Endocrine, *vol. 11, no. 2, 157–164, October 1999* 0969–711X/99/11:157–164/$12.00

The Effects of Histone Acetylation on Estrogen Responsiveness in MCF-7 Cells

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Because histone acetylation is implicated in the facilitation of specific gene transcription, the effect of increasing histone acetylation on the expression of an endogenous gene was investigated. The ability of trichostatin A (TSA), a histone deacetylase inhibitor, to potentiate the estradiol (E_2) induction of progesterone receptor (PR) levels in MCF-7 cells was studied. Although TSA alone had no effect on PR synthesis, measured by a whole-cell binding assay with [^{3}H]R5020, TSA potentiated the effect of 10^{-11} *M* E_2 such that 10 ng of TSA/mL approximately doubled the hormone response. When TSA was removed from the cells after various incubation times (24 and 48 h) by successive washings with TSA-free medium, it was determined that TSA was required throughout the 96-h incubation period in order to achieve maximal potentiation for the E_2 response. In addition, TSA potentiated E_2 induction of pS2 mRNA. These results suggested that the estrogen receptor (ER) complex might alter histone acetylation as part of the gene activation process. To test this directly, MCF-7 cells were incubated for 48 h with E_2 followed by incubation with sodium [^{3}H]acetate for 1 h. From two-dimensional polyacrylamide gel electrophoresis, an increase in total acetate incorporation into histones in estrogen- treated cells compared to control was observed as well as a preferential increase in the mono- and diacetylated histone H4. Experiments with lysine-specific antiacetylated H4 antibodies suggest a preferential increase in acetylation at lysine 16, but not 5, 8, or 12. The results of this study support an important role for histone acetylation in the mechanism of action of the ER.

Key Words: Estrogen receptors; histones; histone deacetylase inhibition; histone acetylation; estrogen action; MCF-7 cells.

Received May 10, 1999; Revised June 29, 1999; Accepted July 23, 1999.

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Introduction

Histones, once thought to serve as nothing but cellular packing materials for chromatin, have been implicated as vital participants in both repressing and facilitating activation of many genes *(1)*. DNA wraps roughly twice around the nucleosomal core, which is composed of two of each of the histones H2A, H2B, H3, and H4. Activator proteins at upstream activator sequences directly or indirectly cause the histone core particles to dissociate from the initiation site. Therefore, the DNA strands are opened and transcription is initiated. It has been observed that transcription is often accompanied by the addition of acetyl groups to histone tails *(2)*. Such additions would be expected to neutralize the positively charged tails, which in turn could potentially disrupt the interaction of tails with negatively charged DNA, unfolding the nucleosomes, freeing TATA boxes from nucleosomes, and allowing the initiation of transcription. In addition, acetylation of histones at specific lysine sites has been implicated as a transducing signal for transcription factors *(3,4)*.

High levels of histone acetylation are associated with transcriptionally active chromatin *(5,6)*. It has been deduced that not only the degree but the order of acetylation and/or the specific lysines acetylated may be potentially significant *(7)*. In general, as the N-terminal lysines of H4 become acetylated, the positive-charge property of this region is lost and the affinity that this portion of the molecule has for negatively charged DNA is reduced *(8)*. The important functional role of the N-terminus of H4 may lie in the control of transcription, specifically in modulating the access of transcription factors *(9–11)*. It has also been shown that the displacement of a nucleosome core can be a direct result of binding by regulatory factors *(9,12)*. It has been speculated that there may be different acetyltransferases for each acetyl site on H4 tails. Also, histone acetylation may give preference for further histone posttranslational modifications, such as adenosine 5'-diphosphate-ribosylation *(13)*, phosphorylation *(14)*, and methylation *(15)*.

Steroid hormones, which induce changes in cell proliferation and differentiation, exert their effects through specific intracellular steroid hormone receptors. On ligand

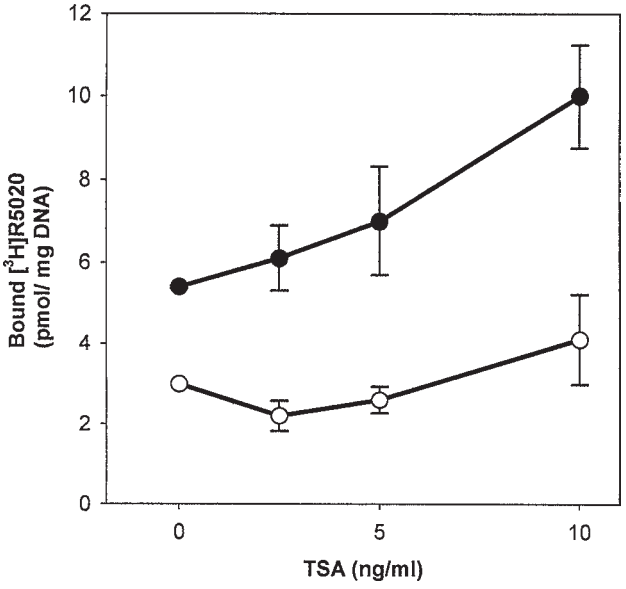


Fig. 1. TSA potentiates E_2 induction of PR. MCF-7 cells were treated with 2.5–10 ng of TSA/mL in the absence (○) or presence (●) of 10^{-11} *M* E_2. PRs were measured at 96 h with a whole-cell binding assay utilizing [^{3}H]R5020 ± 200-fold excess R5020 to measure specific binding. Data are expressed as the mean ± SE of four separate experiments.

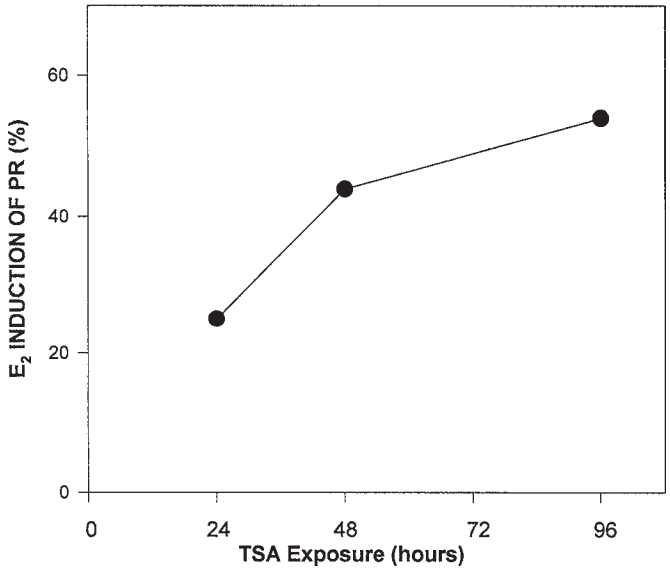


Fig. 2. TSA is necessary throughout E_2 incubation for maximal potentiating effect. MCF-7 cells were treated with 5 ng of TSA/mL for the first 24 or 48 h or the full 96 h. E_2 was present throughout the 96-h period prior to PR measurement. Data are from one of two experiments with similar results. Data are expressed as the percentage of increase in PR levels compared to cells treated with E_2 in the absence of TSA.

binding, these receptors act as hormone-dependent transcription factors by interaction with steroid hormone response elements (SREs) or through protein:protein: DNA complexes. One model put forth to explain the role of steroid receptors in gene transcription suggests that these receptors are involved in chromatin remodeling *(16–18)*.

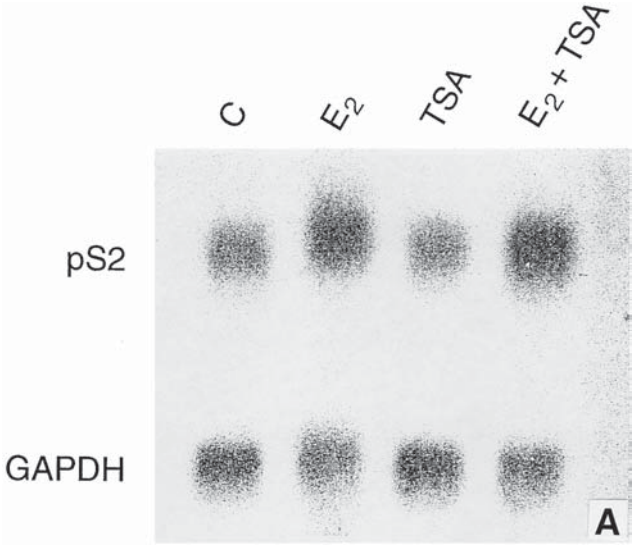
Recent studies have demonstrated that several coregulators associate with steroid receptors and facilitate the transactivation of target genes, or alternatively repress transcription of genes *(9,19–21)*. Coactivators appear to enhance the assembly of basal transcription factors, allowing increased transcription rates by RNA polymerase II. The finding that the cAMP response element-binding protein (CBP), steroid receptor coactivator-1 (SRC-1), and p300/CBP-associated factor associate with each other as well as steroid receptors and contain intrinsic histone acetyltransferase (HAT) activity has led to models *(9,19,20)* that suggest that receptors bound to their respective SREs alter chromatin structure through association with these coactivators with HAT activity. Conversely, a complex of factors that includes a histone deacetylase, HDAC-1, is involved in the repression of target genes *(9,22)*. Thus, chromatin remodeling involving HATs vs HDACs are involved in hormone-induced gene activation/repression. However, the function of a nuclear receptor is limited by the accessibility to its regulatory DNA sequences as well as to regulatory proteins *(23)*, and steroid receptors have been shown to bind histones and nucleosomes and therefore alter chromatin structure *(24)*. It has also been reported that some promoters are inaccessible to cognate binding proteins and that an inducible ligand can increase promoter accessibility in a receptor-dependent fashion, which is postulated to be a result of rapid alterations in chromatin structure *(23)*. Thus, response element accessibility, promoter accessibility, and stabilization of protein/protein/DNA interactions by ligand-induced receptors may involve common parameters, such as nuclear matrix receptor binding factors or nucleosomal proteins (core histones).

The present study investigates the potential for trichostatin A (TSA), a histone deacetylase inhibitor, to potentiate the actions of estrogens on MCF-7 cellular responses and the ability of estrogens to cause acetylation of specific histones and lysine-specific acetylation of H4. The results of these studies support an important role for histone acetylation in the action of estrogen induction of gene expression.

Results

Effect of TSA on Progesterone Receptor Induction by Estradiol

Various concentrations of estradiol (E_2) were tested to determine the dose range for induction of progesterone receptor (PR) as measured by binding of [^{3}H]R5020 in a whole-cell binding assay. Maximal induction occurred with a dose of 10^{-10} *M* E_2, and a 70% increase in PR was obtained with 10^{-11} *M* E_2 (data not shown). Experiments were then performed to determine the effect of various concentrations of TSA on PR levels when MCF-7 cells were grown in the presence of 10^{-11} *M* E_2, a submaximal



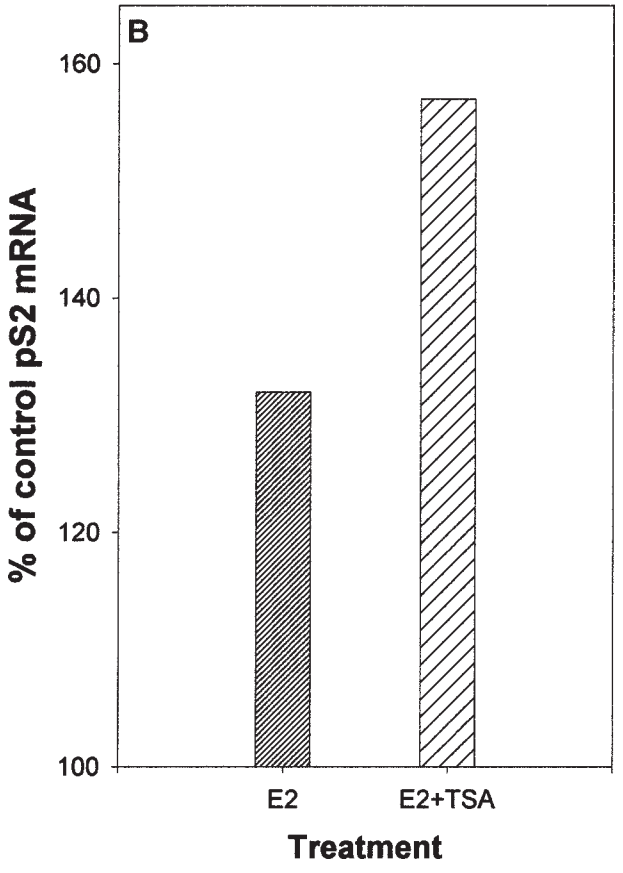


Fig. 3. TSA potentiates the E_2-induced expression of pS2 mRNA. **(A)** Representative autoradiogram of expression of pS2 mRNA. MCF-7 cells were treated with vehicle (lane 1), 10^{-11} *M* E_2 (lane 2), 5 ng/mL of TSA (lane 3) or TSA plus E_2 (lane 4) for 48 h. mRNA was isolated, and the mRNA (5 µg) was analyzed by Northern blot analysis with the pS2 cDNA probe. GAPDH cDNA probe was used to correct for loading. Data are from one of two experiments with similar results. **(B)** PhosphorImager analysis of data in (A).

dose for 96 h. TSA alone had no effect on PR levels in the absence of E_2; however, increasing concentrations of TSA (2.5–10 ng of TSA/mL) potentiated the E_2 effect. The addition of 10 ng of TSA/mL caused an ~100% increase in PR levels compared with E_2 alone (Fig. 1).

To determine whether short-term exposure to TSA was sufficient to potentiate E_2 responsiveness, TSA (5 ng of TSA/mL) was removed by extensive washing of the cells after 24 and 48 h (Fig. 2), with E_2 treatment continuing for 96 h. The greatest potentiation by TSA of the effect of E_2 on PR levels was obtained with continuous treatment with TSA. If TSA was present only for the first 24 h, the potentiation effect was decreased by ~50%.

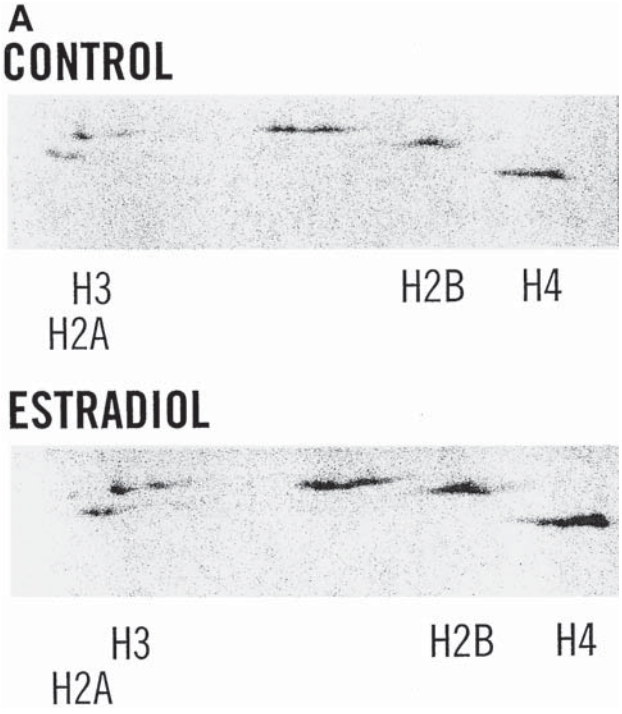
Effect of TSA on Expression of the pS2 Gene

To determine the effect of TSA on the specific induction of pS2 mRNA, Northern blot analysis was performed. Results demonstrated that treatment of cells with TSA alone had no effect on mRNA compared to that seen in untreated cells. However, TSA potentiated the effect obtained with a low dose of E_2 (Fig. 3), resulting in an ~80% increase compared with results obtained with E_2 alone.

Incorporation of [^{3}H]acetate into Histones

Histone isoforms isolated from E_2-treated MCF-7 cells were resolved on 1-D acid-urea-Triton (AUT) gels, and Coomassie Brilliant Blue R-250 staining demonstrated that multiple forms of histones H4 and H2B can be seen in control and E_2-treated cells (data not shown). To determine the effect of E_2 on histone acetylation, cells treated with vehicle or E_2 for 48 h were incubated with sodium [^{3}H] acetate for the last hour of incubation. To quantitate the degree of [^{3}H]acetate incorporation, 2-D AUT-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels were run. As can be seen in an autoradiogram from a representative experiment (*see* Fig. 4A), the four acetylated forms of H4 were clearly resolved. Densitometry analysis of the separated isoforms indicated that in each of these experiments the incorporation of [^{3}H]acetate increased in all four isoforms of H4 (mono-, di-, tri-, and tetra-acetylated H4) in the presence of E_2. In addition, the mono- and diacetylated forms of H4 predominated. In all experiments, equality of protein loading was confirmed by densitometry of Coomassie-stained gels (Fig. 4B). Figure 5 shows the mean percentage of increase in the various acetylated forms of histone H4 from three separate experiments. There was a 42–55% increase compared to control in all acetylated forms of H4.

E_2 also caused a minimal increase (~14%) in the incorporation of [^{3}H]acetate into histone H2B. Since the various isoforms of H2B were not resolved well enough in all experiments to quantitate individual isoforms, we quantitated the radioactivity of the total H2B isoforms. The monoacetylated isoform of H3 was clearly measurable, and E_2 caused an increase in acetylation in all three experiments with an average of 29% compared to control (Fig. 5).



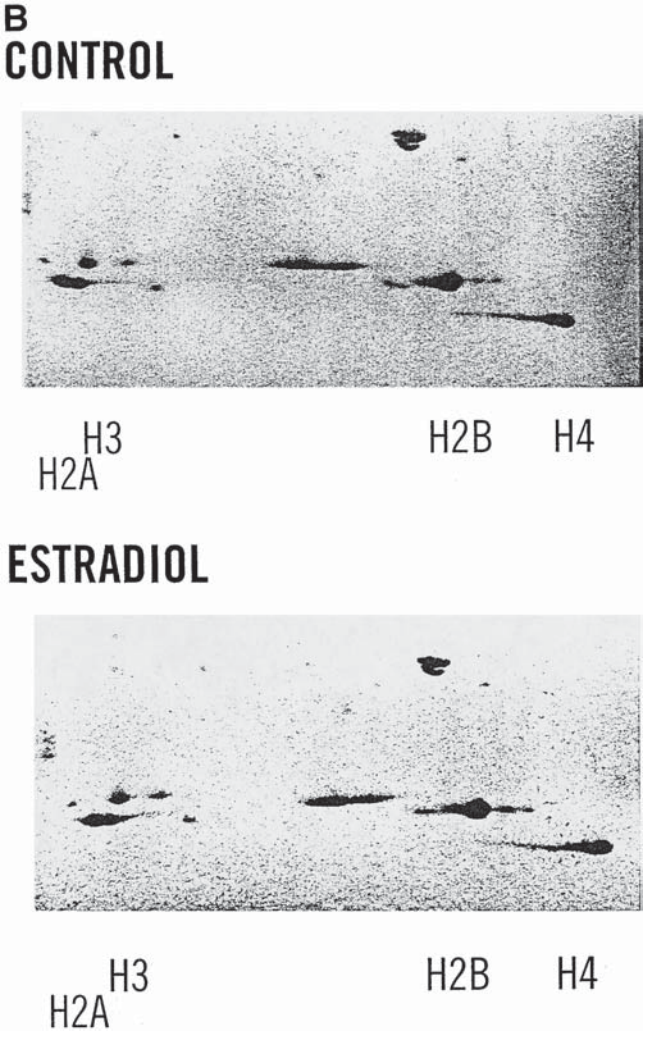


Fig. 4. Acetylated forms of histone H4 increase in response to E_2. MCF-7 cells were incubated with 10 n*M* E_2 for 48 h. The cells were incubated with 0.1 mCi of sodium [^{3}H]acetate/mL for the final hour. The histones were extracted from the nuclei and resolved using 2-D AUT-SDS-PAGE. **(A)** Autoradiogram of a representative 2-D gel of three separate experiments demonstrating the separation of the acetylated forms of H4, H2B, and H3. **(B)** Coomassie staining of gels in (A).

There were no estrogen-induced changes in acetate incorporation in H2A. Therefore, not all core histones demonstrated the same degree of change in acetate incorporation, demonstrating specific effects of estrogen on histone acetylation.

Lysine-Specific Acetylation

The results shown in Fig. 5 indicate the degree of increased acetylation of each H4 isoform induced by E_2 during a 1-h pulse with [^{3}H]acetate. However, the positions of the lysines in the N-terminal tail that were acetylated cannot be determined in these experiments. For example, the increase in acetylation in the diacetylated H4 can be owing to several combinations of acetylated lysines at positions 5, 8, 12, or 16. Therefore, we resolved histones on 1-D AUT gels and transferred the proteins to a polyvinylidene difluoride (PVDF) membrane for immunoblotting. Using Turner's (Serotech, Oxford, England) epitope-specific antiacetylated H4 antibodies *(25,26)*, we were able to determine the position-specific lysines acetylated after E_2 treatment.

There was no consistent change in acetylated lysines in positions 5, 8, and 12 with E_2 treatment (data not shown); however, lysine 16 consistently showed estrogen-induced increases in acetylation (Fig. 6), especially in the mono- (~40% increase) and diacetylated (~90% increase) isoforms. To quantitate acetylated lysine 16 in the tri- and tetra-acetylated forms, overexposed autoradiograms were used (not shown). The antibody to lysine 16 labeled $H4Ac_3$ and $H4Ac_4$ weakly. Since H4Ac4 must be acetylated at position 16, the antibody to that position must be inhibited by some conformational change induced by the acetylation of the other sites, i.e., lysines in positions 5, 8, and 12. This has also been noted for antisera to lysine 8 in human promyeloid HL60 cells *(25)* and in yeast *(27)*.

To ascertain whether the response to E_2 was specific, cells were incubated with 0.01 and 1 n*M* E_2 as well as 1 nM E_2 plus 0.1 μ*M* ICI 182,780. As can be seen in Fig. 7, the higher dose of E_2 demonstrated a greater degree of acetylated isoforms of lysine 16 compared to the lower dose, and incubation with the antiestrogen brought the response back to control levels, indicating that the response was dose dependent and mediated via the estrogen receptor (ER).

Discussion

In addition to steroid hormone receptor association with coactivators such as CBP and SRC-1 *(16)*, ERs have been

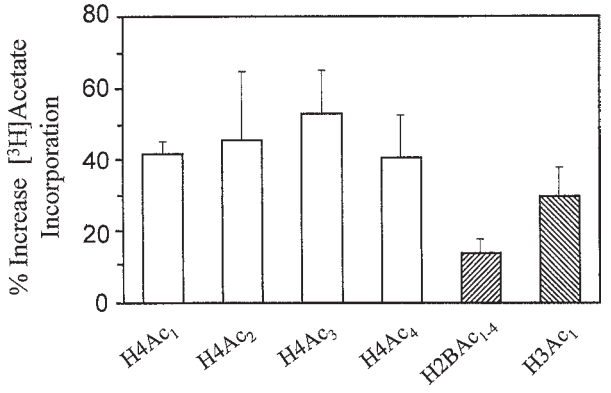


Fig. 5. Estrogen differentially affects the incorporation of [^{3}H] acetate into various histones. Densitometry analysis of autoradiograms of the 2-D gels demonstrates that E_2 caused an increase in the acetylated isoforms of H4 and the combined acetylated isoforms of H2B and monoacetylated H3. The isoforms of H2B were not clearly resolved in all experiments; therefore, the total acetylated isoforms were calculated. Only the monoacetylated isoform of H3 was clearly visible in all experiments. Data are from three separate experiments and are expressed as mean percentage of increase compared to cells incubated with vehicle alone.

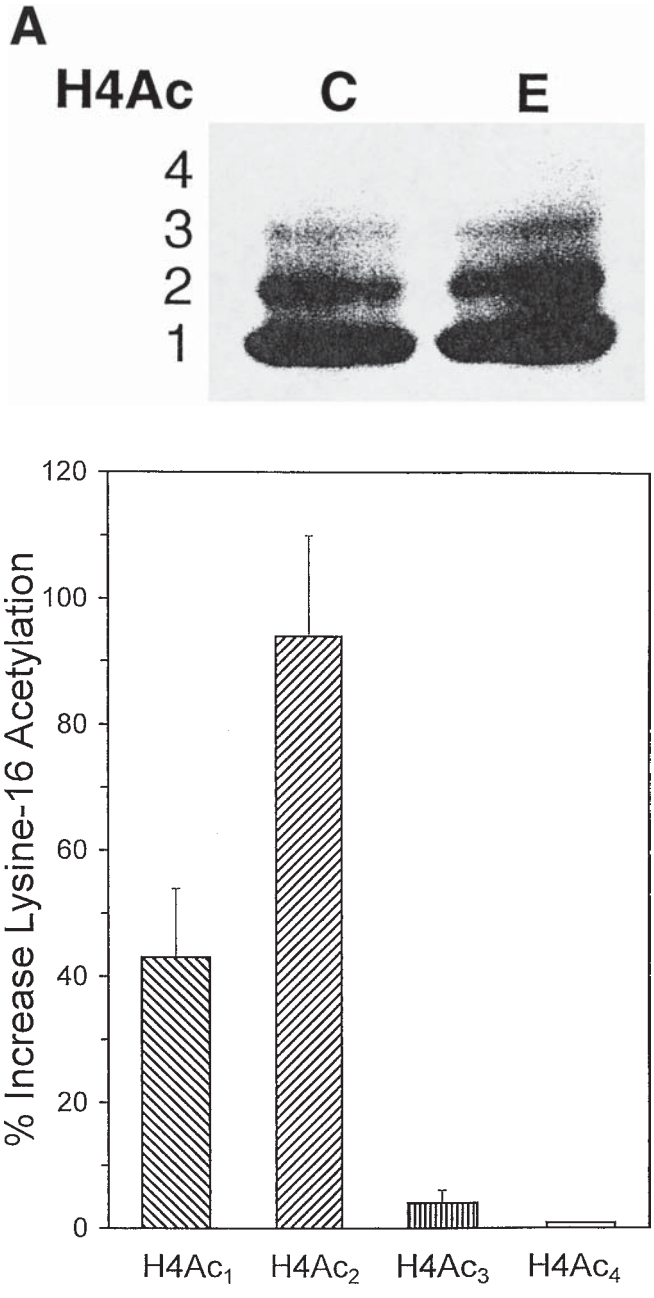


Fig. 6. Estrogen induces an increase in lysine 16 acetylation of histone H4. Histones isolated from control (C) or E_2-treated (E) cells were separated by 1-D AUT gel electrophoresis and the proteins were analyzed by Western immunoblot using position-specific antiacetylated H4 antibodies as described in Methods. **(A)** Representative experiment demonstrating detection of acetylated lysine 16 by ECL; **(B)** Summary of percentage of increase in lysine 16 acetylation. Data were from three separate experiments.

localized to the nuclear matrix *(28)*. ERs have also been reported to bind various histones with high affinity, again suggesting that ligand-induced changes in chromatin stucture are required for steroid hormone induction of gene expression. Kallas et al. *(29)* reported that ERs bind strongly to calf thymus histones H2B and H2A, moderately to histones H3 and H4, and poorly to histone H1 *(29)*. Conversely, Kiyotaka et al. *(30)* reported that glucocorticoid receptors have a higher affinity for H3 and H4 than H2A and H2B. Both groups of investigators postulated that nuclear salt-resistant (0.4 *M* KCl) steroid receptors represent histone-bound receptors. We *(31)* and others *(32)* had previously shown that the nuclear salt-resistant form of the ER is formed in vivo by estrogens but not by antiestrogens. We have also shown that ERs specifically bind unique subsets of H2B and H4, but not H3 *(33)*.

Precedent exists not only for the interaction of various steroid hormone receptors with co-activators or individual histones but also with the nucleosome as a whole. Sequence-specific positioning of nucleosomes over steroid-inducible promoters has also been shown to be important for the chromatin structural changes that occur on ligand binding *(18,22)*. During the process of gene activation, it has been demonstrated that nucleosomal structure can be disrupted. This process is measured as increased sensitivity to nucleases and alterations in the typical nucleosomal structure (i.e., nucleosome ladder). Although the increased sensitivity to nucleases is consistently observed on gene activation, interpretations vary as to whether nucleosomes are disrupted or simply rearranged *(22,34)*.

In the present study, we have demonstrated that TSA potentiated estrogen responsiveness of an endogenous gene in MCF-7 cells. Studies have shown that histone acetylation activates gene expression and can have effects on the expression of specific genes, but does not affect gene expression generally *(35,36)*. TSA, an inhibitor of histone deacetylation, has become an important tool for studies of histone acetylation *(37)*. TSA in low concentrations was shown to activate different genes *(38–40)*, induce

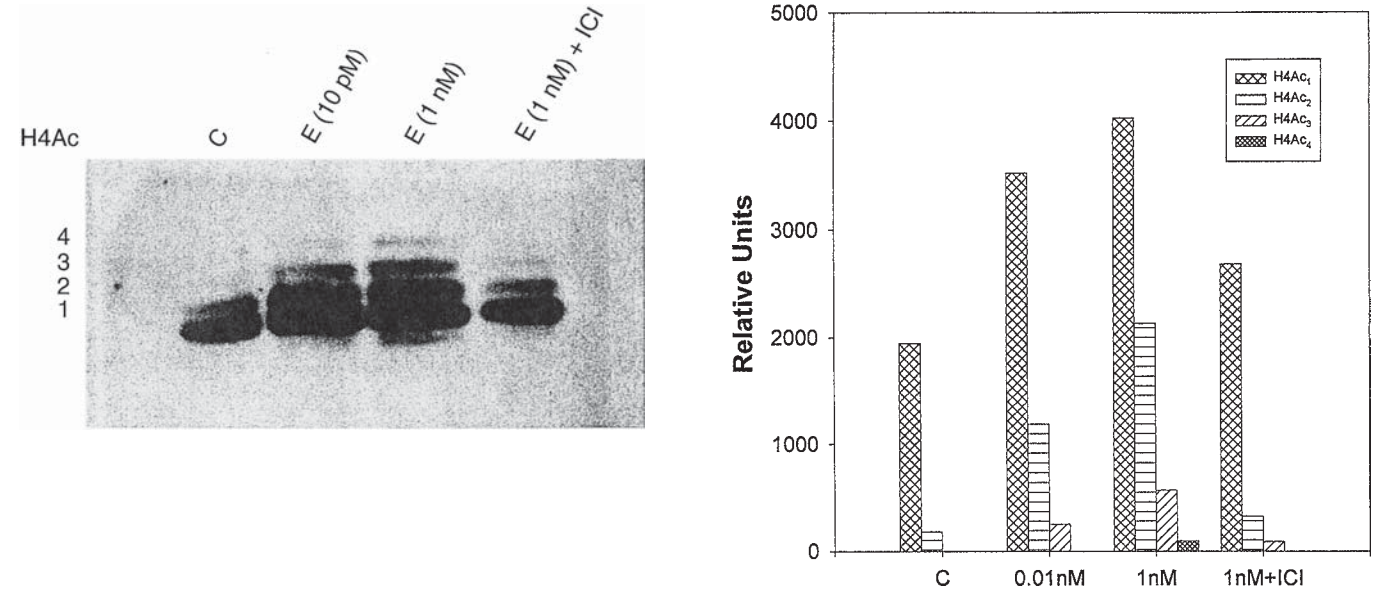


Fig. 7. The estrogen-induced increase in lysine 16 acetylation is blocked by antiestrogen. Histones isolated from control (C), or 0.01 or 1 n*M* E_2-treated cells, or cells treated with 1 n*M* E_2 plus 0.1 μ*M* antiestrogen ICI 182,780 were treated as described in Fig. 6. **(A)** Detection of acetylated lysine 16 by ECL of a representative experiment of two separate experiments. **(B)** Densitometry analysis of immunoblot.

cell differentiation *(41)*, and potentiate transcription from the MMTV promoter by glucocorticoids *(42)*. We recently reported that TSA potentiated the dioxin-induced expression of CYP1A1 *(43)* in primary cultures of hepatocytes. In the present study, increased histone acetylation caused indirectly by TSA was correlated with a potentiation of estrogen-induced PR levels, an endogenous gene in the context of normal chromatin. Others have reported that TSA potentiated PR action on a reporter gene *(9)*; however, correct nucleosomal structure with transiently transfected reporter genes has been questioned *(44)*. We also demonstrated that TSA potentiated E_2 induction of the pS2 mRNA in MCF-7 cells. Estrogen stimulation of the pS2 genes in breast cancer cells has been well characterized *(45)*.

To probe the dynamic acetylation on the N-terminal tails of histones, tritiated acetate was incorporated into histones by incubation of the MCF-7 cells with [^{3}H]acetate. This approach has been used to study the effects of various agents on the dynamics of histone acetylation *(46,47)*. The distribution of acetyl groups determined from the amount of tritiated acetate into each histone isoform can also be determined *(48,49)*. In addition, the activity of HATs in cells as well as the effects of histone acetylation on DNase I sensitivity of genes can be assessed by this method. In our study, [^{3}H]acetate incorporation into the various histone isoforms allowed us to determine estrogen effects on histone acetylation.

There seems to be a general correlation between histone dynamic acetylation and increased transcription activity *(17,50)*. Recently, this has been refined to show that increased acetylation of specific lysines on histone tails can be correlated with specific chromosomal domains and/or functions. For instance, using antibodies to H4 acetylated at lysines 5, 8, 12, or 16 and immunofluorescence, preferential acetylation of the lysine at position 16 on histone H4 in Drosophila polytene chromosomes occurred on the ¥ chromosome in the male but not the female *(51)*. By contrast, histone H4 acetylated at lysine 12 was highly enriched in *Drosophila* centric heterochromatin *(52)*. In addition, H4 acetylated at lysine 12 is relatively enriched in chromatin containing the silent mating type genes in yeast *(53)*. Also, immunoprecipitation analysis of mammalian chromatin from a human lymphoblastic cell line displayed a modest increase in H4 acetylated at lysine 16 in all types of coding DNA *(54)*. In our study estrogen induced a substantial and specific increase in acetylated lysine 16 on H4, which would support the latter finding.

Thus, steroid hormone action in vivo requires changes in chromatin structure, and specific histone proteins appear to be involved. With the multiplicity of variants and posttranslational modifications possible for the eight core histones (especially lysine-specific posttranslational acetylation), the potential for specific regulation of genes through steroid hormone receptor and specific histone octamer interactions is profound.

Materials and Methods

Chemicals

All chemicals were from Sigma (St. Louis, MO) unless specified. TSA, a gift from Dr. Yoshida, was purified by high-performance liquid chromatography (HPLC) with methanol:water (7:3, v/v) as the solvent system. E_2 and TSA were dissolved in dimethyl sulfoxide (DMSO) and

were diluted in complete medium with the final concentration of DMSO <0.01%. Sodium [^{3}H]acetate (20 Ci/mmol) in ethanol was from American Radiolabeled Chemicals (St. Louis, MO). [^{3}H]R5020 (80 Ci/mmol) was from Dupont NEN (Boston, MA).

Cell Culture and PR Measurement

MCF-7 cells were obtained from American Type Culture Collection (Rockville, MD) and were maintained in minimum essential medium (MEM) containing nonessential amino acids, phenol red (10 μg/mL), 10 m*M* HEPES, insulin (6 ng/mL), penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% charcoal-stripped calf serum. Cells were plated at a density of 10^5 cells/well in 12-well plates in estrogen-free (phenol red–free) medium 3 d prior to treatment with media containing the test compounds at the indicated concentrations, with daily medium changes. After various times of treatment, the cells were incubated with 10 n*M* [^{3}H]R5020 ± 200-fold excess R5020 for 30 min at 37°C to measure specific binding. The cells were then washed three times with 1 mL of Hank's balanced salt solution (HBBS) containing 2 mg of bovine serum albumin/mL followed by two washes with 2 mL of HBSS. The washed cells were dissolved in 10 m*M* EDTA, pH 12.3 (37°C); neutralized with 0.77 *M* KH_2PO_4; and sonicated. Aliquots were taken for determination of bound [^{3}H]R5020 by scintillation counting and the measurement of DNA. To assess the effect of the time of TSA treatment on E_2 responsiveness, cells were incubated with 10^{-11} *M* E_2 for 96 h and 5 ng of TSA/mL for 24 and 48 h, at which time the cells were washed with fresh medium to remove the TSA, and the incubation continued with medium containing E_2.

Northern Blot Analysis

To determine the effect of TSA on E_2 induction of the pS2 gene, cells were treated with 5 ng of TSA/mL, 10^{-11} *M* E_2, and TSA plus E_2 for 48 h. mRNAs were extracted by using the fast-track mRNA isolation kit (InVitrogen, San Diego, CA). The mRNAs and Gibco RNA markers (Life Technologies, Grand Island, NY) were separated on a 1.5% agarose gel, transferred onto a nylon membrane by capillary action, and bound to the membrane by ultraviolet crosslinking. The membrane was then prehybridized at 42°C overnight. [^{32}P]-Labeled pS2 cDNA probe (Random Primed Labeling Kit, Boehringer Mannheim, Indianapolis, IN) was added to the prehydridized membrane and hybridized at 42°C overnight. The hybridized membrane then was washed and visualized by autoradiography and quantified by a PhosphorImager. The GAPDH cDNA probe was used as a loading control. The pBS-GAPDH plasmid was a generous gift of Dr. T. J. Chambers (Saint Louis University, St. Louis, MO) and was prepared by subcloning a full length glyceraldehyde phosphate dehydrogenase cDNA into the Bluescript plasmid (Stratagene, LaJolla, CA).

Histone Acetylation

MCF-7 cells were plated at a density of 2×10^6 cells/10 cm plate and incubated in MEM minus phenol red with vehicle or 10 n*M* E_2 for 48 h. Sodium [^{3}H]acetate (0.5 mCi) was added to each plate and incubated for the final hour at 37°C. The reaction was stopped by removing the media, followed by washing the cells with media containing 0.1 m*M* sodium acetate and 10 m*M* sodium butyrate. The cells were harvested from the plates by scraping into HEGD buffer (25 m*M* HEPES, 1.5 m*M* EDTA, 10% glycerol, and 1 m*M* dithiothreitol, pH 7.6) followed by centrifugation at 1000*g* for 5 min at 4°C. The cells were washed twice in 14 mL of HEGD buffer and then were suspended in 14 mL of HED buffer (HEGD without glycerol) for 10 min at 4°C. Following centrifugation, the nuclear pellets were homogenized in 2.5 mL of HEGD buffer; mixed thoroughly with 12 mL of a solution containing 0.08 *M* NaCl and 0.02 *M* EDTA, pH 6.3; and allowed to sit on ice for 20 min. The chromatin solution was centrifuged at 3000*g* for 10 min to obtain the chromatin. The histones were extracted with 0.4 *N* sulfuric acid. After dialysis (mol wt cutoff: 8000) in 0.1 *N* acetic acid (100-fold vol) followed by water (300-fold vol, four times) and 5% acetonitrile in water (100-fold vol), samples were analyzed for protein content *(55)*. The histones were lyophilized and dissolved in sample buffer prepared for AUT-PAGE, and 30-μg samples were resolved by 17.5% 1-D AUT-PAGE according to the procedure of Davie *(56)*. The histones were detected with Coomassie Brillant Blue R-250. Equality of protein loading between control and experimental lanes was confirmed by densitometry. To better resolve the histone isoforms, 2-D AUT-SDS gels were run. A lane from the 1-D AUT gel was layered on a 1.0-mm Tris-glycine SDS gel *(57)* containing a 4.0% stacking gel and a two-step separating gel (8.4% and 15%). The gels were run at 200 V for 8 h. The incorporation of [^{3}H]acetate into the histone isoforms was visualized by autoradiography and quantitated by densitomitry.

Western Blot Analysis for Position-Specific Lysine Acetylation

To determine the effect of E_2 on position-specific lysine acetylation in histone H4, the histones were resolved on 1-D AUT gels and electrotransferred in CAPS buffer *(58)* onto PVDF membranes. The N-terminal tail lysines in the mono-, di-, tri-, and tetra-acetylated H4 were detected using rabbit antihistone H4–acetylated 5, 8, 12, or 16 antibodies *(24,25)* and goat antirabbit horseradish peroxidase conjugate followed by the ECL Western Blotting Kit obtained from Amersham (Arlington Heights, IL).

Ackowledgments

This work was supported by the National Institute of Environmental Health Sciences, ES05968; and the National Institutes of Diabetes and Digestive and Kidney Diseases, DK52478.

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