Steroid Receptor Coactivator-1 Coactivates Activating Protein-1-mediated Transactivations through Interaction with the c-Jun and c-Fos Subunits*

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Steroid receptor coactivator-1 (SRC-1) specifically bound to the transcription factor AP-1 subunits c-Jun and c-Fos, as demonstrated by the yeast two-hybrid test and glutathione S-transferase pull down assays. The c-Jun and c-Fos binding sites were localized to the C-terminal subregion of SRC-1 (amino acids 1101–1441) that encompasses the previously described histone acetyltransferase and receptor-binding domains. In mammalian cells, SRC-1, similar to the previous results with CBP-p300 (Arias, J., Alberts, A. S., Brindle, P., Claret, F. X., Smeal, T., Karin, M., Feramisco, J., and Montminy, M. (1994) Nature 370, 226–229; Bannister, A. J., and Kouzarides, T. (1995) EMBO J. 14, 4758–4762), potentiated the AP-1-mediated transactivations in a dose-dependent manner and derepressed the mutual inhibitions between nuclear receptors and AP-1. Furthermore, coexpression of p300 further enhanced this SRC-1-potentiated level of transactivations. Thus, we concluded that at least two distinct coactivator molecules may cooperate to regulate AP-1-dependent transactivations and mediate transrepression between AP-1 and nuclear receptors in vivo.

The activation protein-1 (AP-1) transcription factors are immediate early response genes involved in a diverse set of transcriptional regulatory processes (1). The AP-1 complex consists of a heterodimer of a Fos family member and a Jun family member. This complex binds the consensus DNA sequence (TGAGTCA) (termed AP-1) sites found in a variety of promoters (2, 3). The Fos family contains four proteins (c-Fos, Fos-B, Fra-1, and Fra-2) (4–6), whereas the Jun family is composed of three (c-Jun, Jun-B, and Jun-D) (7–10). Fos and Jun are members of the bZIP family of sequence-specific dimeric DNA-binding proteins (11). The C-terminal half of the bZIP domain is amphipathic, containing a heptad repeat of leucines that is critical for the dimerization of bZIP proteins (12, 13). The N-terminal half of the long bipartite helix is the basic region that is critical for sequence-specific DNA binding (14–16).

Transcription coactivators bridge transcription factors and the components of the basal transcriptional apparatus (17). Functionally conserved proteins CREB-binding protein (CBP) and p300 have been shown to be essential for the activation of transcription by a large number of regulated transcription factors, including nuclear receptors (18–21), CREB (22–24), NFκB (25, 26), basic helix-loop-helix factors (27), STATs (28, 29), and AP-1 (30, 31). In particular, the nuclear receptor superfamily is a group of ligand-dependent transcriptional regulatory proteins that function by binding to specific DNA sequences named hormone response elements in promoters of target genes (reviewed in Ref. 32). Transcriptional regulation by nuclear receptors depends primarily upon a ligand-dependent activation function, AF-2, located in the C terminus and predicted to undergo an allosteric change upon ligand binding (32). Consistent with this, CBP and p300 have been found to interact directly with nuclear receptors in a ligand- and AF-2-dependent manner (18–21). In addition, a series of factors that exhibit ligand- and AF-2-dependent binding to nuclear receptors have been identified both biochemically and by expression cloning. Among these, a group of highly related proteins have been shown to form a complex with CBP and p300 and enhance transcriptional activation by several nuclear receptors, i.e. steroid receptor coactivator-1 (SRC-1) (20, 33), xSRC-3 (34), AIB1 (35), TIF2 (36), RAC3 (37), ACTR (38), TRAM-1 (39), and p/CIP (40). Interestingly, SRC-1 (41) and its homologue ACTR (38), along with CBP and p300 (42, 43), were recently shown to contain potent histone acetyltransferase activities themselves and associate with yet another histone acetyltransferase protein p/CAF (44). In contrast, it was shown that SMRT (45) and N-CoR (46), nuclear receptor corepressors, form complexes with Sin3 and histone deacetylase proteins (47, 48). From these results, it was suggested that chromatin remodeling by cofactors may contribute, through histone acetylation-deacetylation, to transcription factor-mediated transcriptional regulation.

In light of the fact that SRC-1 is capable of forming a complex with CBP and p300 that in turn coactivate AP-1 (30, 31), we tested whether SRC-1 itself participates in the AP-1-mediated transactivations as well. Herein, we show that 1) SRC-1 specifically binds to the AP-1 components c-Jun and c-Fos, 2) SRC-1 coactivates the AP-1-mediated transactivations, 3) p300 synergizes with SRC-1 in this coactivation, and 4) SRC-1 relieves the transrepression between nuclear receptors and AP-1. These results suggest that at least two distinct coactivator molecules (i.e. SRC-1 and p300) may cooperate to regulate AP-1-dependent transactivations and mediate transrepression between AP-1 and nuclear receptors in vivo.

EXPERIMENTAL PROCEDURES

Plasmids—LexA, B42, T7, or GST vectors to express fragments of SRC-1 (SRC-A through SRC-E as depicted in Fig. 1) were used as described previously (49). Polymerase chain reaction-amplified fragments of c-
Jun and c-Fos were subcloned into EcoRl-SalI restriction sites of the LexA fusion vector pEG202PL (50) and EcoRl-XhoI restriction sites of the B42 fusion vector pJG4-5 (50), the GST fusion vector pGEX4T (Amersham Pharmacia Biotech) or the CMV/T7 vector pcDNA3 (Invitrogen, San Diego, CA). The expression vectors for p300 (kind gift from Dr. David M. Livingston, Dana Farber Cancer Institute, Boston, MA) and SRC-1 (kind gift from Dr. Ming Tsai, Baylor College of Medicine, Houston, TX), along with the transfection indicator construct pRSV-β-gal, the AP-1-responsive reporter construct (TRE)2-TK-Luc, and the T3-responsive reporter construct TREPal-TK-Luc, were as described previously (23, 41, 51, 52).

**Yeast Two-hybrid Test**—For the yeast two-hybrid tests, plasmids encoding LexA fusions and B42 fusions were cotransformed into Saccharomyces cerevisiae EGY48 strain containing the LacZ reporter plasmid, SH16-34 (50). Plate and liquid assays of β-gal expression were carried out as described (50, 52, 53). Similar results were obtained in more than two similar experiments.

**GST Pull Down Assays**—The GST fusions or GST alone was expressed in Escherichia coli, bound to glutathione-Sepharose-4B beads (Amersham Pharmacia Biotech), and incubated with labeled proteins expressed by in vitro translation by using the TNT-coupled transcription-translation system, with conditions as described by the manufacturer (Promega, Madison, WI). Specifically bound proteins were eluted from beads with 40 mM reduced glutathione in 50 mM Tris (pH 8.0) and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography as described (52).

**Cell Culture and Transfections**—CV1 cells were grown in 24-well plates with medium supplemented with 10% fetal calf serum for 24 h and transfected with 100 ng of LacZ expression vector pRSV-β-gal and 100 ng of a reporter gene (TRE)2-TK-Luc or TREPal-TK-Luc, along with increasing amount of expression vectors for SRC-1 or p300. Total amounts of expression vectors were kept constant by adding decreasing amounts of pcDNA3 to transfections containing increasing amounts of the SRC-1 or p300 vector. After 12 h, cells were washed and refed with Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. Cells were harvested 24 h later, luciferase activity was assayed as described (54), and the results were normalized to the LacZ expression. Similar results were obtained in more than two similar experiments.

**RESULTS AND DISCUSSION**

**Interactions of SRC-1 with c-Jun and c-Fos**—We have recently found that c-Jun and c-Fos interacts with a full-length xSRC-3 (34), a Xenopus homologue of the nuclear receptor coactivator SRC-1 (20, 33), but not with a partial xSRC-3 that lacks the C-terminal region encompassing the previously defined histone acetyltransferase- and receptor-binding domains.2 Similarly, a full-length SRC-1 readily interacted with c-Jun and c-Fos in yeast (Table I). To localize the interaction domain, we examined LexA and B42 proteins fused to a series of SRC-1 fragments we recently described (49) (Fig. 1). Consistent with an idea that c-Jun and c-Fos interact with SRC-E, coexpression of a B42 fusion to the full-length c-Jun and c-Fos further stimulated the LexA/SRC-E-mediated LacZ expression, whereas coexpression of B42 alone was without any effects (Table I). In contrast, the LacZ expressions mediated by LexA fusions to SRC-A, -B, -C, or -D were not further stimulated by coexpression of B42/c-Jun or B42/c-Fos. Similar results were also obtained with B42 fusions to SRC-1 fragments and LexA fusions to c-Jun and c-Fos, in which coexpression of the B42/SRC-E and LexA/c-Jun or LexA/c-Fos pair efficiently stimulated the LacZ reporter expression (data not shown).

To further characterize these interactions in vitro, GST alone and GST fusions to c-Fos or c-Jun were expressed, purified, and tested for interaction with in vitro translated luciferase and SRC-1. The radiolabeled SRC-1 interacted with GST/c-Jun and GST/c-Fos, but not with GST alone (Fig. 2A). In contrast, the radiolabeled luciferase did not bind any of the GST proteins, as expected. In agreement with the yeast two-hybrid results (Table I), only SRC-E, among various SRC-1 fragments, specifically interacted with GST/c-Jun and GST/c-Fos but not with GST alone (Fig. 2B). These results, along with the yeast results, suggest that SRC-1 directly associates with c-Jun and c-Fos through a subregion of SRC-1 containing the histone acetyltransferase and receptor-binding domains (i.e. SRC-E).

**Cotransfection of SRC-1 Stimulates AP-1-mediated Transactivations**—To assess the functional consequences of these interactions, SRC-1 was cotransfected into CV1 cells along with a reporter construct (TRE)2-TK-Luc. This reporter construct, previously characterized to efficiently mediate the AP-1-dependent transactivations in various cell types, consists of a minimal promoter from the thyminidine kinase gene and four upstream consensus AP-1 sites (51). Increasing amounts of cotransfected SRC-1 enhanced the reporter gene expressions in an SRC-1 dose-dependent manner, with cotransfection of 100 ng of SRC-1 increasing the activation approximately 4-fold (Fig. 3). Consistent with the reports that CBP and p300 are transcription coactivators of AP-1 (30, 31), increasing amounts of cotransfected p300 also had stimulatory effects on the re-

**Table I**

| LexA fusions to | SRC-A | SRC-B | SRC-C | SRC-D | SRC-E |
|----------------|-------|-------|-------|-------|-------|
| B42/β-gal      | −     | +     | −     | −     | −     |
| B42/c-Jun      | −     | ++    | −     | −     | −     |
| B42/c-Fos      | −     | +++   | −     | −     | −     |

**Fig. 1. Schematic representation of the SRC-1 constructs.** The full-length human SRC-1 (20, 33) and a series of five SRC-1 fragments are as depicted. The nuclear receptor-interacting (Receptor), CBP-p300-interacting (p300), basic helix-loop-helix/PAS (bHLH/PAS), serinederthreonine-rich (S/T-rich), and glutamine-rich domains (Q), along with the recently identified histone acetyltransferase domain (HAT) (41) and the NF-kB component p65-binding domain (p50) (48), are as indicated. The amino acid numbers for each construct are shown.

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2 S.-K. Lee and J. W. Lee, unpublished observations.
mediated transactivations. CV1 cells were transfected with 50 ng of 
(TRE)4-TK-Luc as indicated. Cotransfection of 50 ng of c-Fos activated 
amounts of SRC-1 or p300 expression vectors along with a reporter gene 
AP-1. SRC-1 (Nuclear Receptors and AP-1—which was also shown to 
be a coactivator of AP-1 (30, 31). This synergy is believed to 
reflect a cooperative recruitment of two different coactivator 
molecules (i.e. CBP and p300) (18–21). Finally, competition for a 
limiting amount of these molecules should be involved with 
cross-talks between distinct signaling pathways such as the 
well defined antagonisms between the nuclear receptor- 
and AP-1-mediated transactivations (55).

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