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The nuclear hormone receptor coactivator SRC-1 is a specific target of p300

TSO-PANG YAO, GREGORY KU, NAIDONG ZHOU, RALPH SCULLY, AND DAVID M. LIVINGSTON*

Dana–Farber Cancer Institute and Harvard Medical School, 44 Binney Street, Boston, MA 02115

Contributed by David M. Livingston, June 17, 1996

ABSTRACT **p300 and its family member, CREB-binding protein (CBP), function as key transcriptional coactivators by virtue of their interaction with the activated forms of certain transcription factors. In a search for additional cellular targets of p300/CBP, a protein–protein cloning strategy, surprisingly identified SRC-1, a coactivator involved in nuclear hormone receptor transcriptional activity, as a p300/CBP interactive protein. p300 and SRC-1 interact, specifically, *in vitro* and they also form complexes *in vivo*. Moreover, we show that SRC-1 encodes a new member of the basic helix–loop–helix-PAS domain family and that it physically interacts with the retinoic acid receptor in response to hormone binding. Together, these results implicate p300 as a component of the retinoic acid signaling pathway, operating, in part, through specific interaction with a nuclear hormone receptor coactivator, SRC-1.**

p300 was originally identified by virtue of its ability to bind the viral oncoprotein, E1A (1). A pleiotropic and, likely, important role for p300 in cellular homeostasis is reflected by the fact that its physical association with E1A correlates with the ability of the latter to transform primary cells, to block cellular differentiation along a variety of pathways, and to alter the activity of certain transcriptional enhancer elements (for review, see ref. 2).

p300 consists of at least two closely related family members: p300 and the CREB-binding protein (CBP) (3). CBP was originally cloned by virtue of its specific binding to the protein kinase A-activated form of CREB (4). Microinjection of CBP antibody blocked the transcriptional activity of CREB, indicating that CBP contributes to CREB function in a major way (5). This observation, along with the ability of p300 to relieve E1A-mediated enhancer suppression, has led to the hypothesis that p300/CBP family members function as specific transcription factor coactivators (6).

Remarkably, p300/CBP can interact with other important transcription factors as well. For example, p300/CBP has recently been implicated in the activity of c-fos, c-myb, and MyoD (7, 8, 33). Thus, it appears that p300/CBP expresses its spectrum of activities, at least in part, by participating in and modulating multiple transcriptional pathways. Therefore, identifying additional p300 interactive proteins should assist in understanding how p300/CBP function contributes to multiple aspects of cellular homeostasis.

Nuclear hormone receptors, including retinoic hormone receptor [e.g., retinoic acid receptors (RARs) and retinoic X receptors], thyroid hormone receptors, and steroid hormone receptors (e.g., estrogen receptor), constitute a large family of ligand-dependent transcription factors (9). Although biochemical and genetic studies have revealed much of the molecular detail regarding how nuclear receptors specifically recognize and interact with its target DNA element (10), only recently have workers begun to gain insight into the mechanism underlying ligand activation of nuclear receptor transcription activity.

Specifically, it has been shown that, upon ligand binding, a specific set of proteins are recruited to the hormone bound (i.e., activated) receptor (11–13). It was hypothesized that these multiprotein complexes assemble and become transcriptionally active in response to hormone binding. Identifying and understanding how individual components of these complexes function are part of the key to understand how nuclear receptors modulate transcription. Recently, one of the nuclear receptor-binding proteins, steroid receptor coactivator-1 (SRC-1), was identified and cloned. Consistent with the proposal that it mediates nuclear receptor function, SRC-1 can potentiate the transcription activities of multiple nuclear receptors (14).

By using a yeast two-hybrid screening strategy followed by mammalian cell analyses, we have identified SRC-1 as a specific p300-binding protein. Full-length SRC-1 cDNA was cloned, and its protein product was found to be a member of basic helix–loop–helix (bHLH)–PAS (Per/AHR/SIM homology) family. We have also detected a ligand-dependent interaction of SRC-1 with RAR. These results implicate p300/CBP in the nuclear hormone receptor signaling pathway, at least in part, by virtue of its ability to interact with a known coactivator, SRC-1.

MATERIALS AND METHODS

***In Vitro* Protein-Binding Assay.** p300 interactive factor (IF) 8 isolated from a yeast two-hybrid screen was expressed as a glutathione *S*-transferase (GST) fusion protein bound to glutathione–Sepharose beads and incubated with various ^{35}S-labeled p300 proteins in binding buffer (HYB 75) and then washed in NETN (250 mM NaCl) as described (15). The bound material was analyzed by SDS/PAGE followed by autoradiography. For GST–RAR and mSRC-1 interaction, the following modifications were introduced. GST–RAR was first incubated with 1–3 μM of all trans-retinoic acid at room temperature for 30 min before the addition of labeled mSRC-1 proteins. Nonidet P-40 was omitted from all binding reactions, since it interfered with the GST–RAR-mSRC-1 interaction. One hundred fifty millimoles and 250 nM NaCl NET (NETN without nonidet P-40) were used as binding and washing buffer, respectively.

Yeast Two-Hybrid and Phage Library Screening. For the yeast two-hybrid screen, a fusion between Gal4 DNA binding domain and amino acid 1031–2414 of p300 was constructed (PAS–p300) and used as bait to screen an E17 mouse embryonic library (Clontech) as described (16). Twelve specific

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Abbreviations: CBP, CREB-binding protein; RAR, retinoic acid receptor; bHLH, basic helix–loop–helix; GST, glutathione *S*-transferase; PR, progesterone receptor; h, human; m, murine; IF, interactive factor.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. U64828).

*To whom reprint requests should be addressed.

clones were isolated, and two clones, IF-8 and IF-42, encode overlapping polypeptides.

To obtain full-length cDNA clone, an intact IF-8 fragment was used to screen a mouse pre-B cell library [a generous gift of A. D'Andrea and Y. Zhu (Dana–Farber Cancer Institute)]. Both strands of the longest clone of 5.4 kb were fully sequenced. The presence of a PAS domain in full-length mSRC-1 was confirmed by sequencing of an independent mSRC clone (ΔC in Fig. 4). The compiled sequence comparison for the bHLH and PAS domain was generated by the sequence alignment program, DNA Star (DNAStar, Madison, WI).

Plasmids. All p300 deletion mutants and expression plasmids have been described (6), except for BsaHIΔSphI which was constructed by digesting the pSK–BsaHI plasmid with *Sph*I and then self-ligated. mSRC-1 deletion mutants were constructed by deleting or subcloning various fragments from the full-length clone (see Fig. 4 for a schematic diagram. Details of these constructs are available upon request.).

Antibodies, Immunostaining, and Immunoprecipitation. Antibody to mSRC-1 was raised by injecting rabbits with the GST–IF-8 fusion protein described in Fig. 1. Western blot analysis was done by first performing an immunoprecipitation using either mSRC-1 antibody or preimmune serum, followed by a standard immunoblotting, using the same mSRC-1 antibody. For coimmunoprecipitation experiments, U2OS cells transfected with mSRC-1 and p300 expression plasmids were lysed and processed as stated above except that a p300 monoclonal antibody AC240 was used in the first immunoprecipitation experiment.

For immunostaining, cultured cells grown on coverslips were fixed in 3% paraformaldehyde and then processed for antibody staining. Antibodies were used at the following dilutions: affinity-purified mSRC-1 antibody was used at 1:250 dilution; monoclonal antibody, RW128, was used for p300 staining at a 1:1000 dilution; and a p130 antibody (Santa Cruz Biotechnology) was used at 1:500 dilution. Cell nuclei were visualized by Hoechst 33258 staining. The images in Fig. 3 *A–F* were obtained with a confocal microscope (Zeiss).

Northern Blot Analysis. A mouse tissue Northern blot (Clontech) was performed according to manufacturer's protocol (Clontech). IF-8 fragment was used as the probe.

RESULTS

To isolate p300 interactive proteins, we used a Gal4 DNA-binding domain fused to residues 1031–2414 of p300 as bait in a yeast two-hybrid screening assay. Two positive clones, termed P300 IF-8 and IF-42, were found to encode overlapping polypeptide products of the same gene. To further investigate the nature of the IF-8–p300 interaction, IF-8 was expressed as a GST fusion protein that was then tested for its ability to bind to *in vitro* translated p300. Indeed, GST–IF-8 specifically bound to full-length P300 but not to an unrelated protein, cyclin B (data not shown). Deletion of p300 amino terminal residues 1–1868 (Fig. 1, lanes 1–4) had no effect on the binding of p300 to GST–IF-8, indicating that the C-terminal ≈500 residues of p300 are sufficient to mediate binding. However, a further deletion of 200 residues from the N terminus of this fragment completely eliminated its binding to GST–IF-8 (Fig. 1, lane 5). None of the p300 deletion mutants bound to GST alone (lanes 7–11). These results show that p300 can specifically interact with IF-8 through its C-terminal ≈500 residues.

We next used IF-8 as a probe in an effort to isolate a full-length cDNA clone. The longest positive clone from the ensuing screen that carried a poly(A) tail at its 3′ end was fully sequenced. The DNA sequence of this cDNA contains a single open reading frame encoding a protein of 1405 residues with a predicted molecular mass of 153 kDa (Fig. 2*A*). When this sequence was analyzed by comparison with a DNA data base,

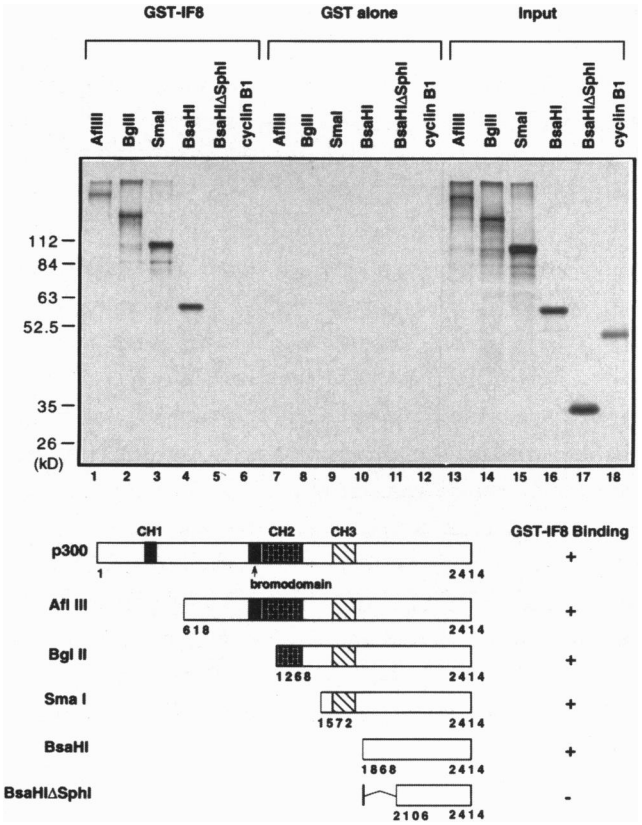

FIG. 1. Specific interaction between GST–IF-8 and p300 via the C-terminal region of p300. (*Upper*) GST–IF-8 (lanes 1–5) but not GST alone (lanes 7–11) binds full-length (data not shown) and various N-terminal deletion mutants of ^{35}S-labeled, *in vitro* translated (IVT) p300. GST–IF-8 does not bind IVT cyclin B (lane 6). (*Lower*) Deletion mutants of p300 with key functional domains schematically diagrammed are shown. One fifth of the input of the IVT p300 mutants and cyclin B1 used in the indicated reaction mixtures are shown in lanes 13–18. CH, cysteine/histidine rich domain (6). Note that BasH1ΔSphI is an N-terminal deletion of the *Bsa*HI mutant protein that eliminates binding to GST–IF-8 (lane 5).

a striking sequence homology to the recently identified human (h) SRC-1 (hSRC-1) (14) was apparent. Indeed, full-length IF-8 and hSRC-1 were 92% identical throughout the reported hSRC-1 sequence (Fig. 2*A*). This degree of identity indicates that IF-8 is likely the mouse (m) homologue of hSRC-1; thus, it was renamed mSRC-1.

Interestingly, mSRC-1 contains an additional 362 N-terminal residues that are novel and were not reported in the hSRC-1 sequence. Since the complete mSRC-1 polypeptide includes residues that precede the putative hSRC-1 initiation methionine (see Fig. 2*A*), the reported hSRC-1 likely represents a partial cDNA clone.

Certain structural motifs were detected in mSRC-1 and were absent in hSRC-1. In particular, the newly identified N terminus of mSRC-1 bears strong homology to a PAS domain (Fig. 2 *A* and *B*). PAS domains have been shown to function as dimerization motifs and were originally identified in several nuclear proteins including *P*eriod (Per), *A*ryl hydrocarbon receptor (AHR) and its heterodimeric partner ARNT, and *S*ingle Minded (Sim; refs. 17–20). Also similar to a subgroup of PAS family members (Sim, AHR, and ARNT), mSRC-1 has a well-conserved bHLH domain at the N terminus of the PAS domain (Fig. 2*B*). Moreover, like SRC-1, all of the above noted PAS domain-containing proteins have a glutamine-rich domain in their C-terminal region, further strengthening the concept of kinship and the likelihood that they share a common mode of activity.

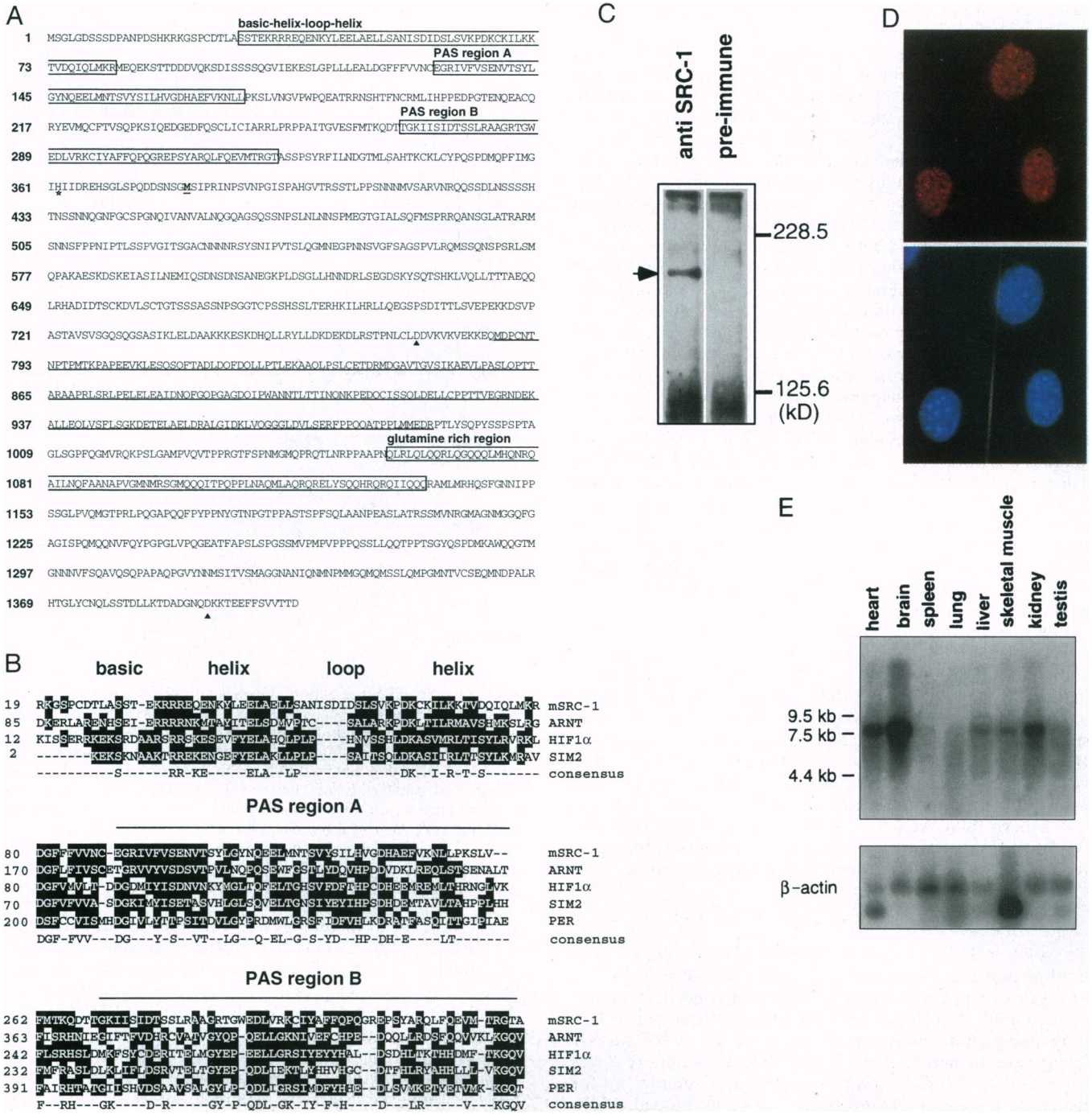

FIG. 2. The p300 interactive clone, IF-8, shares sequence homology with hSRC-1 and encodes a new member of the PAS domain family. (*A*) Protein sequence of full-length IF-8 (mSRC-1). The sequence of the initially isolated IF-8 is underlined, and the limits of IF-42 sequence identity are marked by solid triangles. bHLH, PAS, and glutamine-rich domains are in boxes. The previously designated (14) hSRC-1 initiation methionine is in underlined boldface type. We note that 19 amino acids (the first amino acid is marked by an asterisk) preceding the putative hSRC-1 initiation methionine are also present in mSRC-1. Sequences of hSRC-1 and mSRC-1 diverged in the last C-terminal 14 amino acids of mSRC-1, where an alternatively spliced form of mSRC-1 was also identified (data not shown). (*B*) Compiled sequence comparison between mSRC-1 and members of the PAS family. Note that Per does not contain a bHLH region. Identical and conserved residues are shaded. Consensus residues are defined when three of five of the PAS domain and three of four of the bHLH domain residues are identical. (*C*) Western blot. Anti-mSRC-1 antibody but not preimmune serum identified a single band of ≈170 kDa in Hela cell extracts. This band can be detected in both mouse and human cell lines (data not shown). (*D*) mSRC-1 is a nuclear protein. (*Upper*) Affinity purified anti mSRC-1 antibody stains cell nuclei in 10T 1/2 mouse cells. (*Lower*) Cell nuclei identified by Hoechst staining. (*E*) Tissue Northern blot analysis. Loading control (actin) is shown in the bottom half of the figure.

Consistent with initial binding analysis (Fig. 1), a GST–p300 C-terminal region fusion protein bound full-length SRC-1, specifically, through the IF-8 domain (data not shown). This demonstrates that p300 can interact with full-length SRC-1. Polyclonal antibody raised against mSRC-1 recognized a single protein band of ≈170 kDa close to the predicted molecular mass of mSRC-1 (Fig. 2*C*). Consistent with its ability to bind to p300, a known nuclear element, affinity-purified SRC-1 antibody specifically stained cell nuclei (Fig. 2*D*). The mRNA for mSRC-1 was present in all of the murine tissues examined, although the level varied among the organs tested, with brain and heart containing much higher levels than spleen and lung

(Fig. 2*E*). These results show that SRC-1 is a ubiquitously expressed gene.

In a search for more direct evidence that p300 and SRC-1 interact *in vivo*, we took advantage of the observation that, when transfected into cultured cells, p300 forms readily identifiable, nuclear dot-like structures (6). We, thus, asked whether cotransfection of mSRC-1 resulted in both proteins colocalizing to these dot structures. As shown in Fig. 3*B*, when p300 and mSRC-1 expression plasmids were cotransfected into cells, p300, indeed, formed multiple dots, as shown by antibody staining. Remarkably, when the distribution of mSRC-1 was examined in the same cells by specific immunostaining, it showed the same distribution as p300 (Fig. 3*A*). That both proteins had colocalized was shown by the ability of these images to be superimposed (Fig. 3*C*). Importantly, transfection of the mSRC-1 expression plasmid alone did not show such a nuclear staining pattern, rather it displayed a uniform nuclear staining, supporting the idea that mSRC-1 was recruited to the nuclear dots by p300 (data not shown). As a control, p300 was also cotransfected with an expression plasmid encoding the nuclear pocket protein, p130. These two proteins are not known to interact. While the p300 signal was dot-like, the p130 signal was uniform and did not concentrate in these dot structures (Fig. 3 *D* and *E*). Similarly, when another nuclear protein, p21, was tested, again, no colocalization with p300 was detected (data not shown). These results indicate that p300 and mSRC-1 can form nuclear complexes. Consistent with the colocalization data, antibody specific to p300 can coimmunoprecipitate SRC-1 under similar transfection conditions (Fig. 3*G*).

hSRC-1 was originally isolated by virtue of its ability to interact with the progesterone receptor (PR) in a ligand-dependent manner, and it can synergize with several other nuclear receptor members in reporter assays (14). Since p300 is a key participant in multiple cellular differentiation processes (R. Eckner, T.-P.Y., E. Oldread, and D.M.L., unpublished work), we asked whether mSRC-1 could interact with the RAR, the key receptor mediating the effects of retinoic acid in multiple differentiation processes (21).

To this end, we asked whether mSRC-1 can bind RAR in a ligand- dependent manner. As shown in Fig. 4, in the absence of the ligand, all trans-retinoic acid, full-length mSRC-1 did not bind GST–RAR (lane 1). Remarkably, upon addition of all trans-retinoic acid, prominent binding of mSRC-1 to GST–RAR was observed (lane 2).

Surprisingly, in an analysis of the mSRC-1 structural requirements for RAR binding, an mSRC-1 mutant with a C-terminal deletion mutation that removes most of the sequence reported to mediate the hSRC/PR interaction (14), showed strict ligand-dependent binding to GST–RAR (Fig. 4, lanes 3 and 4). Analysis of additional C-terminal deletion mutants showed that the PR-binding domain is completely dispensable for the ligand-dependent binding of mSRC-1 to GST–RAR (lanes 5, 6, 9, and 10). By contrast, a small polypeptide (residues 569–789) from the N-terminal half of mSRC-1 was sufficient for RAR binding in a ligand-dependent manner (lanes 15 and 16). In keeping with this observation, the C-terminal half of mSRC-1 (termed IF-42) showed only weak and ligand-independent binding (lanes 11 and 12), and the p300 binding sequence, alone, was also inactive (lanes 13 and 14). Furthermore, since a PAS domain deletion mutant retained wild-type-binding activity, this sequence is also dispensable for RAR binding (lanes 7 and 8). Hence, mSRC-1 interacts with RAR in a hormone-dependent manner, and the minimal interaction domain is located in the N-terminal part of the protein.

DISCUSSION

p300/CBP likely plays a series of key regulatory roles in animal cells, given that binding of p300 by E1A leads to perturbation of the cell cycle, blockade of multiple differentiation processes, failure to suppress transformation, and loss of elements of

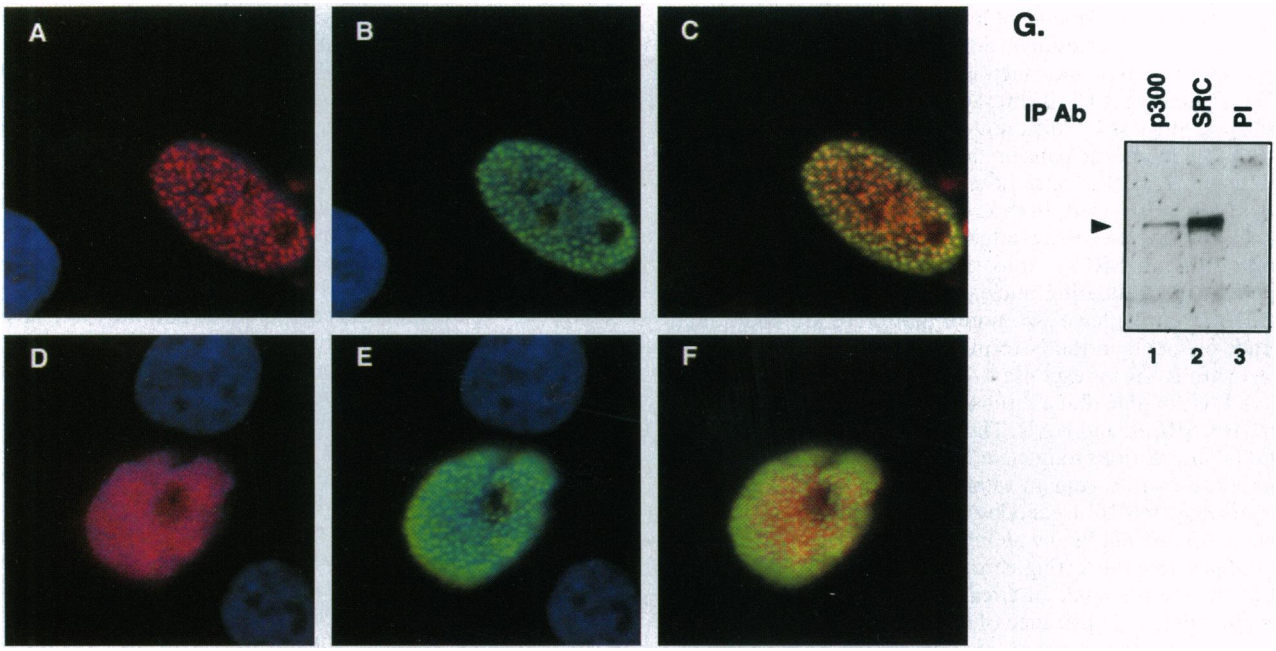

FIG. 3. p300 and mSRC-1 can form complexes in cells. (*A–C*) Colocalization of p300 and mSRC-1. mSRC-1 (*A*, Red) and p300 (*B*, green) form nuclear dots when coexpressed, and these dots are identical as revealed by the resulting yellow image following superimposition of the two (*C*). Overproduction of mSRC-1 alone did not form these dot-like structures (data not shown). (*D–F*) p130 was not recruited to p300-containing dots. p130 shows uniform nuclear staining (*D*, red) while p300 forms nuclear dots (*E*, green). (*F*) We noted that, because of the presence of uniform red staining of p130 in the background, the p300 nuclear dots become yellowish when *D* and *E* are superimposed. Untransfected cell nuclei can be seen as blue in *A*, *B*, *D*, and *E*. We also note that mSRC-1 antibody does not recognize p300 (data not shown). (*G*) Association of p300 and mSRC-1 in coimmunoprecipitation. mSRC-1 can be detected in an anti-p300 monoclonal antibody immune-complex by immunoblotting with a polyclonal antibody against mSRC-1 (lane 1). An immunoprecipitation with mSRC-1 antibody or preimmune serum were loaded and processed as control (lanes 2 and 3, respectively).

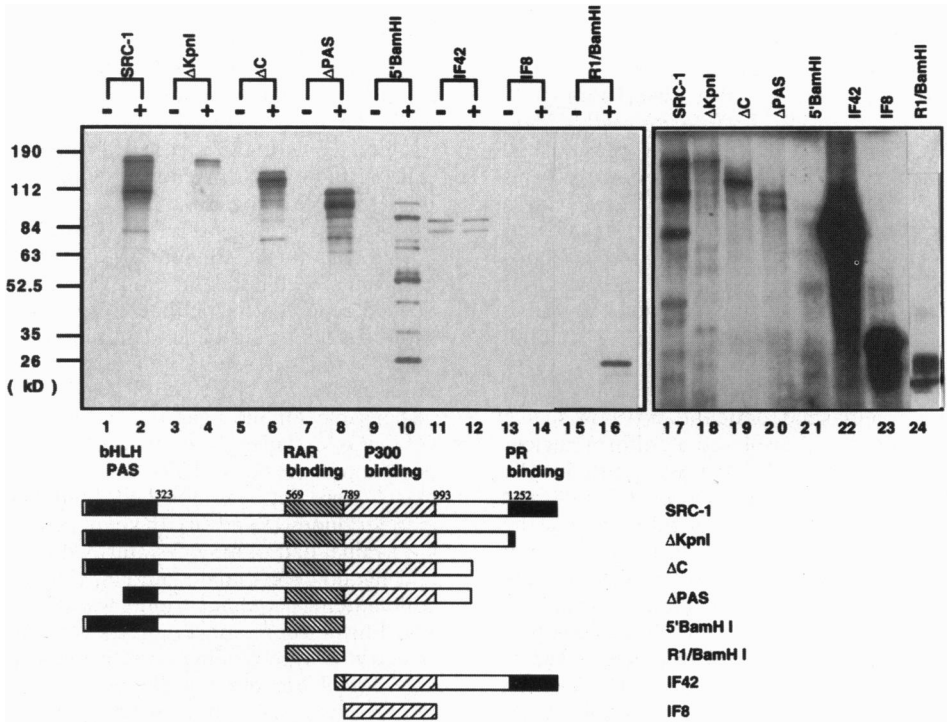

FIG. 4. mSRC-1 interacts with RAR in a ligand-dependent manner. *In vitro* translated mSRC-1 and various deletion mutants were incubated with GST–RAR in the absence (−) or presence (+) of retinoic acid, and the binding of mSRC-1 to GST–RAR was analyzed. The structures of the relevant deletion mutants are summarized in the lower half of the figure. One fifth of the input of each mutant used in the various reaction mixtures is shown in lanes 17–24. PR, PR-binding domain identified in hSRC-1. p300-binding domain and PAS domains are in boxes. We note that complete deletion of the PR-binding region had no effect on mSRC-1 binding to RAR (ΔC, lanes 5 and 6), while an N-terminal deletion mutant (IF42) that retains its PR-binding region showed weak binding considering the much higher input level (compare lane 12 with lane 22). The p300 binding domain (the original IF-8 clone) did not bind RAR (lanes 13 and 14). A small polypeptide (R1/BamH1) in the amino terminal is sufficient to mediate the ligand-dependent interaction (lanes 15 and 16).

transcriptional control (2). The biological activity of p300 family members may depend, at least in part, upon their ability to function as transcriptional adapters through interactions with groups of transcription factors such as CREB and c-*fos* (4, 7). Our finding that p300 interacts with mSRC-1, a known coactivator, suggests a different mode of action, i.e., that p300/CBP may participate in a transcription complex by interacting with a coactivator instead of with the DNA-binding transcription factor itself. In this regard, our data suggests that p300 may be selectively recruited into a receptor-containing complex through SRC-1, thus, implicating p300 as a new component in modulating nuclear receptor transcription activity. However, we have also noted that there are additional sequence elements in the N-terminal region of p300 that can interact with RAR in response to ligand binding (unpublished results). It is possible that a three-way interaction exists among p300/CBP, SRC-1, and RAR. The exact contributions of these individual interactions to nuclear hormone receptor-mediated transcription control remain to be elucidated.

Surprisingly, mSRC-1 encodes a novel bHLH–PAS domain protein. PAS domain family members play numerous essential roles ranging from directing central nervous system development to the maintenance of circadian rhythm regulation (20, 22). Although the significance of the PAS domain in mSRC-1 remains unclear, the fact that this domain has been shown to mediate both heterotypic PAS–PAS and PAS–non-PAS polypeptide interactions (23, 24) leaves open the possibility that SRC-1 may use the PAS domain to interact with other key factors in activated nuclear receptor-containing complexes. Interestingly, mSRC-1 also contains a bHLH domain that is frequently found in transcription factors that bind DNA (25). Indeed, four other PAS family members, including AHR, ARNT, Hif-1, and Sim, have been shown to bind DNA (26–28). This raises the notion that mSRC-1 may also have an intrinsic DNA-binding activity. If so, it will be interesting to know whether such an activity is involved in coactivating nuclear receptor transcription or contributes to other processes in which mSRC- 1 may participate by functioning as a genuine transcription factor. If the latter were true, it would be important to learn whether p300/CBP–SRC interaction contributes to these processes.

Certain transcription factors that mediate different physiological responses to various signaling events are known to communicate and modulate each other's transcriptional activity, a phenomenon known as cross-coupling (29). Interestingly, of the two, well-characterized interactive pathways that involve glucocorticoid receptor, retinoic acid receptor, and AP-1 (30, 31), p300/CBP appears to be the common cofactor (this study and ref. 5). Another well-documented antagonistic interaction involves MyoD and c-jun transcriptional activity and, again, p300/CBP appears to be a key cofactor for both c-jun and MyoD activity (5, 33). Given this critical and highly specific interaction with different key transcription factors and/or cofactors, we speculate that p300/CBP may function as a nodal point in coordinating and communicating different transcription pathways. It is possible that p300/CBP provides a molecular platform that brings together different transcription factors so they may functionally interact either negatively or positively. If so, p300/CBP may represent a novel class of transcriptional modulators that plays an important role in eliciting the cross-coupling phenomenon.

Note. During the preparation of this manuscript, Kamei *et al.* (32) reported similar results on the SRC-1/p300 interaction.

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1. Whyte, P., Williamson, N. M. & Harlow, E. (1989) *Cell* **56,** 67–75.
2. Moran, E. (1993) *Curr. Opin. Genet. Dev.* **3,** 63–70.
3. Arany, Z., Sellers, W. R., Livingston, D. M. & Eckner, R. (1994) *Cell* **77,** 799–800.
4. Chrivia, J. C., Kwok, R. P., Lamb, N., Hagiwara, M., Montminy, M. R. & Goodman, R. H. (1993) *Nature (London)* **365,** 855–859.
5. Arias, J., Alberts, A. S., Brindle, P., Claret, F. X., Smeal, T., Karin, M., Feramisco, J. & Montminy, M. (1994) *Nature (London)* **370,** 226–229.
6. Eckner, R., Ewen, M. E., Newsome, D., Gerdes, M., DeCaprio, J. A., Lawrence, J. B. & Livingston, D. M. (1994) *Genes Dev.* **7,** 869–884.
7. Bannister, A. J. & Kouzarides, T. (1995) *EMBO J.* **14,** 4758–4762.
8. Dai, P., Akimaru, H., Tanaka, Y., Hou, D. X., Yasukawa, T., Kanei, I. C., Takahashi, T. & Ishii, S. (1996) *Genes Dev.* **10,** 528–540.
9. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P. & Evans, R. M. (1995) *Cell* **83,** 835–839.
10. Mangelsdorf, D. J. & Evans, R. M. (1995) *Cell* **83,** 841–850.
11. Halachmi, S., Marden, E., Martin, G., MacKay, H., Abbondanza, C. & Brown, M. (1994) *Science* **264,** 1455–1458.
12. Kurokawa, R., Soderstrom, M., Horlein, A., Halachmi, S., Brown, M., Rosenfeld, M. G. & Glass, C. K. (1995) *Nature (London)* **377,** 451–454.
13. Cavailles, V., Dauvois, S., L'Horset, F., Lopez, G., Hoare, S., Kushner, P. J. & Parker, M. G. (1995) *EMBO J.* **14,** 3741–3751.
14. Onate, S. A., Tsai, S. Y., Tsai, M. J. & O'Malley, B. W. (1995) *Science* **270,** 1354–1357.
15. Kaelin, W. G. Jr., Krek, W., Sellers, W. R., DeCaprio, J. A., Ajchenbaum, F., Fuchs, C. S., Chittenden, T., Li, Y., Farnham, P. J., Blanar, M. A., Livingston, D. M. & Flemington, E. K. (1992) *Cell* **70,** 351–364.
16. Durfee, T., Becherer, K., Chen, P. L., Yeh, S. H., Yang, Y., Kilburn, A. E., Lee, W. H. & Elledge, S. J. (1993) *Genes Dev.* **7,** 555–569.
17. Crews, S. T., Thomas, J. B. & Goodman, C. S. (1988) *Cell* **52,** 143–151.
18. Burbach, K. M., Poland, A. & Bradfield, C. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8185–8189.
19. Reyes, H., Reisz, P. S. & Hankinson, O. (1992) *Science* **256,** 1193–1195.
20. Jackson, F. R., Bargiello, T. A., Yun, S. H. & Young, M. W. (1986) *Nature (London)* **320,** 185–188.
21. Kastner, P., Mark, M. & Chambon, P. (1995) *Cell* **83,** 859–869.
22. Thomas, J. B., Crews, S. T. & Goodman, C. S. (1988) *Cell* **52,** 133–141.
23. Huang, Z. J., Edery, I. & Rosbash, M. (1993) *Nature (London)* **364,** 259–262.
24. Gekakis, N., Saez, L., Delahaye, B. A., Myers, M. P., Sehgal, A., Young, M. W. & Weitz, C. J. (1995) *Science* **270,** 811–815.
25. Murre, C., McCaw, P. S., Vaessin, H., Caudy, M., Jan, L. Y., Jan, Y. N., Cabrera, C. V., Buskin, J. N., Hauschka, S. D., Lassar, A. B., Weintraub, H. & Baltimore, D. (1989) *Cell* **58,** 537–544.
26. Swanson, H. I., Chan, W. K. & Bradfield, C. A. (1995) *J. Biol. Chem.* **270,** 26292–26302.
27. Wang, G. L., Jiang, B. H., Rue, E. A. & Semenza, G. L. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5510–5514.
28. Muralidhar, M. G., Callahan, C. A. & Thomas, J. B. (1993) *Mech. Dev.* **41,** 129–138.
29. Schule, R. & Evans, R. M. (1991) *Trends Genet.* **7,** 377–381.
30. Yang, Y. H., Chambard, J. C., Sun, Y. L., Smeal, T., Schmidt, T. J., Drouin, J. & Karin, M. (1990) *Cell* **62,** 1205–1215.
31. Schule, R., Rangarajan, P., Yang, N., Kliewer, S., Ransone, L. J., Bolado, J., Verma, I. M. & Evans, R. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6092–6096.
32. Kamei, Y. L., X., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S., Heyman, R. A., Rose, D. W., Glass, C. K. & Rosenfeld, M. G. (1996) *Cell* **85,** 403–414.
33. Eckner, R., Yao, T.-P., Oldread, E. & Livingston, D. M. (1996) *Genes Dev.*, in press.