Sequential recruitment of steroid receptor coactivator-1 (SRC-1) and p300 enhances progesterone receptor-dependent initiation and reinitiation of transcription from chromatin

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Employing a cell-free chromatin transcription system that recapitulates progesterone receptor (PR)-mediated transcription *in vivo*, we have investigated further the coactivator functions of steroid receptor coactivator-1 (SRC-1) in terms of its functional domains as well as cooperation with other coactivators in PR transactivation. By analyzing wild-type and mutant SRC-1 with liganded PR in the chromatin transcription system *in vitro*, the basic helix–loop–helix/Per-Arnt-Sim domain, the p300-binding domain, and the carboxyl-terminal region (containing the PR-binding site) of SRC-1 were shown to be important for PR transactivation. Although in context of a synthetic promoter its histone acetyltransferase activity was nonessential for PR-mediated transcription, SRC-1 was observed to act synergistically with p300 to enhance PR transactivation from chromatin. Moreover, SRC-1 and p300 were found to function cooperatively to increase the efficiency of productive transcription initiation and reinitiation. Further analysis of synergism between SRC-1 and p300 revealed an obligatory ''sequential'' recruitment of SRC-1 and p300 to liganded PR. Efficient recruitment of p300 required the presence of SRC-1. In addition, functional analysis of SRC-2 and SRC-3 coactivators indicated that the SRC family modulated PR transactivation from chromatin by a similar mechanism.

The steroid hormone progesterone is involved in the regulation of cellular events including reproduction, differentiation, and development (1, 2). The biological action of progesterone is mediated by the progesterone receptor (PR), which belongs to the nuclear receptor (NR) superfamily of transcription factors (3–5). In the absence of progesterone, a transcriptionally inactive form of PR exists in an oligomeric complex with heat shock proteins and other cellular proteins (6). After hormone binding, the receptor undergoes a conformational change, resulting in dissociation from the oligomeric complex, translocation to the nucleus, dimerization, and binding to progesterone-responsive elements (PREs) within the promoter regions of target genes (3–5). When bound to the PRE, the receptor may modulate target gene transcription directly by contact with components of the transcription machinery (7) or indirectly via coregulators such as steroid receptor coactivator (SRC)-1 (ref. 8) and p300/CREB-binding protein (CBP; refs. 9 and 10).

The SRC family consists of three closely related members: SRC-1/p160/NCoA-1, SRC-2/TIF2/GRIP1/NCoA-2, and SRC-3/ACTR/pCIP/RAC3/AIB1/TRAM-1 (11, 12). These coactivators are used for transcriptional activation not only by NRs but also by other select transcription factors (11, 13, 14). The SRC proteins contain multiple conserved regions including a basic helix–loop–helix (bHLH) domain, two Per-Arnt-Sim (PAS) domains, and LXXLL motifs (NR boxes) responsible for the interaction with NRs (15, 16). The amino-terminal bHLH/PAS domain is the most highly conserved region among the SRC family members. It has a striking sequence similarity with those from the bHLH/PAS family of transcription factors and is thought to mediate protein–protein interactions (11, 12). Although it has been shown that this region contains the binding site for several transcription factors (13, 14), its function in mediating NR transcriptional activity remains unclear. In addition, SRC-1 contains protein interaction domains responsible for the association with basal machinery such as TATA box-binding protein and transcription factor (TF)-IIB (17), coactivators such as p300/CBP (18, 19), and p300/CBP-associated factor, which have histone acetyltransferase (HAT) activity (20), as well as the protein methyltransferase CARM1 (21). Besides interactions with downstream targets, the carboxyl termini of SRC-1 (20) and ACTR (22) possess HAT activity. Studies of interactions with the receptors revealed that the carboxyl terminus of SRC-1 interacts with the AF2 domain of PR in a ligand-dependent manner (23). By using our cell-free chromatin template-based transcription system, we have shown previously that PR-mediated transcriptional activation is accompanied by histone acetylation as well as recruitment/stabilization of the preinitiation complex. Nevertheless, the issue of whether the intrinsic HAT activity of SRC-1 itself is essential for PR transactivation, as well as the roles of its other functional domains in the transcriptional process in a chromatin context, was not determined.

In an attempt to further understand the molecular basis for the SRC-1 coactivation of PR, we used a cell-free transcription system with chromatin templates and analyzed the effects of wild-type or mutant versions of SRC-1 on transcriptional activation by PR. We show here that multiple functional regions of SRC-1 are important for the PR-mediated transcriptional process in a context of chromatin. We also show that SRC-1 acts synergistically with p300 to enhance PR-dependent transcription by promoting the formation of preinitiation complex as well as increasing the frequency of reinitiation. Interestingly, our data indicate that a specific sequential order of recruitment of coactivators is critical for efficient PR-dependent transactivation *in vitro*.

Materials and Methods

Purification of Recombinant Proteins. Purification of His-tagged human PR B isoform and full-length FLAG-tagged SRC-1 was

Abbreviations: PR, progesterone receptor; NR, nuclear receptor; PRE, progesterone-responsive element; SRC, steroid receptor coactivator; CBP, CREB-binding protein; bHLH, basic helix–loop–helix; PAS, Per-Arnt-Sim; TF, transcription factor; HAT, histone acetyltransferase.

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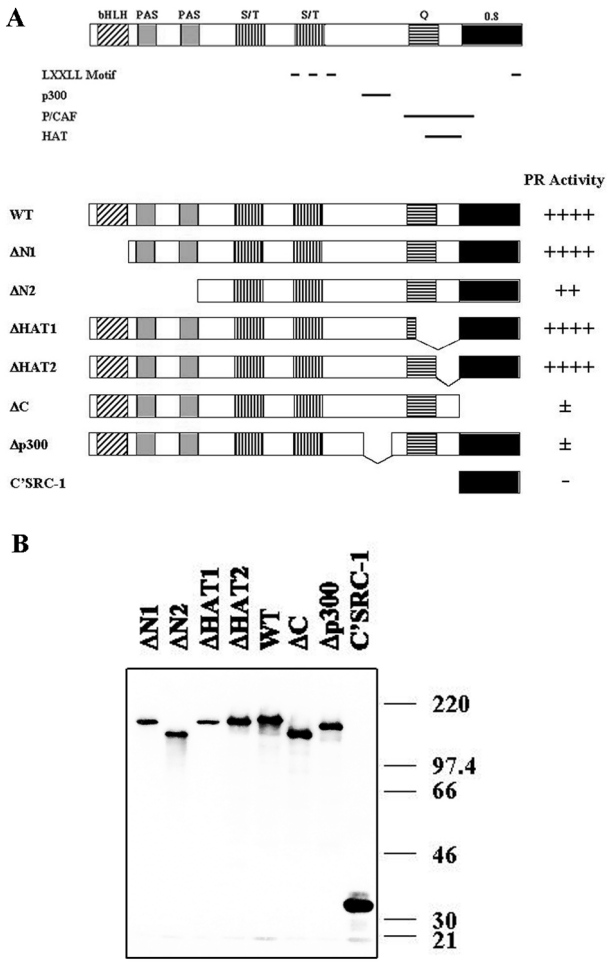

Fig. 1. Wild-type and mutant SRC-1 proteins. (*A*) Schematic representation of wild-type (WT) and mutant forms of SRC-1. Specific domains of SRC-1 are depicted: bHLH domain, PAS domain, serine- and threonine-rich regions (S/T), glutamine-rich region (Q), SRC-1 (0.8) fragment, which serves as a dominant negative inhibitor of receptor functions, LXXLL motifs, p300-binding region, p300/CBP-associated factor-binding region (P/CAF), and HAT domain. (*B*) Expression and purification of wild-type and mutant SRC-1 proteins. FLAG-tagged wild-type and mutant SRC-1 proteins were expressed in injected *Xenopus* oocytes and affinity-purified with a monoclonal antibody against the FLAG epitope. The purified recombinant proteins were analyzed on a 7.5% polyacrylamide-SDS gel and subjected to Western blot analysis by using an anti-FLAG antibody.

described previously (24). Full-length FLAG-tagged SRC-2 and SRC-3 were purified in the same way as SRC-1. Human His-tagged p300 was prepared by infection of Sf9 cells with a recombinant baculovirus kindly provided by L. Kraus (Cornell University, Ithaca, NY) and purified as described (25). A series of SRC-1 mutants described in the legend of Fig. 1 were constructed by inserting PCR-amplified fragments into the appropriate restriction sites in the full-length FLAG-tagged SRC-1 cDNA and then cloning into the pSP64 vector (Promega). The integrity of all PCR-amplified DNA fragments as well as the flanking region around the insertion sites was analyzed by DNA sequencing. The mutant SRC-1 proteins were synthesized in *Xenopus* oocytes and purified as described for wild-type SRC-1. The deleted regions of SRC-1 are as follows: ΔN1 lacks amino acid 1–93, ΔN2 lacks amino acid 1–324, ΔHAT1 lacks amino acid 1,107–1,216, ΔHAT2 lacks amino acid 1,138–1,216, ΔC lacks amino acid 1,217–1,441, and Δp300 lacks amino acid 913–977. C′SRC-1 contains amino acid 1,217–1,441.

Chromatin Assembly and *in Vitro* Transcription. Nucleosomal arrays were assembled on the plasmid $pPRE_3$-E_4 (24) DNA with assembly extracts derived from *Drosophila* embryos (24). PR B isoform, ligand, and/or SRC-1 was added after the chromatin assembly was complete. The reaction mixtures were incubated for an additional 30 min to allow interactions of these proteins with the chromatin templates and formation of proper protein complexes.

In vitro transcription reactions were performed essentially as described previously (24). Chromatin template (100 ng) was incubated at room temperature with HeLa cell nuclear extract (20 μg) for 30 min to allow the formation of transcription preinitiation complexes. Subsequently, transcription was initiated by the addition of rNTPs (0.5 mM final concentration), and the templates were transcribed for 1 h at 30°C. The resulting transcripts were detected by primer extension analysis. All experiments were performed at least three times to ensure reproducibility. Quantitation of the data were carried out by a PhosphorImager (Molecular Dynamics).

Protein–Protein Interactions. Assays to determine interactions between wild-type or mutant SRC-1 proteins and full-length p300 proteins were performed with injected *Xenopus* oocytes as described (24). Briefly, *Xenopus* oocytes (stage VI) were coinjected with mRNAs for p300 and wild-type or mutant FLAG-tagged SRC-1 and incubated for 1 day at 18°C to allow the synthesis of proteins. After the incubation, the oocytes were washed with lysis buffer [20 mM Hepes (pH 7.9)/150 mM KCl/20% glycerol/0.5 mM EDTA (pH 8.0)/0.1% Nonidet P-40/2 mM DTT/0.5 mM phenylmethylsulfonyl fluoride] three times and homogenized in a proper volume of lysis buffer by pipetting. Cell debris and lipids were removed from the cell lysate by centrifugation for 10 min. The supernatants were mixed with 10 μl of anti-FLAG M_2 affinity resin (Sigma) and incubated at 4°C with gentle rotation for 3 h. After low speed centrifugation to remove the supernatants, the resin was washed four times with 1 ml of lysis buffer and resuspended in 10 μl of 2× SDS loading buffer. The samples were subjected to SDS-PAGE and Western blot analysis with anti-p300 and anti-SRC-1 antibodies. To assess the recruitment of p300 to PR, the chromatin template was incubated with PR and progesterone and p300 or SRC-1 for 30 min before the addition of the remaining cofactors. Subsequently, the mixture was incubated with anti-PR antibody and protein A/G-Sepharose beads. After incubation at 4°C for 1 h with rocking, the beads were washed five times with the binding buffer. Bound proteins were eluted with 2× SDS loading buffer and analyzed by SDS-PAGE and Western blot with anti-p300 and anti-SRC-1 antibodies.

Results

We previously established an *in vitro* transcription system by using chromatin templates in which SRC-1 coactivates PR directly in a ligand-dependent manner (24). To investigate contributions of SRC-1 functional domains to coactivation of PR in an *in vitro* transcription assay, we generated a series of mutant forms of human SRC-1. As shown in Fig. 1*A*, the mutations are focused mainly on the conserved protein motifs as well as regions of SRC-1 that were identified to be important for associations with other transcription factors. All mutants of SRC-1 were FLAG-tagged, synthesized in *Xenopus* oocytes, and affinity-purified as described previously (ref. 24; Fig. 1*B*).

As shown in Fig. 2, the deletion of the bHLH domain (ΔN1) exhibited an activation of PR-dependent transcription comparable to that of wild-type SRC-1. Deletion of the two adjacent PAS domains (ΔN2) in addition to the bHLH domain resulted in a significant reduction of SRC-1-induced transactivation. These data indicate that the composite bHLH/PAS domain of

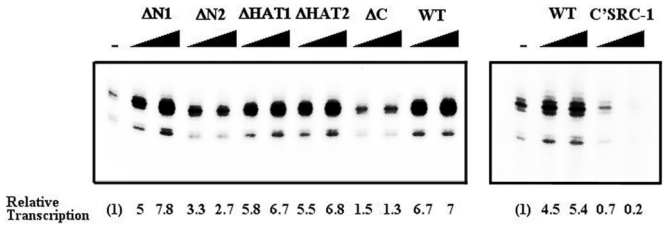

Fig. 2. Transcriptional coactivator activity of wild-type and mutant SRC-1 proteins with PR. $pPRE_3$-E_4 plasmid was assembled into chromatin. Where indicated, purified PR B isoform, progesterone (P), and purified wild-type (WT) or mutant SRC-1 were added to preassembled chromatin and then subjected to *in vitro* transcription analysis. The final concentrations of PR B isoform, progesterone , and SRC-1 (WT or mutant) in the transcription reactions were 15 nM, 10^{-7} M, and 0.5–2 nM, respectively. Relative transcription levels determined by PhosphorImager scanning are listed below each lane. All experiments were performed at least three times and had similar results. In all transcription assays, lane 1 represents the activity of PR in the presence of hormone.

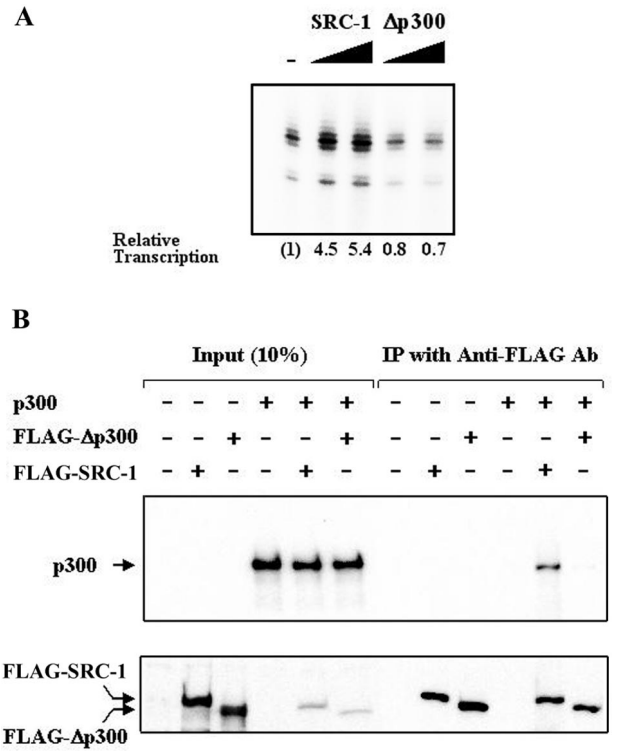

Fig. 3. The p300-binding region of SRC-1 is required for its coactivator function. (*A*) Deletion of the p300-binding region failed to enhance PR transactivation. Chromatin assembly and *in vitro* transcription reactions were performed as described for Fig. 2. (*B*) Deletion of the p300-binding region of SRC-1 abolished the interaction between p300 and SRC-1. *Xenopus* oocytes were coinjected with either wild-type or mutant SRC-1 and p300 mRNA as indicated. Whole oocyte extracts were incubated with anti-FLAG M_2 beads. Bound proteins were analyzed by Western blot assay for p300 or SRC-1.

SRC-1 is required for optimal coactivation of ligand-dependent PR function in a context of chromatin.

Histone acetylation has been shown previously in our laboratory to be important for hormone-dependent transcriptional activation by PR (24, 26). Moreover, SRC-1 contains intrinsic HAT activity (20). To investigate whether the intrinsic HAT activity of SRC-1 is necessary for PR-mediated transcription in a chromatin context, we assessed the effects of the SRC-1 mutants ΔHAT1 and ΔHAT2, which lack overlapping regions of the HAT domain, on PR transactivation. As illustrated in Fig. 2, ΔHAT1 and ΔHAT2 enhanced PR-mediated transcription from chromatin templates to an extent comparable with that of the wild-type SRC-1. Because the deleted regions contain the defined HAT domain of SRC-1 (20), these data indicate that in a context of the PRE-driven minimal promoter, intrinsic HAT activity is dispensable for SRC-1-enhanced transcription by PR from chromatin templates.

We next analyzed the role of the SRC-1 carboxyl terminus in its coactivator functions. Consistent with our previous findings, deletion of the SRC-1 carboxyl-terminal region, which contains PR-interacting domain (ΔC, Fig. 2), abolished its coactivation potential. Because it was shown previously that the carboxyl terminus of SRC-1 acted as a dominant negative inhibitor of PR transactivation (8), we then examined the effect of C′SRC-1, the carboxyl-terminal fragment of SRC-1, on PR-dependent transcription *in vitro* (Fig. 2). We found that the presence of C′SRC-1 resulted in a substantial inhibition of SRC-1-dependent transcriptional activity in a context of chromatin, confirming the dominant negative properties of the carboxyl terminus. Thus, these data indicate the important role of the SRC-1 carboxyl terminus in mediating PR transcriptional activation.

We next used the SRC-1 mutant (Δp300) to assess the role of the p300-binding region in SRC-1 function. As shown in Fig. 3*A*, as compared with the wild-type SRC-1, mutant Δp300 failed to potentiate the PR-mediated transcription. Therefore, these results emphasize the role of the p300-interacting domain in the enhancement of PR transactivation by SRC-1 on chromatin.

To confirm that the deletion of the potential p300-binding region abolished the interaction between SRC-1 and p300, we examined the binding of full-length p300 to the mutant SRC-1 (Δp300). In this experiment, *Xenopus* oocytes were coinjected with mRNAs of full-length p300 and FLAG-tagged wild-type or mutant SRC-1, and then FLAG-SRC-1 and associated proteins were copurified with anti-FLAG affinity beads. As shown in Fig. 3*B*, the p300 protein was observed to bind efficiently to the wild-type SRC-1, whereas there was no detectable binding of p300 to the SRC-1 mutant Δp300. Thus, the deletion of this interior peptide sequence suppresses SRC-1-induced transactivation activity mainly through elimination of the interaction between SRC-1 and p300.

Coactivator p300/CBP has been shown in our laboratory to act synergistically with SRC-1 to enhance PR-mediated transcription in transient transfection assays (10). To test whether p300 can synergize with SRC-1 to activate PR-dependent transcription in a chromatin context, the full-length human p300 protein was overexpressed in a baculovirus expression system and affinity-purified (Fig. 4*A*) and then assayed in our *in vitro* transcription system (Fig. 4*B*). The addition of recombinant p300 protein alone resulted in a modest but significant enhancement of PR transcription (Fig. 4*B*, lane 2), whereas the presence of both SRC-1 and p300 resulted in a synergistic activation compared with p300 or SRC-1 alone (Fig. 4*B*, lane 5 versus lane 2 or 3). Moreover, this synergistic effect was detected among a wide range of concentrations of SRC-1 and p300 (data not shown). To demonstrate that the p300-binding region is required for the synergism between SRC-1 and p300, we tested the ability of SRC-1 mutant, Δp300, to effect transcription by PR and p300. As shown in Fig. 4*B*, Δp300 failed to synergize with p300 to enhance the transcriptional activity of PR (lane 6). These results further substantiate that the p300-binding region of SRC-1 is important for the synergistic activation by SRC-1 and p300. Taken together, these data suggest that recruitment of p300 is critical for efficient SRC-1 coactivator function.

To determine whether the temporal sequence of recruitment of coactivators by PR was important for this synergistic activation, we varied the order of addition of SRC-1 and p300 during the preincubation period before the start of transcription. Sur-

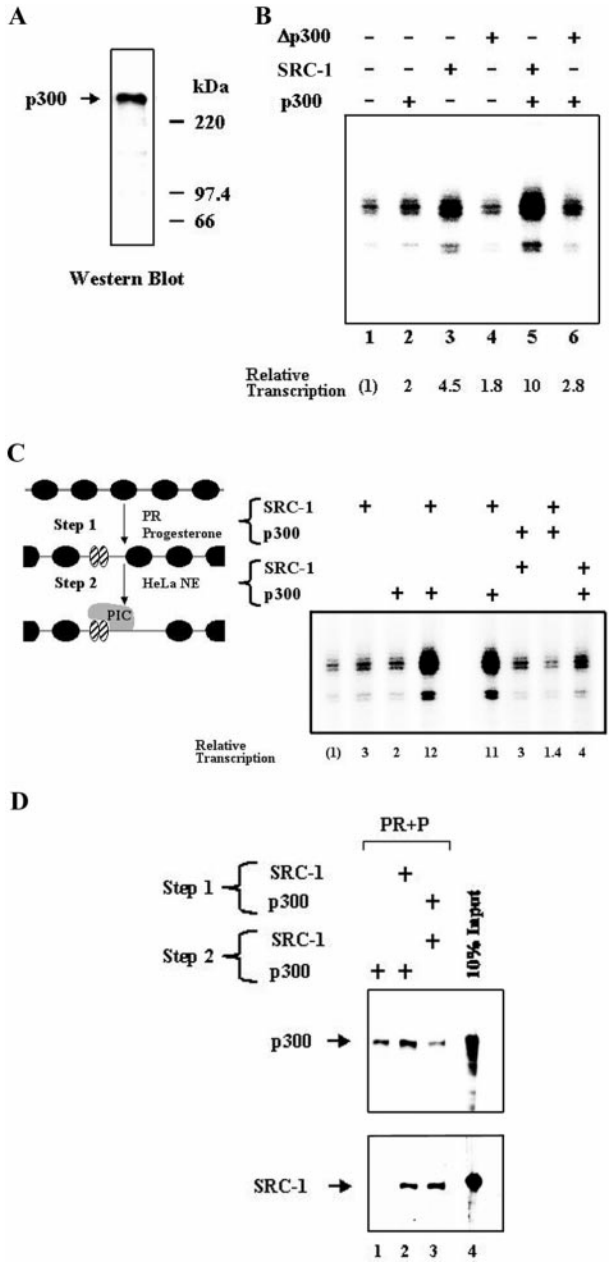

Fig. 4. Sequential recruitment of SRC-1 and p300 by PR was critical for their synergistic activation. (*A*) Purification of human p300 from baculovirus-infected Sf9 cells. The full-length His_6-tagged p300 was overexpressed in Sf9 cells by using a baculovirus expression system and purified by nickel-nitrilotriacetic acid affinity chromatography. The recombinant protein was subjected to Western blot analysis with a monoclonal antibody against p300. (*B*) Deletion of the p300-binding region abolishes the synergistic activation between SRC-1 and p300. Chromatin assembly and *in vitro* transcription reactions were performed as described in the Fig. 2 legend. Where noted, purified SRC-1 or Δp300 were added with PR and progesterone to preassembled chromatin, and exogenous p300 was added 30 min later. The final concentrations of p300 and SRC-1 (wt or Δp300) in the transcription reactions were 1.5 nM and 1 nM, respectively. Results shown here are representative of three independent experiments. (*C*) The importance of sequential recruitment of coactivators in PR transactivation. Transcription was performed as described in *B* with PR, progesterone, SRC-1, and p300. Additions of SRC-1 or p300 to the transcription reactions were as indicated at either the same time with PR and progesterone (step 1) or 30 min later with HeLa nuclear extract (step 2). Results are representative of three independent experiments. (*D*) More p300 is recruited to PR in the presence of SRC-1. The chromatin template was incubated with PR, progesterone (P), and recombinant p300 or SRC-1, before the addition of remaining factors as indicated. Specially bound p300 and SRC-1 to liganded PR were detected by Western blotting.

prisingly, the synergistic activation required an incubation of PR-bound chromatin templates with SRC-1 *before* the addition of p300 (Fig. 4*C*). In contrast, preincubation with p300 before the addition of SRC-1 or with p300 and SRC-1 together did not lead to further PR-dependent transcriptional enhancement, indicating that the prior presence of SRC-1 is required for subsequent efficient recruitment of p300 by PR. To examine this phenomenon further, we assessed the binding of p300 to liganded PR. In these experiments, purified p300 was incubated with liganded PR in the presence or absence of SRC-1, and then associated p300 proteins were coprecipitated with anti-PR antibody. As shown in Fig. 4*D*, some p300 protein bound to liganded PR in the absence of SRC-1 (lane 1). When liganded PR was preincubated with SRC-1 before the addition of p300, an increased amount of p300 was coprecipitated with liganded PR (lane 2 versus 1). In comparison, less p300 was recruited to PR when PR was preincubated with p300 before the addition of SRC-1 (lane 3 versus 2), while similar amounts of SRC-1 were present in the PR-associated complex in both conditions. A lesser amount of p300 associated to PR is not due to the instability of proteins, because more p300 protein was present in the supernatant after precipitation than that when SRC-1 was added before p300 (data not shown). Thus, consistent with results from transcription assays (Fig. 4 *B* and *C*), our data indicate that binding of SRC-1 to liganded PR seems to be an initial critical step, such that recruitment of SRC-1 by liganded PR establishes an appropriate interaction surface to mediate efficient recruitment of p300, which is critical for efficient SRC-1 coactivator function.

By using the detergent Sarkosyl, we investigated the step(s) in the transcription process, which was regulated synergistically by SRC-1 and p300, we assessed the ability of these cofactors to enhance the efficiency of transcription in a single round of transcription as well as their ability to promote transcriptional reinitiation. Preinitiation complexes were formed on the chromatin templates, and transcription was initiated by the addition of rNTP. Sarkosyl (0.1% final concentration) was added immediately after the initiation of transcription. Because it effectively prevents reinitiation but allows elongation by transcriptionally engaged RNA polymerase, the presence of Sarkosyl limits transcription to a single round and reveals the efficiency of the transcription initiation process (25). By measuring the amount of transcripts synthesized in a single round of transcription (+Sarkosyl) as well as in multiple rounds of transcription (−Sarkosyl), we were able to estimate the average number of transcription rounds from the ratio between RNA synthesized in the absence and presence of Sarkosyl. As shown in Fig. 5, in a single round of transcription the presence of both SRC-1 and p300 further enhanced ligand-dependent transcription by PR (about 6-fold), indicating that they acted together to increase the assembly of productive preinitiation complexes. Analysis of the number of transcription rounds revealed approximately 8 rounds of transcription in the presence of PR + progesterone (Fig. 5). The addition of SRC-1 alone or p300 alone failed to increase the number of transcription rounds, suggesting that either one alone stimulates transcription primarily by enhancing the formation of productive preinitiation complexes rather than by promoting transcription reinitiation. In contrast, there was a significant increase of rounds of transcription (an average of 19 rounds) in the presence of both SRC-1 and p300. This result indicated that SRC-1 and p300 function to synergistically promote the assembly of transcription complexes to facilitate reinitiation as well as to increase the efficiency of productive transcription initiation.

We next examined the ability of two other SRC family members (SRC-2 and SRC-3) to coactivate PR transcription *in vitro*. Similar to SRC-1, both SRC-2 and SRC-3 were synthesized in *Xenopus* oocytes and affinity-purified (Fig. 6*A*). The effects of three family members on PR-dependent transcription were

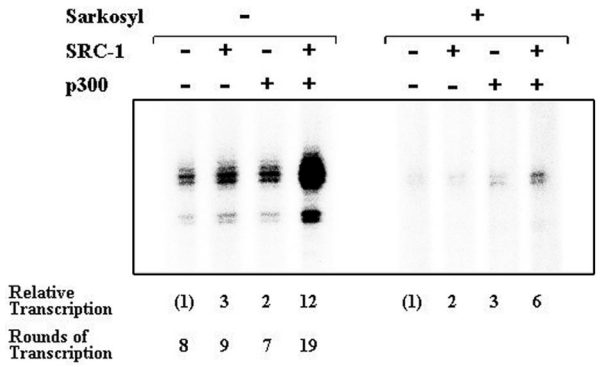

Fig. 5. SRC-1 and p300 enhance transcription synergistically by promoting transcription initiation as well as reinitiation. Transcription on *in vitro* assembled chromatin templates was carried out as described for Fig. 4*B* with the indicated components. Where indicated, transcription was limited to a single round by the addition of Sarkosyl (0.1%) 10 sec after initiation of transcription. For each condition, the number of rounds of transcription was calculated by dividing the amount of transcription in the absence of Sarkosyl (multiple rounds) by that in the presence of Sarkosyl (single round). Data are the means from two independent experiments.

analyzed in the cell-free system. As shown in Fig. 6*B*, both SRC-2 and SRC-3 were able to enhance transcriptional activity of PR from chromatin to a similar extent as compared with SRC-1. When combinations of either two or three members were added simultaneously, they failed to further enhance PR-dependent transcriptional activity from the PRE-driven minimal promoter as compared with either one alone (data not shown). This result suggests that SRC family members function redundantly within the context of our PR-dependent *in vitro* transcription system. Because the p300-binding region is conserved among SRC family members, we also tested whether either SRC-2 or SRC-3 could act synergistically with p300 to enhance transcription *in vitro*. Similar to SRC-1, both SRC-2 and SRC-3 were able to enhance PR-mediated transcription with p300 in a synergistic manner (Fig. 6*C*). Thus, these results indicate that in a context of chromatin, the SRC family modulates PR-dependent transcription from a PRE-minimal promoter by a similar mechanism: recruitment of p300 to promote transcription initiation and reinitiation.

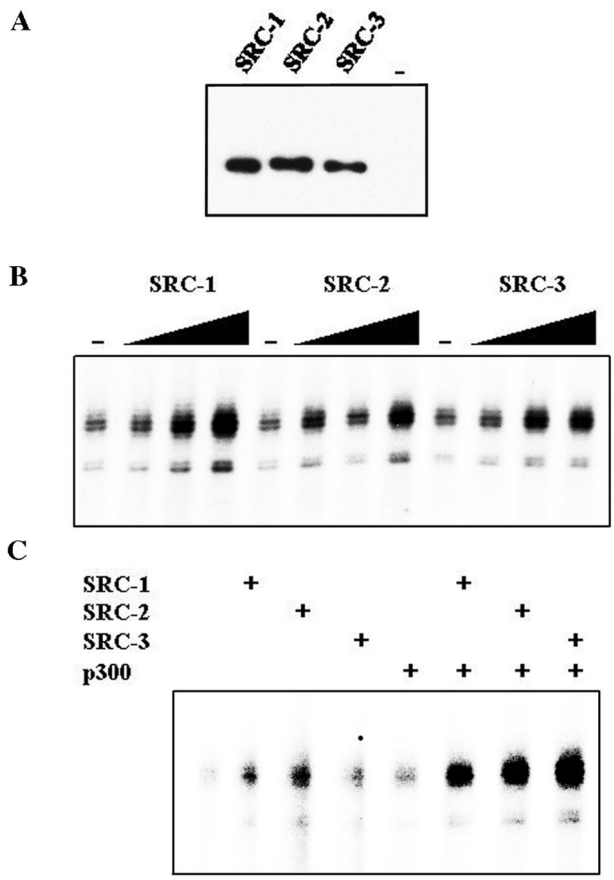

Fig. 6. SRC family acts synergistically with p300 to enhance PR transactivation. (*A*) Purification of SRC family proteins. FLAG-tagged SRC-1, SRC-2, and SRC-3 proteins were expressed in injected *Xenopus* oocytes and affinity-purified as indicated in Fig. 1*B*. The purified recombinant proteins were subjected to Western blot analysis by using anti-FLAG antibody. (*B*) SRC-1, SRC-2, and SRC-3 coactivate PR-dependent transcription to a similar extent. Chromatin assembly and *in vitro* transcription reactions were performed as described for Fig. 2. Where indicated, purified SRC-1, -2, or -3 (0.5–2 nM) was added to preassembled chromatin. (*C*) SRC-1, -2, and -3 synergize with p300 to enhance PR transactivation. Chromatin assembly and *in vitro* transcription reactions were performed as described for Fig. 4*B*.

Discussion

Regions of SRC-1 Required for PR Transactivation. The results of this study demonstrate that in a context of a PRE-driven minimal promoter, multiple distinct subregions of SRC-1 are required for the PR transcriptional activation from chromatin (summarized in Fig. 1*A*). These regions include the bHLH/PAS domain and the p300-binding region as well as the carboxyl-terminal region.

The deletion of the bHLH/PAS domain led to a significant reduction of SRC-1 coactivation. Because the bHLH/PAS domain is the most conserved region among the SRC family members, it has been proposed to be involved in the formation of multimeric complexes among SRC family members (11). Moreover, recent studies have revealed it functions as a protein-interacting motif to recruit SRC-1 to different transcription factors (13, 14). It is possible that this region may be important for the interaction(s) of SRC-1 with intermediary factors to establish a functional coactivator complex. However, the data presented here could not distinguish whether both bHLH and PAS domains or PAS domain alone are required for SRC-1 coactivator functions. It will be interesting in the future to further define the important amino acids within bHLH/PAS domains and identify potential factors bound to this region to better understand the mechanism underlying SRC-1 coactivation.

The carboxyl terminus of SRC-1 has been shown to be responsible for interaction with the ligand-bound AF2 domain of PR (23). The importance of the SRC-1 carboxyl-terminal region for its enhancement of PR transactivation was indicated by the following evidence. First, the deletion of the carboxyl terminus resulted in an almost complete loss of SRC-1 transcriptional activity. Second, the addition of the carboxyl-terminal fragment (C′SRC-1) led to a great reduction in PR-dependent transcriptional activity. Thus, the carboxyl terminus of SRC-1 is of primary importance in mediating transcriptional activation by PR.

The central portion of SRC-1 is indispensable for its coactivator function, because the deletion of this region abolished SRC-1-enhanced transcriptional activation. On the basis of the previous findings that p300 binds to SRC-1 through this region, we speculate that this activation occurred through the interaction of SRC-1 with p300. Indeed, the deletion of this region eliminated interactions with p300. Consistent with a previous study indicating that the SRC-binding region of p300 was important for transcriptional activation by estrogen receptor (27), our data suggest that the interaction with p300 through this region is required for efficient SRC-1 coactivator activity.

SRC-1 possesses HAT activity (20), which acetylates histones

in vitro. Deletions of the HAT domain did not affect SRC-1-enhanced transactivation from the synthetic PRE-linked minimal promoter, indicating that in a context of this artificial promoter, the SRC-1 HAT activity is dispensable for PR-mediated transcription from chromatin templates. Previous studies suggest that differential HATs within the same coactivator complexes are required for the transactivation of different classes of transcription factors in a context of different promoters (12). We could not exclude the possibility that SRC-1 HAT activity plays an important role in its coactivator functions in a context of different promoters as well as different transcription factors. The role of SRC-1 HAT activity in the transcriptional process remains to be assessed in a context of more complex natural promoters as well as different transcription factors.

Synergism Between SRC and p300. In the present study, we demonstrated that SRC-1 and p300 potentiated PR-mediated transcription from chromatin templates in a synergistic manner. Our experiments reveal that the chronology of SRC-1 and p300 recruitment to chromatin-bound receptor affects their synergistic activation of PR function (Fig. 4*C*). Our data suggests that SRC-1 must first bind to PR and form an appropriate conformational surface for subsequent binding and maximal activation of p300.

In further support of our hypothesis, deletion of the p300 interaction domain of SRC-1 abolished the synergistic activation (Fig. 4*B*). Additional biochemical evidence indicated that effective recruitment of p300/CBP to the receptor required the presence of SRC-1 (Fig. 4*D*; ref. 28). In total, these data suggest that liganded PR recruits SRC-1 to form an interaction platform for the efficient recruitment of p300.

It has been found that p300 has a potent HAT activity, and its HAT activity is important for estrogen receptor-mediated transcription (27). Moreover, p300 has been shown to interact with components of the basal transcription machinery. Thus, SRC-1 enhances PR-dependent transcription presumably by recruitment of p300, which then facilitates association with general transcription factors and/or remodeling of chromatin structure. In addition, because several sequence motifs within the p300-binding region are highly conserved among SRC family members, we analyzed the effects of two other SRC family members on PR transactivation in the cell-free transcription system. Our data revealed that similar to SRC-1, SRC-2 and SRC-3 enhanced PR transactivation from chromatin and act redundantly in this assay system. Nevertheless, they all acted synergistically with p300 (Fig. 6). In agreement with a previous report (29), the addition of exogenous p300 alone resulted in only a modest increase in transcription. It should be noted that some endogenous p300 is already present in the HeLa cell extract used in the transcription system (data not shown).

Our present study revealed that either SRC-1 or p300 alone was able to increase transcription initiation rather than promote transcription reinitiation, which was consistent with previous data (25). In addition, an interesting observation in this study is that the synergistic activation by SRC-1 and p300 has a duel function in both transcription initiation and reinitiation. It has been found that both SRC-1 and p300 interact with TFIIB and TATA box-binding protein as well as the RNA polymerase II complex (11, 12). Therefore, it is likely that the association between SRC-1 and p300, and perhaps other factors, forms a proper interaction surface to recruit efficiently the RNA polymerase II complex, leading to the increase of preinitiation complex formation. Moreover, it may stabilize the complex containing TFIID and/or TFIIB bound at the promoter to promote reinitiation. Because a significant amount of nonproductive transcription initiation by RNA polymerase II exists (30), it is possible that SRC-1 and p300 might increase the ratio of productive versus nonproductive transcription by efficient formation of productive transcription initiation complexes.

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