Accelerated Publication

Steroid Hormone Receptor-mediated Histone Deacetylation and Transcription at the Mouse Mammary Tumor Virus Promoter*

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Acetylation of lysines in histones H3 and H4 N-terminal tails is associated with transcriptional activation and deacetylation with repression. Our studies with the mouse mammary tumor virus (MMTV) promoter in chromatin show significant levels of acetylation at promoter proximal and distal regions prior to transactivation. Upon activation with glucocorticoids or progestins, promoter proximal histones become deacetylated within the region of inducible nuclease hypersensitivity. The deacetylation lags behind the initiation of transcription, indicating a role in post-activation regulation. Our results indicate a novel mechanism by which target promoters are regulated by steroid receptors and chromatin modification machinery.

The association of histone acetylation with transactivation, and deacetylation with repression, was first suggested in 1964 by Allison et al. (1). Such associations are now well documented in numerous studies (2–4), including several with steroid hormone-regulated promoters (5–7). Acetylation of specific lysine residues in histone N-terminal tails decreases their net positive charge, which has been proposed to cause an electrostatic repulsion between the histones and the negatively charged phosphate backbone of DNA (8) that results in a more open chromatin conformation. There are, however, data that conflict with this simple model. Mutskov et al. (9) have shown that hyperacetylated and hypoacetylated histone tails can associate almost equally well with DNA in chromatin at physiological salt concentrations. Mizuguchi et al. (10) show that hyperacetylation of histones alone does not stimulate transcription from the adenovirus E4 promoter. An alternative model proposes that the pattern and nature of histone tail modification provides a "code" that can be recognized by specific factors that then associate with the tail, thereby determining the functional consequence of the histone modification (11). The two models are not mutually exclusive.

Circumstantial evidence suggests that transactivation of some promoters is associated with deacetylation. Treatment with histone deacetylase (HDAC) inhibitors, which results in hyperacetylation of histones, can decrease rather than increase transactivation at some promoters or result in no change at others (12). The HDAC inhibitor sodium butyrate inhibits transactivation of the ovalbumin promoter by estrogen receptors (ERs) (13), and of the tyrosine aminotransferase promoter (14) and the MMTV-LTR by glucocorticoid receptors (GRs) (15). Furthermore, Deckert and Struhl (16) have recently shown that in Saccharomyces cerevisiae both acetylated and deacetylated histones H3 and H4 can be associated with transcriptionally active promoters.

Our results demonstrate that at the MMTV-LTR there is a significant level of basal acetylation at the promoter when it is inactive that decreases during hormone activation. This differs from what has generally been found at mamalian gene promoters characterized thus far (7, 17), but it has been described at some yeast promoters (Refs. 16, 18, and others). The experiments we describe significantly advance our understanding of the role of deacetylation in transcriptional regulation at the MMTV-LTR. The results suggest a mechanism by which steroid hormone-targeted promoter activity is regulated by the acetylation state of histones in addition to other proteins found at the promoter in response to stimuli that induce transactivation.

EXPERIMENTAL PROCEDURES

Cell Culture—The cell lines used in these experiments, 1470.2 and 3017.1, are derived from the same mouse adenocarcinoma parent line, C127i, have multiple copies of stably integrated MMTV-LTR (19, 20), and constitutively express mouse GR. Cells were grown in Dulbecco’s modified Eagle’s medium containing either 10% FBS or charcoal-stripped 10% FBS for 16–20 h prior to treatment with the synthetic glucocorticoid dexamethasone (Dex) (100 nm) or the progestin R5020 (30 nm).

Chromatin Immunoprecipitation (ChIP) Assay—Following treatment with hormone, cells were cross-linked with 0.5% formaldehyde at 37 °C for 10 min, nuclei were isolated, and the DNA was digested to predominantly monosomes with micrococcal nuclease (0.1 units/µg nucleic acid) at 37 °C for 10 min. Chromatin immunoprecipitation with antibodies to acetylated histones H3 and H4 was done essentially as described by the Upstate Biotechnology ChIP protocol (Upstate Biotechnology Inc., Lake Placid, NY). After overnight incubation at 4 °C, protein A-Sepharose was added for 3 h to pull out immune complexes. Washes were also as described but with the addition of 0.5% deoxycholate following cross-link reversal by incubation at 65 °C for 4–6 h. DNA was purified by repeated phenol/chloroform extraction and quantified by fluorimetry (Amersham Pharmacia Biotech). All steps starting with the isolation of nuclei to the cross-link reversal step were done in the presence of 5 mM sodium butyrate. DNA was analyzed by PCR (20 cycles) with primers that amplify MMTV nucleosome B (100-base pair

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1 The abbreviations used are: HDAC, histone deacetylase; MMTV, mouse mammary tumor virus; LTR, long terminal repeat; ER, estrogen receptor; GR, glucocorticoid receptor; PG, progestosterone receptor; ChIP, chromatin immunoprecipitation; FBS, fetal bovine serum; PCR, polymerase chain reaction; HRE, hormone response element; DEX, dexamethasone; Nuc, nucleus.

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To better understand the mechanisms that underlie chromatin remodeling and transactivation by steroid hormone receptors, we investigated the acetylation status of histones H3 and H4 in the MMTV promoter in response to steroid hormone receptor activation. The MMTV-LTR has six well-defined positioned nucleosome families, designated nucleosomes (Nuc) A–F. NucA overlaps the TATA region and the transcription start site, and NucF is the farthest 5′ to the transcription start site (−1 kilobase) (Fig. 1A) (22, 23). Four GR binding sites or hormone response elements (HREs) are located in the NucB region (Fig. 1A). A nucleosome hypersensitive site develops across NucB in response to glucocorticoid treatment, which indicates chromatin reorganization or remodeling at this nucleosome (24–26). Just 5′ to NucB there are two HREs in the NucC region, and nucleosome hypersensitivity extends into this nucleosome (26, 27). Nucleosome hypersensitive sites in promoters are generally associated with histone acetylation and with transcriptionally active promoters (28–30).

We determined the acetylation level of histones H3 and H4 at NucB and NucF, located inside and outside the hypersensitive site, respectively (25). A difference in the acetylation status of histones at these two nucleosomes can indicate whether any observed changes are associated with ATP-dependent chromatin remodeling. The mouse adenocarcinoma cell line 1470.2 was treated with 100 nM Dex for 1 h. Nuclei were isolated and subjected to micrococcal nuclease digestion. Actuation status was determined using ChIPs assays with antibodies to acetylated lysines in H3 (Lys-9 and -14) and H4 (Lys-5, -8, -12, and -16). There is a significant level of basal acetylation of histone N-terminal tails H3 and H4 at both NucB and NucF prior to formaldehyde cross-linking and processed as described in Fig. 1 legend. Relative levels of acetylated H3 (shaded bars) and acetylated H4 (hatched bars) are indicated on the left y axis. Levels of MMTV transcription (broken line) are expressed as fold induction with basal transcription (0 min) set to a value of 1 and are indicated on the right y axis. n = 3–6 for the ChIP analysis and 3–5 for the run-on analysis; error bars represent S.E.

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However, transcription has already reached its maximum. Deacetylation reaches its maximum at the 50% level after 30 min of treatment and persists after transcription decreases. By 15 min of treatment, histone acetylation at NucF is somewhat higher than that observed in untreated cells, as shown in Fig. 1, and does not change significantly at any time point (data not shown). These results indicate that deacetylation at NucB is associated with hormone-induced transcription but lags behind transcriptional activation, as deacetylation is still decreasing when transcription is maximal.

The progesterone receptor (PR) is closely related to the GR, binds to the same HREs, and can activate transcription from the MMTV-LTR. Like GR, stably expressed PR is able to induce hypersensitivity at the MMTV promoter. Miziguchi et al. (10) report that histone hyperacetylation at an in vitro assembled chromatin template is not sufficient to cause increased transcription but synergistically facilitates transcription induced by the binding of an activator and the activity of the ATP-dependent chromatin remodeling factor, NURF (nucleosome remodeling factor). Additionally, maximal histone deacetylation at the MMTV promoter is achieved just prior to the decrease in transcription, which suggests that deacetylation plays a role in the down-regulation of activated transcription, consistent with the generally observed correlation between deacetylation and transcriptional repression. HDAC inhibitors can, however, decrease rather than increase transcription at the MMTV promoter (15) but not in a time-frame consistent with repression. It is thus possible that the HDAC inhibitors target a non-histone protein in which acetylation is inhibitory to transcriptional initiation. Acetylation of the non-histone coactivator protein ACTR (activator of thyroid and retinoic acid receptors) coincides with inhibition of ER-mediated transcription (5).

The dynamic pattern of histone acetylation we observed at the MMTV promoter is different from two other systems examined in kinetic detail. Experiments with the estrogen-responsive cathepsin D and the pS2 promoters (5, 7) show low levels of histone acetylation prior to treatment with estradiol, which then rise and reach a peak just prior to the maximum of transcription at 60 min. Elevated levels of acetylation then persist to some degree as transcription declines and increases again. Reinke et al. (18) describe a transient hyperacetylation at the PHO8 promoter in yeast that occurs prior to chromatin remodeling and transcriptional activation. However, unlike our observation at the MMTV-LTR, levels of acetylation did not drop below those observed prior to activation. The variety of acetylation patterns and the dynamics of timing observed in these promoter systems suggests that different genes utilize histone and non-histone protein acetylation in distinct ways to regulate transcriptional activity. The mechanisms by which acetylation functions in transcriptional regulation are likely more complex than simply electrostatic repulsion between acetylated histones and DNA, or a histone code that directs non-histone proteins to the promoter, and are not yet fully understood.

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REFERENCES

1. Allfrey, V., Faulkner, R. M., and Mirsky, A. E. (1964) Proc. Natl. Acad. Sci. U. S. A. 51, 786–794
2. Wolfe, A. P., and Guschin, D. (2000) J. Struct. Biol. 129, 102–122
3. Struhl, K. (1996) Genes Dev. 12, 599–606
4. Grunstein, M. (1997) Nature 389, 349–352
5. Chen, H., J., L. R., Xie, W., Wilpitz, D., and Evans, R. M. (1999) Cell 98, 675–686
6. DiRenzo, J., Shang, Y., Phelan, M., Sif, S., Meyers, M., Kingston, R. E., and Brown, M. (2000) Mol. Cell. Biol. 20, 7541–7549
7. Shang, Y., Hu, X., DiRenzo, J., Lazar, M. A., and Brown, M. (2000) Cell 103, 843–852
8. Hong, L., Schrotz, G. P., Matthews, H. R., Yao, P., and Bradbury, E. M. (1993) J. Biol. Chem. 268, 305–314
9. Mutsukov, V., Gerber, D., Angelov, D., Ausio, J., Workman, J., and Dimitrov, S. (1998) Mol. Cell. Biol. 18, 6293–6304
10. Miziguchi, G., Vassilev, A., Tsukiyama, T., Nakatani, Y., and Wu, C. (2001) J. Biol. Chem. 276, 14773–14783
11. Strahl, B. D., and Allis, C. D. (2000) Nature 403, 41–45

\(^2\) C. L. Smith, unpublished observations.
12. Van Lint, C., Emiliani, S., and Verdin, E. (1996) Gene Expr. 5, 245–253
13. McKnight, G. S., Hager, L., and Palmiter, R. D. (1980) Cell 22, 469–477
14. Plesko, M., Hargrove, J. L., Granner, D. K., and Chalkley, R. (1983) J. Biol. Chem. 258, 13728–13744
15. Bresnick, E. H., John, S., Berard, D. S., LeFebvre, P., and Hager, G. L. (1996) Proc. Natl. Acad. Sci. U. S. A. 87, 3977–3981
16. Deckert, J., and Struhl, K. (2001) Mol. Cell. Biol. 21, 2726–2735
17. Cheung, P., G., T. K., Cheung, W. L., Sassone-Corsi, P., Denu, J. M., and Allis, C. D. (2001) Mol. Cell 5, 905–915
18. Reinke, H., Gregory, P. D., and Horz, W. (2001) Mol. Cell 7, 529–538
19. Smith, C. L., Woldford, R. G., O’Neill, T., and Hager, G. L. (2001) Mol. Endocrinol. 15, 956–971
20. Charron, J., Richard-Foy, H., Berard, D. S., Hager, G. L., and Drouin, J. (1989) Mol. Cell. Biol. 9, 3227–3131
21. Pennie, W. D., Hager, G. L., and Smith, C. L. (1995) Mol. Cell. Biol. 15, 2125–2134
22. Fragoso, G., John, S., Roberts, M. S., and Hager, G. L. (1995) Genes Dev. 9, 1933–1947
23. Fragoso, G., and Hager, G. L. (1997) Methods Companion Methods Enzymol. 4, 246–252
24. Payvar, F., DeFranco, D., Firestone, G. L., Edgar, B., Wrangle, O., Okret, S., Gustafsson, J. A., and Yamamoto, K. R. (1983) Cell 35, 381–392
25. Richard-Foy, H., and Hager, G. (1987) EMBO J. 6, 2321–2332
26. Fragoso, G., Pennie, W. D., John, S., and Hager, G. L. (1998) Mol. Cell. Biol. 18, 3633–3644
27. Fletcher, T. M., Ryu, B.-W., Baumann, C. T., Warren, B. S., Fragoso, G., John, S., and Hager, G. L. (2000) Mol. Cell. Biol. 20, 6466–6475
28. Hebbes, T. R., Thorne, A. W., and Crane-Robinson, C. (1988) EMBO J. 7, 1395–1402
29. Hebbes, T. R., Clayton, A. L., Thorne, A. W., and Crane-Robinson, C. (1994) EMBO J. 13, 1823–1830
30. Urnov, F. D., and Wolffe, A. P. (2001) Mol. Endocrinol. 15, 1–16
