results in an equivalent enhancement of transcription.

Abbreviations: MMTV, mouse mammary tumor virus; TSA, (R)-trichostatin A; CAT, chloramphenicol acetyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MPE-Fe(II), methionyl-propyl-EDTA-Fe(II); FACS, fluorescence-activated cell sorter.

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MATERIAL AND METHODS.

Hyperacetylation Drugs. Sodium butyrate (Merck) was used as a 100 mM stock solution in water. TSA, a gift of T. Beppu (Tokyo), was dissolved in ethanol and stored as a 100 mM stock solution at −20°C.

Cell Culture. The cell line C127-BPVMTCV (6) contains ∼100 episomal copies of the plasmid MMTV-CAT encompassing MMTV-LTR sequences of the GR strain from −631 to +125 relative to the transcriptional start site. These cells were grown in DMEM with 10% fetal calf serum. The cell line T47D-MTVL (6) is a derivative of T47D cells containing a single copy of plasmid pAGE5MMTV-Lu, which harbors a neomycin resistance gene with an simian virus 40 promoter, and a luciferase gene under the control of an MMTV-LTR fragment from the C3H strain of 1.4 kb length. These cells were grown in RPMI 1640 medium containing 10% fetal calf serum and 700 μg/ml of G418 (GIBCO/BRL).

Reporter Gene Assays. Chloramphenicol acetyltransferase (CAT) activities were usually performed with 100 ng of cytoplasmic extract protein as described (28), and the results were quantitated using an imaging scanner (United Technologies-Packard). Luciferase assays were performed as described (29). Cells were washed twice with cold PBS and lysed with Triton-containing lysis buffer. Equal amounts of extracts were assayed for luciferase activity in a Berthold (Nashua, NH) luminometer (LB 953).

RNase Mapping. Radioactive riboprobes were synthesized from pGEM vectors containing MMTV sequences from −72 to +111 (GR strain) or from −140 to +91 (C3H strain) according to published protocols (30). Total cytoplasmic RNA was isolated by the CsCl method, and 5 μg of RNA were used for the analysis. After overnight hybridization at 42°C, the samples were digested with RNase T1 and RNase A for 1 h at 37°C. Following proteinase K treatment for 15 min, the samples were phenol extracted and run on a 6.5% sequencing gel. Fragments of pBR322 digested with HpaII and 5′-labeled with [γ-32P]ATP were used as size markers.

Run-Off Analysis. The run-on assays with isolated nuclei were performed as described (31). The newly synthesized RNA samples were isolated according to Greenberg and Ziff (32). For detection of specific RNAs, 10 μg of denatured plasmids pMMTV-CAT and pGAPDH (glyceraldehyde-3-phosphate dehydrogenase) were spotted onto PALL B membrane and used for hybridization in 6× standard saline citrate (SSC), 0.5% SDS, 10× Denhardt’s solution, and 100 μg/ml of denatured herring sperm DNA with 106 cpm of synthesized RNA.

DNase I Digestion. For DNase I analysis, 105 cells were preincubated with vehicle or with 5 or 50 ng/ml TSA for 6 h, followed by incubation for additional 30 min with either ethanol or 10−7 M dexamethasone. The cells were scraped in PBS, pelleted, resuspended in 0.5 ml DNase I digestion buffer (33), adjusted to 0.2% Nonidet P-40, subjected to 20 strokes of Dounce homogenization, and treated with 10–80 units DNase I (Boehringer Mannheim) for 2 min at room temperature. Reactions were stopped by adding EDTA to 12.5 mM and SDS to 0.5%. DNA was purified as described (6).

Methidium-propyl-EDTA-Fe(II) [MPE-Fe(II)] Cleavage. Analysis of nucleosome positioning on the MMTV promoter sequences with MPE-Fe(II) in control and TSA-treated cells was performed as described (34). Nuclei from control cells and from cells treated with TSA were prepared as described above and resuspended in MPE cleavage buffer (15 mM Tris-HCl, pH 7.4/15 mM NaCl/60 mM KCl/1 mM EDTA/0.25 M Sucrose). MPE-Fe(II)-cleavage was performed with 25 μM MPE-Fe(II) for 5, 15, and 30 min at room temperature.

Indirect End-Labeling Analysis. MPE-Fe(II)- or DNase I-cleaved genomic DNA samples were restricted with EcoR I, resolved in 1.2% agarose gels, blotted onto PALL B nylon membrane, and probed with the EcoR I/BgII fragment (6). DNA cleaved with SsrI (position −108) and either EcoRI or HindIII served as an internal size marker (6). Quantitative evaluation was performed with a PhosphorImager (Molecular Dynamics).

Core Histone Preparation and Analysis of Histone Acetylation. Cells were washed with cold PBS and cell nuclei were prepared (6). Histone H1 and nonhistone proteins were eliminated with MgCl2 washings, and acid-soluble core histones were extracted with H2SO4 as described (35). All manipulations were performed in the presence of 10 mM sodium butyrate. Histones were acid precipitated, washed with acetone/thiodiglycol/HCl (97:2:1), followed by acetone/thiodiglycol (98:2), and vacuum dried. Histones were analyzed on acetic acid/urea/Tris-gels as described (36).

Fluorescence-Activated Cell Sorter (FACS) Analysis. Analysis of TSA effects on cell cycle were performed as described (37). Cells were trypsinized, resuspended in PBS, and fixed by adding 3 volumes of cold (−20°C) EtOH. DNA was stained with Hoechst 33258 (Sigma). FACS analysis was performed with a FACStar®Plus (Becton Dickinson).

RESULTS

Low Concentrations of Inhibitors Enhance Basal and Glucocorticoid-Induced Transcription from an Episomal MMTV Promoter. We have recently established a cell line, C127-BPVMTVC, that carries ∼100 copies of a bovine papilloma virus-based episomal vector containing a fragment of the MMTV-LTR driving the CAT gene of Escherichia coli (6). This cell line contains glucocorticoid receptor and responds to dexamethasone treatment with a rapid induction of MMTV transcription. When these cells were treated for 24 h with various concentrations of sodium butyrate we observed an enhancement of CAT activity with 0.5 and 1 mM butyrate, and a reduction at concentrations of 4 mM and higher (Fig. 1A, −DEX (Upper), and data not shown). When the cells received in addition 0.1 μM dexamethasone during the last 12 h of incubation, we observed an enhanced CAT activity in the absence of butyrate, which was further increased at low concentrations of butyrate, with an optimum at 1 mM, and decreased at higher concentrations (+DEX in Fig. 1A Upper, and data not shown).

A similar effect was observed when TSA was used to inhibit histone deacetylase activity. The optimal inducing TSA concentration was found to be 16 nM for both basal and glucocorticoid-induced transcription in C127-BPVMTCV cells, whereas a 10-fold higher concentration drastically inhibited CAT activity in the absence or presence of dexamethasone (Fig. 1A Lower). The effect of TSA is not due to its influence on the cell cycle, as both inhibiting and activating concentrations of TSA led to a similar and moderate accumulation of cells in G1 (Table 1).

To assess the transcriptional activity of the MMTV promoter, we quantitated specific transcripts by RNase protection. Treatment of C127-BPVMTCV cells with low concentrations of TSA for 24 h resulted in a maximal increase of MMTV transcripts in the absence or in the presence of hormone (Fig. 1B Left). In both cases the stimulation was already evident after 6 h of incubation with TSA (Fig. 1C). High concentrations of TSA (50 ng/ml or 165 nM) inhibited basal and hormone induced transcription (Fig. 1B Right). Thus, the changes in CAT activity following treatment with the inhibitors reflect changes in accumulation of transcripts from the MMTV promoter.

The rate of transcription from the MMTV promoter was measured in nuclear run-on assays. A comparison with the GAPDH internal standard shows that treatment with low concentration of TSA resulted in enhanced rates of transcription both in nuclei from hormone naive cells and in nuclei from cells treated with dexamethasone (Fig. 1D). High concentrations of TSA inhibited transcription in both cases.

Fig. 1. Dose dependence of butyrate and TSA effects on basal and dexamethasone-induced MMTV promoter expression in C127-BPVMTVC cells. Cells were incubated with the indicated concentrations of butyrate or TSA for 2 h, followed by 10⁻⁷ M dexamethasone (+ DEX) or ethanol (− DEX) for additional 12 h. CAT activity was determined with 100 ng of cytoplasmic extract as described (38). (A) Quantitative representation of CAT activities expressed as % conversion to acetylated chloramphenicol (see Materials and Methods). (B) Effect of TSA on transcription from the MMTV promoter in C127-BPVMTVC cells. Data show the time dependence of the TSA effect on the steady-state levels of MMTV promoter transcripts in C127-BPVMTVC cells. Cells were incubated without TSA or with 5 ng/ml (16 nM) or 50 ng/ml (165 nM) TSA for the times indicated. After an additional time of 30-min incubation with 10⁻⁷ M dexamethasone (+ DEX) or ethanol (− DEX), the cells were harvested, and total RNA was prepared and analyzed by RNase mapping. Correctly initiated MMTV transcripts result in RNase-resistant fragments of 111 bp length. The positions of size markers (pBR322 digested with HpaII) are indicated on the right. (C) Quantitative evaluation of the levels of MMTV transcript. The gel used for the autoradiogram shown in B was quantitated with a Phosphorimager. (D) Rate of RNA synthesis in C127-BPVMTVC cells treated with TSA. Cells were treated with the indicated concentrations of TSA for 12 h, followed by induction with 10⁻⁷ M dexamethasone or ethanol for 60 min. Run-off analysis was performed according to Greenberg and Ziff (32). For hybridization, 10 μg of the plasmids pMMTV-CAT (MMTV) and pGAPDH (GAPDH) were used.

We conclude from this set of experiments that subsaturating concentrations of inhibitors enhance the rate of transcription of episomal MMTV promoters approximately to the same level as achieved with dexamethasone. In glucocorticoid-induced cells, these relatively low concentrations of inhibitors further enhance the rate of MMTV transcription. However, higher inhibitor concentrations, which have been used in most previous studies (24), repress both basal and hormone induced transcription from the MMTV promoter.

Low Concentrations of Inhibitors Enhance Basal and Progestin-Induced Transcription from a Chromosomally Integrated MMTV Promoter. To test whether the effects of inhibitors of histone deacetylases were limited to episomal vectors and glucocorticoid induction we analyzed another recently established cell line, T47D-MTVL, that carries a single copy of the complete MMTV-LTR driving the luciferase gene (6). This cell line contains high levels of the progesterone receptor and responds to addition of progesterin with a rapid stimulation of MMTV transcription (6).

Treatment of T47D-MTVL cells with butyrate or TSA had a similar effect on MMTV promoter activity as described above for C127-BPVMTVC cells: low concentrations enhanced basal and induced transcription, whereas higher concentrations inhibited both basal and hormone-induced promoter activity (Fig. 2 and data not shown). The effect of butyrate in the absence of added hormone was not due to the presence of low concentrations of hormone in the culture medium, nor to activation of the receptor in the absence of ligand, as it was not influenced by the progestin antagonist RU486 (data not shown), which is known to prevent binding of the hormone receptor to the MMTV promoter (29). Thus the stimulatory effect of butyrate takes place independently of ligand activated receptor.

The optimal inducing concentration of TSA was found to be 165 nM (50 ng/ml) and inhibition of MMTV transcription was observed at 330 nM TSA (Fig. 2A). At the inducing concent-

![Table 1. Analysis of TSA effects on cell cycle distribution of T47D-MTVL cells](image)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% cell in various phases of the cell cycle</th>
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<tr>
<td>− TSA</td>
<td>62.9 16.6 20.6</td>
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<tr>
<td>+ 50 nM TSA</td>
<td>72.4 15.6 12.0</td>
</tr>
<tr>
<td>+ 100 nM TSA</td>
<td>77.2 11.5 11.2</td>
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Cells treated with ethanol (−TSA), or 50 or 100 nM TSA for 12 h were trypsinized, resuspended in PBS, and fixed by adding 3 volumes of cold (−20°C) EtOH. DNA was stained with Hoechst 33258 (Sigma) and FACS analysis was performed with a FACStarPlus (Becton Dickinson).

![Fig. 2. Effect of TSA on basal and progesterin-induced expression of a chromosomally integrated MMTV-luciferase reporter](image)

(A) Dose dependence of the TSA effect on expression of the MMTV promoter in T47D-MTVL cells. Cells were treated with the indicated concentrations of TSA for 12 h. Treatment with either ethanol or 2 × 10⁻⁸ M R5020 was continued for additional 6, 12, and 24 h. After incubation the cells were harvested with Triton-lysine buffer and luciferase activity was determined. (B) Time dependence of the TSA effect on mRNA levels in T47D-MTVL cells. Cells were treated with 50 ng/ml TSA (165 nM) for the times indicated and induced with either ethanol or 2 × 10⁻⁸ M R5020 for 30 min. Total cellular RNA was prepared and analyzed by RNase mapping using a riboprobe that contains MMTV sequences of the C3H strain from +91 to −140. Correctly initiated transcripts protect a 91-bp fragment from RNase digestion. The position of the correct transcript is indicated by an arrow.
Transcription, TSA pretreatment enhanced the accumulation of transcripts from the MMTV promoter in hormone naive and in progesterin-induced cells (Fig. 2B). The effect of TSA was evident after 6 h, maximal after 12 h, and decreased after 24 h.

These results suggest that moderate acetylation of core histones induces transcription from a chromosomally integrated MMTV promoter. Furthermore, the response to progesterin is enhanced to a similar extent as the response to glucocorticoids, although the quantitative aspects of the response are slightly different in both cell lines.

Maximal Transcriptional Activation Correlates with Moderate Acetylation of Core Histones. To directly compare the effects of the different TSA concentrations on histone acetylation in both cell lines and to correlate histone acetylation with transcriptional activity of the MMTV promoter, core histones from cells treated with activating and inhibiting concentrations of TSA were prepared and the degree of core histone acetylation estimated by electrophoretic analysis on acid–urea polyacrylamide gels that resolve differentially acetylated forms of histones (22). In the absence of inhibitor, the nonacetylated and the monoacetylated forms of H4 are dominant (Fig. 3) in both cell lines. Concentrations of TSA which cause enhanced basal and hormone induced MMTV transcription (5 nM for C127-BPV-MTVC cells and 50 nM for T47D-MTDL cells), led to a similar reduction of the nonacetylated form, while the mono- and diacetylated forms of histone H4 became dominant, with a weak band corresponding to triacetylated H4 (Fig. 3). Exposure of cells to higher concentrations of TSA results in the decrease of the amount of monoacetylated forms of histone H4 while the tri- and tetracetylated forms became dominant (Fig. 3). Changes were also detected in the degree of acetylation of the other core histones, in particular H3, but the individual acetylated forms could not be assigned unambiguously. Similar results were obtained when the effect of various butyrate concentrations was analyzed (data not shown).

We conclude that moderate acetylation of core histones in both cell lines correlates with an enhancement of transcription, while highly acetylated core histones correlate with an inhibition of basal as well as glucocorticoid or progesterin induced MMTV transcription.

Transcriptional Enhancement by Histone Deacetylase Inhibitors Does Not Affect the Hormone Dose Response. To test whether hormones and inhibitors of histone acetylation activate MMTV transcription by the same or independent mechanisms, we analyzed the dose response to increasing concentrations of hormone in the absence and presence of the inducing concentrations of inhibitor. We reasoned that if hormones act by enhancing histone acetylation, one should expect additive effects at low concentration of hormone, but the effect of the inhibitor should diminish as the concentration of hormone is raised and induction reaches optimal values. If, however, hormones and acetylase inhibitors act by independent mechanisms, one would expect the effect of inhibitor to be similar at every concentration of hormone. The results obtained with T47D-MTDL show that R5020 and TSA yield additive effects over the investigated range of hormone concentrations. The shape of the R5020 dose-response curve was not affected by the presence of inducing levels of TSA (data not shown).

TSA Treatment Does Not Change Nucleosome Positioning or Spacing. MMTV sequences are organized in a regular array of nucleosomes (5) that is supposed to contribute to transcriptional repression and may set the stage for hormone induction of transcription (6). Inhibitors of histone deacetylase could act by altering the nucleosomal organization of the promoter. To test this possibility, we analyzed the influence of various TSA concentrations on the nucleosomal organization of the MMTV-LTR. Treatment of isolated nuclei with MPE-Fe(II) was used to determine the translatonal positioning of nucleosomes on the minichromosomes of C127-BPV-MTVC cells (34). As previously reported (6), several nucleosomes were found at defined positions along the MMTV-LTR, as indicated by the regular spacing of MPE-cleavage sites detected in indirect end-labeling experiments (5). The distribution of cleavage sites was not influenced by the pretreatment of cells with various concentrations of TSA (data not shown), suggesting that histone acetylation does not alter the general packaging of MMTV sequences in nucleosomes. Similar results have been reported for MMTV expressing cells after butyrate treatment (39).

Activation by TSA Causes DNase I Hypersensitivity of Nucleosomal DNA Similar To That Generated by Hormone Induction. Glucocorticoid induction of MMTV reporter genes integrated in chromatin leads to the rapid appearance of DNase I hypersensitive sites over a phased nucleosome encompassing the hormone-responsive elements (4, 5). We have recently reproduced these findings in C127-BPV-MTVC cells, and located the DNase I cleavage sites to a region near the dyad of nucleosome B (6). Interestingly, treatment of C127-BPV-MTVC cells with low concentrations of TSA, in the absence of hormonal stimulation, also resulted in the appearance of this DNase I hypersensitive cleavage site (Fig. 4, lanes 5–7). This effect is selective for nucleosome B, as no changes in nuclease sensitivity are detected in the neighboring nucleosomes A and C. The same moderate concentration of TSA also enhanced slightly the hormone induced DNase I cleavage (Fig. 4, lanes 14–16). In contrast, high TSA concentrations which inhibit MMTV transcription did not generate DNase I hypersensitivity at the dyad of nucleosome B in the absence of hormone and completely abolished the hormone induced DNase I cleavage at this site (Fig. 4, lanes 8–10 and 17–19).

We conclude that the concentrations of TSA that activate transcription from the MMTV promoter and lead to moderate histone acetylation cause a local structural change of nucleosome B similar to that detected in cells induced by hormonal treatment.

**DISCUSSION**

The results reported above demonstrate a correlation between the effect of inhibitors of histone deacetylases on the degree
of acetylation of core histones and changes of the transcriptional activity of the MMTV promoter. Low inhibitor concentrations, which lead to accumulation of mono- and diacetylated forms of histone H4, caused an enhancement of MMTV transcription both in the absence and in the presence of the inducing hormones. These effects were observed in GR-positive cells carrying episomal copies of an MMTV reporter plasmid as well as in PR-positive cells carrying a single chromosomal integrated MMTV reporter. The observed effects are not due to accumulation of cells in a particular phase of the cell cycle (18), since activating and inhibiting concentrations of histone deacetylase inhibitors cause weak but qualitatively similar effects on cell cycle distribution of treated cells.

One remarkable finding is that treatment with the inhibitors induces the same type of enhanced sensitivity to DNase I near the nucleosome dyad as found after hormone treatment (6). The effect of the inhibitors in the absence of hormone is not due to the presence of traces of hormone in the culture medium and is probably not mediated by the receptor since it is not blocked by addition of the antihormone RU486, which is known to prevent binding of hormone receptors to the MMTV promoter in vivo (29).

Our findings that high concentrations of butyrate inhibit basal and hormone induced transcription from the MMTV promoter confirm and extend previous reports (24). Moreover, high concentrations of inhibitor do not induce the characteristic changes in conformation of the MMTV nucleosome leading to DNase I hypersensitivity, and prevent their appearance when the cells are treated with hormone. In C127-BPV-MTVC cells, we need relatively low concentrations of the inhibitors to induce tri-and tetraacetylated forms of core histones, and these low doses are sufficient to inhibit transcription from the MMTV promoter. In T47D-MTVC cells, much higher concentrations of the inhibitors are required for inducing the same degree of hyperacetylation, and similarly high concentrations are needed to inhibit MMTV transcription. The good correlations between the effects of butyrate and TSA on histone hyperacetylation and their influence on MMTV transcription in the two cell lines studied strongly suggest that the degree of acetylation of the core histones determines the transcriptional state of the promoter.

In principle there are two main mechanisms by which changes in acetylation could influence MMTV transcription: direct participation of the histone tails in factor binding and indirect effects on nucleosome structure. In any case, our results suggest that acetylation at different sites or to different levels could have opposite effects on promoter accessibility and transcriptional activity (40).

It is conceivable that the positively charged N-terminal histone tails play an active role in recruiting transcription factors or in stabilizing their binding to the MMTV promoter. Indications for an active role of the N-terminal domains of histone H4 in transactivation have been obtained in yeast (41), whereas the N-terminal tail of histone H3 seems to play a role in silencing (42). A direct interaction of the histone tails with regulatory proteins has been recently demonstrated in yeast (43). On the other hand, in vitro nucleosome reconstitution experiments suggest that hyperacetylation of core histones facilitates binding of transcription factors, such as TFIIIA and TATA box-binding protein, to a positioned nucleosome (44). If the histone tails would be directly involved in factor binding to the MMTV promoter, the biphasic nature of the dose response to butyrate and TSA suggests that acetylation of certain sites favors factor binding, whereas extensive acetylation inhibits it. Further elucidation of these processes could be achieved in Saccharomyces cerevisiae, since the MMTV promoter is appropriately organized in nucleosomes in yeast and is subjected to the same transcriptional control as in metazoan cells (45). Thus, one could study the influence of mutations in the acetylated lysines of the histone tails on the behavior of the MMTV promoter.

Since the order of acetylation of the various lysine residues is not random (46, 47), it is possible that the initially acetylated sites make nucleosomal DNA more accessible for factor binding by facilitating a conformational transition of the nucleosome. On the contrary, strong hyperacetylation could generate a nucleosome structure unable to undergo this kind of transition and, thus, incompatible with factor binding. These effects could be direct (48, 49) or mediated by the influence of acetylation on the interaction of core particles with each other.

FIG. 4. Effect of TSA on nucleosome structure of the MMTV promoter. Analysis of DNase I hypersensitivity of the MMTV promoter in C127-BPV-MTVC cells after treatment with 5 ng/ml (16.5 nM) and 50 ng/ml (165 nM) TSA for 12 h. After incubation with either 10⁻⁷ M dexamethasone (DEX) or ethanol for 30 min, cells were collected and subjected to a DNase I digest with 10, 20, and 40 units DNase I and analyzed by indirect end-labeling using an EcoRI/BglII probe. Equally digested genomic DNA was treated with EcoRI. The positions of the nucleosomes A-D and the StuI restriction site are marked on the right. The quantitation of DNase I hypersensitivity is shown at the bottom. The restriction cut of genomic DNA with EcoRI and StuI serves as a control for the maximal signal and was set to 100% (last lane).
and with the linker histones (14, 50, 51). The relevance of histone acetylation in gene activation has been highlighted recently by the discovery that the nuclear histone acetyltransferase type A from Tetrahymena is homologous to the putative transcriptional adaptor Gcn5 (52), originally found in yeast but also present in higher eukaryotes (53).

Regardless of the mechanism of acetylation-mediated activation of the MMTV promoter, it remains to be studied whether hormonal induction does lead to a change in the acetylation state of the MMTV nucleosomes. The similar changes in DNase I sensitivity observed with inducing concentrations of deacetylase inhibitors and with hormones make this speculation tempting, but the lack of influence of the inhibitors on the shape of the hormone induction curve, does not favor this concept. A direct answer to this question awaits the analysis of the MMTV nucleosome before and after hormonal induction in terms of the state of acetylation of its core histones.

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