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Steroid receptor coactivator-1 (SRC-1) enhances ligand-dependent and receptor-dependent cell-free transcription of chromatin

ZHENG LIU, JIEMIN WONG, SOPHIA Y. TSAI, MING-JER TSAI, AND BERT W. O'MALLEY*

Department of Cell Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

Contributed by Bert W. O'Malley, June 24, 1999

ABSTRACT **Progesterone receptor (PR) functions as a transcription factor that modulates the transcription of target genes in response to progesterone and other signals. The transcriptional activity of PR requires the involvement of coactivators such as steroid receptor coactivator-1 (SRC-1). To dissect the role of SRC-1 in PR transactivation, we established an *in vitro* transcription system with chromatin templates, in which PR induced transcription in a ligand-dependent and PRE-dependent manner. In the presence of ligand, purified PR bound to chromatin templates, resulting in chromatin remodeling. With this system, the ability of purified SRC-1 to act as a coactivator of PR was examined. SRC-1 potentiated transcription by ligand-activated PR, whereas it had no effect on transcription in the absence of ligands. As SRC-1 possesses intrinsic histone acetyltransferase activity, we tested the role of acetylation in PR-mediated transcription by using a histone deacetylase inhibitor, trichostatin A (TSA). We found that addition of TSA strongly enhanced PR-dependent transcription on chromatin but not on naked DNA template, and the effects of SRC-1 and TSA on PR transactivation were partially redundant. In addition, SRC-1 was able to potentiate PR transactivation with nonchromatin templates. Thus, our results substantiate a two-step mechanism whereby recruitment of coactivator SRC-1 by the ligand-activated PR *in vivo* leads to (*i*) chromatin remodeling through histone acetylation and (*ii*) recruitment/stabilization of the preinitiation complex.**

Progesterone receptor (PR) is a member of the class A nuclear receptors that bind to palindromic steroid response elements (SREs) as homodimers (for review, see refs. 1–4). PR contains multiple functional domains: the amino-terminal AF1 region, the central DNA-binding domain (DBD), and the carboxyl-terminal ligand-binding domain (LBD), which includes the AF2 region (1–4). Regulation of gene transcription by PR is hormone-dependent. In the absence of hormone, PR is complexed with heat shock proteins. Ligand binding triggers the release of PR from heat shock proteins and efficient translocation of PR to the nucleus (1–3). Direct interactions with DNA response elements (SREs) and with components of the transcription machinery have been postulated to be important for PR transactivation (1, 5–7). Nevertheless, the process of hormone-dependent receptor activation is more complicated. Squelching between different receptor family members in target gene activation assays indicates that limiting intracellular factors are also required to mediate receptor activation (8). Consequently, several receptor coactivators, including the steroid receptor coactivator (SRC) gene family, have been identified and characterized (9–11).

SRC-1 [or p160/NCoA-1 (12, 13)] was cloned in our laboratory as a coactivator that enhances ligand-dependent transactivation by nuclear receptors (14). Two other family members have been subsequently identified, which are structurally and functionally related to SRC-1 (ref. 11 and references therein). SRC-1 has been reported to interact *in vitro* with general transcription factors TBP and TFIIB (15), as well as with general coactivators CBP/p300 (12, 16) and PCAF (17); a dominant-negative mutant of SRC-1 inhibits PR transactivation on DNA *in vitro* (18). Thus SRC-1 appears to be important for assembly of basal transcription factors and transcriptional initiation. The findings that members of the SRC family (17, 19) and the general coactivators CBP/p300 (20, 21) and PCAF (22) possess intrinsic histone acetyltransferase (HAT) activity suggest that ligand-mediated receptor transactivation may also involve remodeling of chromatin structure through targeted histone acetylation by recruited coactivators.

In an attempt to better understand the molecular basis of hormonal activation and the roles of SRC-1 in receptor transactivation, we employed a cell-free transcription system with chromatin templates. Here we report the successful reconstitution of ligand-dependent and PR-dependent transcription using chromatin. We show that addition of liganded PR to preassembled chromatin leads to the reconfiguration of nucleosome structure in the vicinity of its binding site. In addition, we investigate the role of SRC-1 in ligand-regulated transcription by PR. Consistent with our previously proposed hypothesis (18), this study suggests a dual role for SRC-1 in PR-mediated transactivation: SRC-1 is involved in both chromatin remodeling and the process of recruitment/stabilization of general transcription factors.

MATERIALS AND METHODS

Purification of PR_B and SRC-1. The full-length His_6-tagged human PR B isoform (PR_B) was prepared by infection of Sf9 cells with the corresponding recombinant virus as described previously (23), followed by affinity chromatography of the infected whole-cell extract with Ni-nitrilotriacetate (NTA)-affinity resin (Qiagen, Chatsworth, CA) as specified by the manufacturer. Protein concentration was determined by Bradford assay (Bio-Rad), and purity was estimated by SDS/PAGE. Typical yields were ≈10–16 μg of protein per 150-mm plate. Western blot assay was performed with a polyclonal antiserum against PR (24). The DNA-binding property of PR_B was examined by electrophoretic band-shift assay as described previously (25).

Full-length Flag-tagged SRC-1 was prepared from *Xenopus* oocytes by injection of *in vitro* synthesized mRNA encoding Flag-tagged SRC-1 into ≈1,000 stage VI *Xenopus* oocytes (5 ng of mRNA per oocyte), followed by immunoaffinity chromatography of the oocyte lysate with an anti-Flag M2 affinity resin (Kodak/IBI). Flag-SRC-1 proteins were eluted from the resin with 200 μg/ml Flag peptide (Kodak/IBI), dialyzed, and

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Abbreviations: PR, progesterone receptor; HAT, histone acetyltransferase; PRE, progesterone response element; TSA, trichostatin A.

*To whom reprint requests should be addressed. E-mail: berto@bcm.tmc.edu.

stored at −80°C. The yield of Flag-SRC-1 from 1,000 oocytes was estimated to be approximately 5 μg.

Plasmid Construction and Chromatin Assembly. The plasmid pPRE$_3$-E4 was constructed by inserting three copies of the progesterone response element (PRE) from the tyrosine aminotransferase gene (26) into the *Xba*I site, upstream of the adenovirus E4 core promoter in pIE0 (27).

Chromatin assembly reactions were performed with S190 extract derived from postblastoderm *Drosophila* embryos (0–6 hr) (28, 29), core histones, and an ATP-regenerating system as previously described (30). Typically, the components such as T47D cell nuclear extracts, PR$_B$, ligand, and/or SRC-1 were added after the DNA templates were assembled for 4 hr at 27°C. Subsequently, the assembly reaction was carried out for an additional 30 min at 27°C, and the products were subjected to *in vitro* transcription (29) or chromatin structure analysis. Efficiency of assembly was monitored by micrococcal nuclease assay (30); typically, about 8–12 nucleosomal bands with about 160-bp repeat length were observed with the chromatin templates. In experiments to analyze the effect of trichostatin A (TSA) on transcription from chromatin templates (Fig. 4), TSA (3 μM final concentration) was added to the reaction mixtures before the start of assembly of the chromatin templates.

Chromatin Structure Analysis. Chromatin structure was determined by micrococcal nuclease digestion assay as described (30). Newly assembled chromatin was incubated with or without PR$_B$ in the presence or absence of progesterone for 30 min at 27°C. The chromatin mixture was partially digested with micrococcal nuclease in the absence of transcriptional extract. The resulting DNA fragments were analyzed by agarose gel electrophoresis, transferred to nitrocellulose, and sequentially probed with oligonucleotide probes that correspond to the sequence of either the PRE site or a site around 900 bp upstream of the transcription start.

***In Vitro* Transcription.** Certain transcription assays were performed with T47D cell nuclear extracts (31) as previously described (30). When purified PR was used in the transcription reaction, HeLa cell nuclear extract (32) was used instead of T47D nuclear extract. Briefly, 100 ng of chromatin template was incubated at room temperature with T47D or HeLa cell nuclear extract (20 μg) and buffer in a 50-μl reaction (final volume) for 30 min. Subsequently, transcription was initiated by the addition of rNTPs (0.5 mM final), and the templates were transcribed for 1 hr at 30°C. The resulting transcripts were detected by primer extension. In experiments with plasmid DNA as a template (Figs. 2, 4, and 5), T47D nuclear extract was preincubated with or without progesterone (10^{-7} M) for 15 min at 4°C. One hundred nanograms of DNA template was then added along with SRC-1 (or corresponding buffer as a control), or TSA (or corresponding buffer as a control) followed by an additional 30-min incubation at room temperature before the addition of buffer and rNTPs. Each experiment was performed a minimum of three separate times to ensure reproducibility.

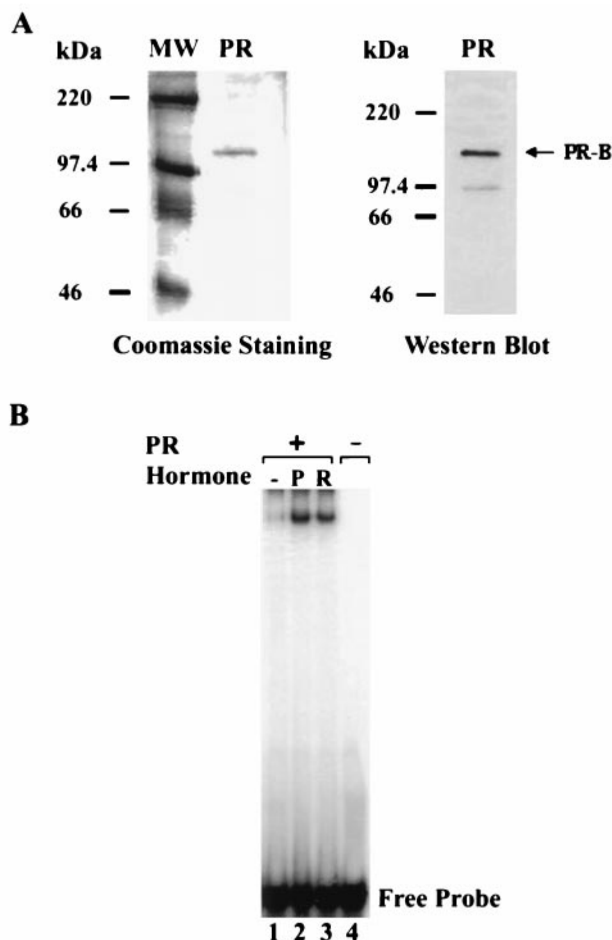

FIG. 1. Purification of human PR$_B$ from baculovirus-infected Sf9 cells. (*A*) The full-length His$_6$-tagged PR$_B$ was overexpressed in Sf9 cells by using a baculovirus expression system and purified by Ni-NTA affinity chromatography. The recombinant protein was analyzed on a 10% polyacrylamide/SDS gel and then subjected to staining with Coomassie brilliant blue R-250 (*Left*) or Western blot analysis with a polyclonal antibody against PR (*Right*). (*B*) DNA-binding activity of purified PR$_B$. Gel retardation experiments were carried out as previously described (31). Recombinant PR$_B$ was preincubated with the control buffer (lane 1), 10^{-7} M progesterone (P, lane 2), or R5020 (R, lane 3) for 15 min at 25°C. Labeled double-stranded PRE oligonucleotides were then added and the reaction mixture was incubated for an additional 15 min. Samples were analyzed on a nondenaturing 5% polyacrylamide gel.

RESULTS

To study the functions of PR *in vitro*, full-length His$_6$-tagged hPR$_B$ was overexpressed in *Spodoptera frugiperda* (Sf9) cells by using a baculovirus expression system. The recombinant proteins purified by affinity chromatography were ligand-free with ≈90% homogeneity (Fig. 1*A*). As shown in Fig. 1*B*, purified hPR$_B$ exhibited ligand-dependent DNA-binding activity, similar to endogenous PR from T47D human breast cancer cells (31).

We have shown previously that PR from T47D cells activates transcription *in vitro* in the absence of any nucleosomal structure (5, 18, 31). Because of the potential importance of chromatin structure in the regulation of PR transactivation, we analyzed the transcriptional activity of PR with chromatin templates. Nucleosomal arrays with proper periodic spacing were reconstituted by using the S190 extract for chromatin assembly (see *Materials and Methods*). The plasmid pPRE$_3$-E4, which contains three copies of a PRE upstream of the adenovirus E4 core promoter, was used as a reporter template, and T47D nuclear extract was employed in the assay to provide endogenous PR and basal transcriptional machinery. As illustrated in Fig. 2 *Left*, when plasmid DNA was used as a template, treatment of T47D nuclear extract with progesterone resulted in a significant induction (>4-fold) in transcription; low levels of transcriptional activity were detected in the absence of hormone. This hormonal induction of transcription was observed over a wide range of concentrations of nuclear extract (data not shown). Hormone-induced transactivation was dependent solely on PRE, as progesterone failed to induce transcription from a promoter lacking PREs, although transcriptional activity was observed in the absence or presence of hormone. These results are consistent with our previous *in*

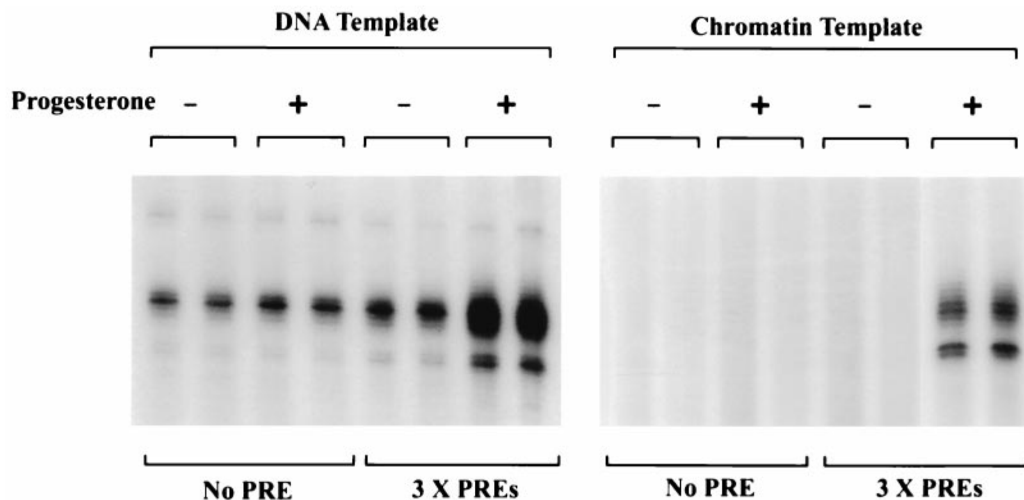

FIG. 2. Ligand-dependent transcription from chromatin (*Right*) and nonchromatin (*Left*) template with T47D nuclear extract. *In vitro* transcription was performed on 100 ng of chromatin or DNA templates by incubating with T47D nuclear extract (20 μg) for 30 min in the presence or absence of progesterone (10^{-7} M) in a final reaction volume of 50 μl as indicated. The primer extension analysis was carried out after the transcription was complete. Where indicated, two different plasmids, $pPRE_3$-E4 (3 X PREs) and pIE0 [which is identical to $pPRE_3$-E4 except for lacking PREs (No PRE)], were used in the experiment. All reactions were performed in duplicate.

vitro studies using a G-free cassette transcription system (5, 18, 31).

When the reporter DNA was assembled into a chromatin structure and T47D nuclear extract was added after the chromatin assembly was complete (Fig. 2 *Right*), no significant transcription from a PRE-driven promoter was observed in the absence of hormone. However, hormone treatment resulted in a potent activation (>20-fold) of transcription from this preassembled chromatin template. The amount of basal transcripts from the chromatin template was <10% of that observed with naked DNA template in the absence of hormone, indicating a repression of gene transcription by the chromatin structure. To test whether this activation depends on the presence of PREs in the reporter template, we examined the transcriptional activity of chromatin template reconstituted from a control promoter that lacks PREs; no transcripts were detected in the absence of PREs (Fig. 2). These results demonstrate that hormone-activated transcription requires specific binding of PR to the chromatin template. Similar results were also obtained with purified PR in the presence of HeLa extract, as will be discussed in a later section (Fig. 4*B*).

To further investigate the molecular basis of PR transcriptional activation from chromatin templates, we tested the effects of PR binding on chromatin structure. Micrococcal nuclease digestion experiments revealed that with a probe located ≈900 bp upstream of the transcription start site (distal probe), similar periodic nucleosomal arrays were observed in either the presence or the absence of PR, and with or without progesterone (Fig. 3 *Left*). In contrast, when the same blot was rehybridized with a probe corresponding to the PRE sequence (promoter probe), fragments derived from subnucleosomes were detected only in the presence of PR and progesterone (Fig. 3 *Right*). Thus, the binding of ligand-activated PR to preassembled chromatin results in a remodeling of nucleosomal structure in the vicinity of its binding sites.

To investigate the function of SRC-1 as a coactivator of PR *in vitro*, we examined the ability of SRC-1 to enhance ligand-mediated transactivation by PR in our cell-free system. Using *Xenopus* oocytes, we synthesized and purified full-length, Flag-tagged SRC-1 (Fig. 4*A*). As shown in Fig. 4*B*, when recombinant PR_B was added to preassembled chromatin, potent activation of transcription was induced by hormone treatment. The addition of exogenous SRC-1 further increased the ligand-dependent activation by PR (>5-fold) on chromatin, whereas it had no effect on transcription in the absence of either hormone or PR. Thus, SRC-1 enhanced the PR-mediated transcription on chromatin in a ligand-dependent manner.

We have published previously that inclusion of a dominant-negative SRC-1 mutant strongly inhibited PR-driven transcription on naked DNA template, suggesting that SRC-1 is necessary for the PR-dependent transcription (18). To further support this conclusion, we assessed the direct effect of purified SRC-1 on PR transcription using a naked DNA template. As shown in Fig. 4*C*, recombinant SRC-1 protein also potentiated PR-dependent transcription from DNA, but only in the presence of hormone (≈3-fold induction), indicating that SRC-1 is involved in the transcription initiation process in the absence of chromatin structure.

Several coactivators (SRC-1, CBP/p300) are proposed to mediate transactivation by nuclear receptors partly through

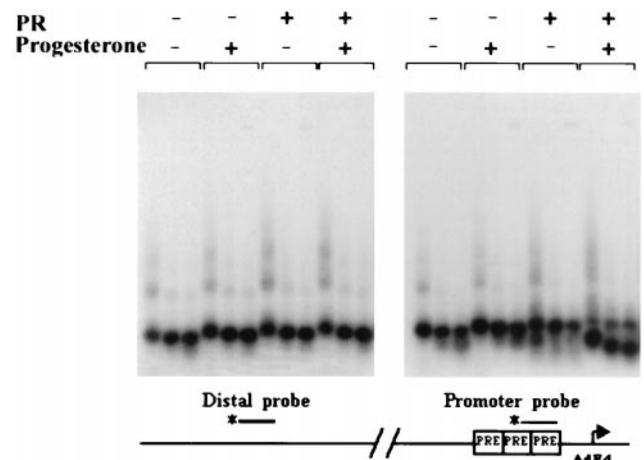

FIG. 3. PR-mediated chromatin remodeling is ligand-dependent. Where indicated, PR_B (6 pmol) and progesterone (10^{-7} M) were added to preassembled chromatin (200 ng). After 30-min incubation, the chromatin templates were subjected to micrococcal nuclease digestion. Reaction mixtures shown in each set of three were incubated with micrococcal nuclease (4 units) for 1, 5, and 10 min at room temperature, respectively. Blots were hybridized to a distal probe (located ≈900 bp upstream of transcription initiation site) to assess the regularity of the nucleosomal array in bulk chromatin (*Left*), and with a promoter probe (corresponding to the PRE sequence) to analyze the disruption in nucleosome array near the PR-binding sites (*Right*).

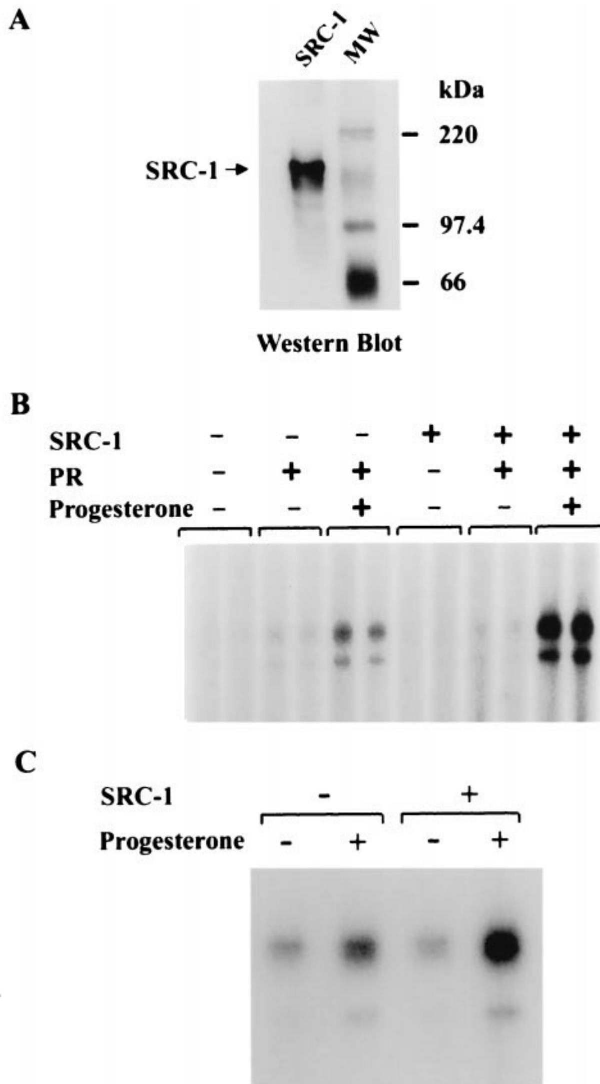

FIG. 4. SRC-1 functions as a coactivator of PR *in vitro*. (*A*) Expression and purification of SRC-1. Flag-tagged SRC-1 was expressed in injected *Xenopus* oocytes and affinity-purified with a monoclonal antibody against the Flag epitope. The purified recombinant protein was analyzed on a 7.5% polyacrylamide/SDS gel and subjected to Western blot analysis using a polyclonal antibody against SRC-1. (*B*) SRC-1 enhances ligand-dependent transcription by purified PR_B on chromatin *in vitro*. $pPRE_3$-E4 was assembled into chromatin. Where indicated, purified PR_B, progesterone, and SRC-1 were added to preassembled chromatin, followed by a 30-min incubation at 27°C. The samples were then subjected to *in vitro* transcription analysis. The final concentrations of PR_B, progesterone, and SRC-1 in the transcription reactions were 15 nM, 10^{-7} M, and 1 nM, respectively. All reactions were performed in duplicate. (*C*) SRC-1 stimulates PR-dependent transcription from nonchromatin template *in vitro*. *In vitro* transcription was performed with $pPRE_3$-E4 DNA template in hormone-untreated or hormone-treated T47D nuclear extract (10 μg) in the presence or absence of exogenous SRC-1, as noted. The final concentration of SRC-1 in the transcription reactions was 1 nM.

their intrinsic HAT activity (see Introduction for references). Moreover, histone acetylation has been implicated to play a major role in chromatin remodeling and transcriptional activation for many genes (32–36). It is likely that ligand-bound PR recruits coactivators with intrinsic HAT activity to acetylate target nucleosomal histones, leading to the observed chromatin reconfiguration. To assess whether histone acetylation is important for PR transcriptional activity, we tested the effect of TSA, a potent inhibitor of histone deacetylase, in our

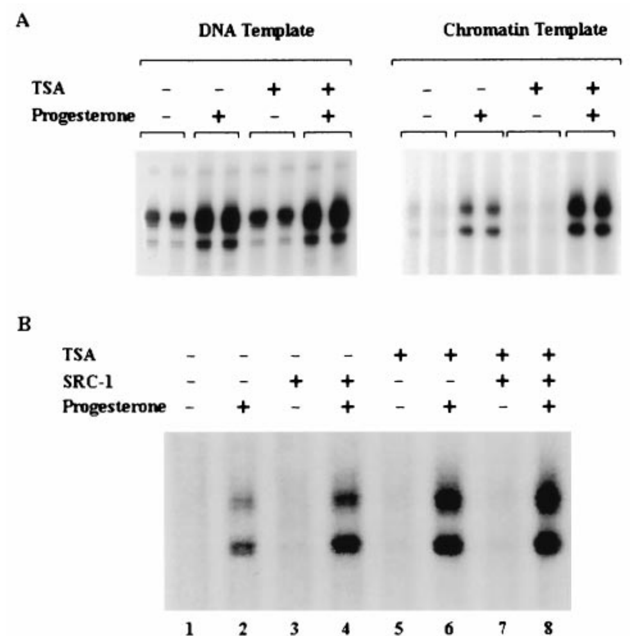

FIG. 5. Effect of TSA on PR-mediated transcription. (*A*) Addition of the histone deacetylase inhibitor TSA strongly induced ligand-dependent transcriptional activation by PR *in vitro*. (*Right*) Addition of TSA before the start of chromatin assembly enhances PR transcription on chromatin templates *in vitro*. $pPRE_3$-E4 was assembled into chromatin with or without TSA, as noted, for 4 hr at 27°C. The samples were incubated with T47D nuclear extract and progesterone (or corresponding buffer as a control) for 30 min at 27°C, then subjected to *in vitro* transcription analysis. (*Left*) TSA has no effect on PR-dependent transcription from nonchromatin $pPRE_3$-E4 DNA template. Parallel transcription reactions were carried out in T47D nuclear extract with or without progesterone in the presence or absence of TSA, as indicated above the lanes. The $pPRE_3$-E4 plasmid was used as a DNA template in the experiment. All reactions were performed in duplicate. (*B*) The effects of SRC-1 and TSA on PR-mediated transcription from chromatin are partially redundant. Reactions were carried out as in *A Right*, with or without SRC-1 (1 nM), in the presence or absence of TSA, as indicated above each lane.

cell-free system. TSA has been shown previously to induce PR transactivation *in vivo* (18). As illustrated in Fig. 5*A Right*, the addition of TSA prior to chromatin assembly potentiated the ligand-dependent transcription by PR. In contrast, TSA did not affect significantly the basal or the ligand-induced PR transcriptional activity from nucleosome-free templates (Fig. 5*A Left*). These results suggest that HAT proteins may play an important role in PR-mediated transactivation.

To investigate whether the HAT activity of SRC-1 is necessary for PR-mediated transactivation through chromatin remodeling, we assessed the effects of SRC-1 and TSA on ligand-induced transcription. As shown in Fig. 5*B*, as expected, SRC-1 and TSA stimulated the PR-dependent transcription, respectively. However, when both SRC-1 and TSA are present, we did not see a synergistic activation of PR-dependent transcription. Instead, we observed only a small amount of additional activity as compared with individual molecules alone (see lane 8 vs. 6, lane 8 vs. 4), suggesting that the effects of SRC-1 and TSA are partially redundant. This result suggests that in addition to playing its role in the transcriptional initiation process, SRC-1 also contributes to chromatin remodeling through its HAT activity.

DISCUSSION

Studies of hormone-regulated gene transcription in a context of chromatin are relevant because DNA is contained in a

chromatin structure and the critical role of chromatin structure in the regulation of gene expression has been well documented (refs. 37 and 38 and references therein). Genetic studies in yeast have provided evidence of a role for histones in transcriptional regulation, and biochemical approaches have demonstrated a general repression of transcription upon packaging of template DNA into chromatin. However, studies of transcription on naked DNA have revealed stimulation upon the addition of enhancer-dependent DNA-binding proteins. Such findings suggest that sequence-specific transcription factors could function both to counteract chromatin-mediated repression and to act in the absence of histones to facilitate the intrinsic transcriptional process at the proximal promoter. We have postulated that PR is involved in chromatin remodeling as well as in transcriptional initiation (18). Because some of the coactivators possess intrinsic HAT activity and also bind other HATs (17, 20–22), these findings suggest a role for coactivators in chromatin remodeling to facilitate the access of general transcription factors to promoter regions.

Although we reported previously a cell-free transcription system that reproduces transactivation by steroid receptors *in vitro* (23, 39, 40), these biochemical studies were carried out with nonchromatin templates. Consequently, we were not able to explore the mechanisms of PR-regulated transcription on chromatin and the precise role of coactivators in gene activation. In initial temporal priority, a ligand-regulated transcriptional activation using the estrogen receptor has been achieved *in vitro* with chromatin but not with nonchromatin templates (41). A similar observation was reported also in an *in vitro* study of retinoic acid receptor α (RARα)/retinoid X receptor α (RXRα) mediated transcription (42), as well as a study of vitamin D receptor (VDR)-dependent transcription (43). The general aspects of these systems and ours are similar. In the present study, we demonstrated that PR (either purified protein or endogenous protein from T47D cell nuclear extract) induced transcription in a ligand-dependent manner. We further demonstrated that stimulation of transcription by hormone requires specific binding of PR to its cognate recognition sites on chromatin. By comparing transcription from nonchromatin and chromatin templates, we illustrated a general chromatin-mediated repression that was replaced by a strong activation of transcription in the presence of liganded PR. A feature of this study of PR differed from features in two previously reported studies (41, 42), in that hormone-dependent transcription also was observed with nonchromatin templates. Our results are consistent with our earlier *in vitro* studies of PR-mediated transcription (5, 23, 31).

Binding of PR to chromatin templates was PRE-dependent and resulted in chromatin remodeling adjacent to the PR-binding sites. In concert with an *in vivo* study which reported that chromatin binding by PR alone is not sufficient to mediate transcriptional activation in cultured cells (44), our experiments substantiate the finding that binding of PR to chromatin is necessary, but not sufficient, for transactivation. It should be noted that endogenous chromatin-remodeling complexes exist in the chromatin assembly S190 extracts that were used in our experiments. In addition to association with HAT-containing coactivators, hormone-dependent association of PR with the chromatin-remodeling hBRG1 complex has been reported (45). Thus, PR is likely to change the chromatin structure by the recruitment of a combination of coactivators and chromatin-remodeling complexes.

Purified SRC-1 increased our observed PR-dependent transcription on chromatin only in the presence of ligand. In the absence of PR, SRC-1 had no effect on basal transcription. Thus, SRC-1 acts specifically on the activator-dependent transcription of a target gene. As SRC-1 exhibits intrinsic HAT activity (17), we tested the effect of TSA on PR-mediated transcriptional activation to confirm the importance of histone acetylation on hormone-dependent transcriptional activation by PR. The effects of SRC-1 and TSA on PR-mediated transcription are partially redundant, since very little additional activity was observed when both SRC-1 and TSA were present as compared with the presence of either alone. These findings indicate that SRC-1 acts in part to stimulate the ligand-induced transcription by remodeling chromatin templates through its HAT activity. This hypothesis can be tested by using SRC-1 mutants deficient in HAT activity. SRC-1-mediated enhancement of transcription also was observed on nonchromatin templates in the presence of receptor and hormone, consistent with our earlier results obtained with a dominant-negative SRC-1 mutant in a cell-free transcription assay (18). This finding indicates that SRC-1 also plays a role in the actual transcriptional process, in addition to chromatin remodeling. It should be noted that the actual level of stimulation by SRC-1 is likely to be higher than what we have observed because endogenous SRC-1 is already present in the T47D and HeLa nuclear extracts used in the transcription assays. Nevertheless, a 20-fold stimulation of chromatin transcription in the presence of liganded PR, and an additional 5-fold enhancement with added SRC-1, were observed. This $\approx$100-fold range of enhancement of transcription is quite respectable.

In summary, results reported herein more directly support our two-step model for SRC-1-mediated PR transcriptional activation proposed earlier (18). Step 1: liganded PR binds to PREs within chromatin and recruits SRC-1 into the DNA-bound complex; SRC-1 acetylates local histones to "remodel" the nucleosomal structure, thereby facilitating the access of general transcription factors (GTFs) to the target promoter. Step 2: SRC-1 enhances stabilization of the preinitiation complex by direct or indirect interactions with GTFs.

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1. Tsai, M.-J. & O'Malley, B. W. (1994) *Annu. Rev. Biochem.* **63,** 451–486.
2. Beato, M., Herrlich, P. & Schutz, G. (1995) *Cell* **83,** 851–857.
3. Freedman, L. P. (1999) *Cell* **97,** 5–8.
4. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Shutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P. & Evans R. M. (1995) *Cell* **83,** 835–839.
5. Bagchi, M. K., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1991) *Mol. Cell. Biol.* **11,** 4998–5004.
6. Ing, N. H., Beekman, J. M., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1992) *J. Biol. Chem.* **267,** 17617–17623.
7. Beato, M. & Sanchez-Pacheco, A. (1996) *Endocr. Rev.* **17,** 587–609.
8. Meyer, M. E., Gronemeyer, H., Turcotte, B., Bocquel, M. T., Tasset, D. & Chambon, P. (1989) *Cell* **57,** 433–442.
9. Shibata, H., Spencer, T. E., Onate, S. A., Jenster, G., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1997) *Recent Prog. Horm. Res.* **52,** 1–25.
10. Xu, L., Glass, C. K. & Rosenfeld, M. G. (1999) *Curr. Opin. Genet. Dev.* **9,** 140–147.
11. McKenna, N. J., Lanz, R. B. & O'Malley, B. W. (1999) *Endocr. Rev.* **20,** 321–344.
12. Kamei, Y., Xu, L., Heinzel, T., Torchia, T., Kurokawa, R., Gloss, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass, C. K. & Rosenfeld, M. G. (1996) *Cell* **85,** 403–414.
13. Halachmi, S., Marden, E., Martin, G., MacKay, H., Abbondanza, C. & Brown, M. (1994) *Science* **264,** 1455–1458.
14. Onate, S. A., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1995) *Science* **270,** 1354–1357.
15. Takeshita, A., Yen, P. M., Misiti, S., Cardona, G. R., Liu, Y. & Chin, W. W. (1996) *Endocrinology* **137,** 3594–3597.
16. Yao, T. P., Ku, G., Zhou, N., Scully, R. & Livingston, D. M. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 10626–10631.

17. Spencer, T. E., Jenster, G., Burcin, M. M., Allis, C. D., Zhou, J., Mizzen, C. A., McKenna, N. J., Onate, S. A., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1997) *Nature (London)* **389,** 194–198.
18. Jenster, G., Spencer, T. E., Burcin, M. M., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 7879–7884.
19. Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatani, Y. & Evans, R. M. (1997) *Cell* **90,** 569–580.
20. Bannister, A. J. & Kouzarides, T. (1996) *Nature (London)* **384,** 641–643.
21. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H. & Nakatani, Y. (1996) *Cell* **87,** 953–959.
22. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H. & Nakatani, Y. (1996) *Nature (London)* **382,** 319–324.
23. Elliston, J. F., Beekman, J. M., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1992) *J. Biol. Chem.* **267,** 5193–5198.
24. McKenna, N. J., Nawaz, Z., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 11697–11702.
25. Bagchi, M. K., Elliston, J. F., Tsai, S. Y., Edwards, D. P., Tsai, M.-J. & O'Malley, B. W. (1988) *Mol. Endocrinol.* **2,** 1221–1229.
26. Strahle, V., Klock, G. & Schutz, G. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7871–7875.
27. Pazin, M. J., Kamakaka, R. T. & Kadonaga, J. T. (1994) *Science* **266,** 2007–2011.
28. Kamakaka, R. T., Bulger, M. & Kadonaga, J. T. (1993) *Genes Dev.* **7,** 1779–1795.
29. Bulger, M. & Kadonaga, J. T. (1994) *Methods Mol. Genet.* **5,** 241–262.
30. Pazin, M. J. & Kadonaga, J. T. (1998) in *Chromatin: A Practical Approach*, ed. Gould, H. (Oxford Univ. Press, Oxford), pp. 173–194.
31. Bagchi, M. K., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1990) *Nature (London)* **345,** 547–550.
32. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
33. Grunstein, M. (1997) *Nature (London)* **389,** 349–352.
34. Struhl, K. (1998) *Genes Dev.* **12,** 599–606.
35. Kadonaga, J. T. (1998) *Cell* **92,** 307–313.
36. Wade, D. A., Pruss, D. & Wolffe, A. P. (1997) *Trends Biochem. Sci.* **22,** 128–132.
37. Wolffe, A. P. (1994) *Curr. Opin. Genet. Dev.* **4,** 245–254.
38. Paranjape, S. M., Kamakaka, R. T. & Kadonaga, J. T. (1994) *Annu. Rev. Biochem.* **63,** 265–297.
39. Bagchi, M. K., Tsai, S. Y., Weigel, N. L., Tsai, M.-J. & O'Malley, B. W. (1990) *J. Biol. Chem.* **265,** 5129–5134.
40. Klein-Hitpass, L., Tsai, S. Y., Weigel, N. L., Allan, G. F., Riley, D., Rodriguez, R., Schrader, W. T., Tsai, M.-J. & O'Malley, B. W. (1990) *Cell* **60,** 247–257.
41. Kraus, W. L. & Kadonaga, J. T. (1998) *Genes Dev.* **12,** 331–342.
42. Dilworth, F. J., Fromental-Ramain, C., Remboutsika, E., Benecke, A. & Chambon, P. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 1995–2000.
43. Rachez, C., Lemon, B. D., Suldan, Z., Bromleigh, V., Gamble, M., Naar, A. M., Erdjument-Bromage, H., Tempst, P. & Freedman, L. P. (1999) *Nature (London)* **398,** 824–828.
44. Mymryk, J. S. & Archer, T. K. (1995) *Genes Dev.* **9,** 1366–1376.
45. Fryer, C. J. & Archer, T. K. (1998) *Nature (London)* **393,** 88–91.