Steroid Receptor Coactivator-1 Interacts with Serum Response Factor and Coactivates Serum Response Element-mediated Transactivations*

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Steroid receptor coactivator-1 (SRC-1) specifically bound to serum response factor (SRF), as demonstrated by glutathione S-transferase pull down assays, and the yeast and mammalian two-hybrid tests. In mammalian cells, SRC-1 potentiated serum response element (SRE)-mediated transactivations in a dose-dependent manner. Coexpression of p300 synergistically enhanced this SRC-1-potentiated level of transactivations, consistent with the recent finding (Ramirez, S., Ali, S. A. S., Robin, P., Trouche, D., and Harel-Bellan, A. (1997) J. Biol. Chem. 272, 31016–31021) in which the p300 homologue CREB-binding protein was shown to be a transcription coactivator of SRF. Thus, we concluded that at least two distinct classes of coactivator molecules may cooperate to regulate SRF-dependent transactivations in vivo.

The serum response element (SRE)1 (1) is present in the upstream regulatory sequences of a number of immediate early genes such as c-fos (2, 3). The SRE is constitutively occupied by a complex of two proteins, serum response factor (SRF) (4) and ternary complex factor (TCF) (5). SRF belongs to the MADS box family of proteins (6) and recognizes a CArG box in the SRE (7). TCF does not bind autonomously to the element, but requires the assistance of SRF to efficiently contact the DNA (8, 9). The sequence recognized by TCF, located upstream of the CArG box, is in the form of CAGGA, a sequence that binds proteins from the Ets family. Several Ets proteins display a TCF activity on the c-fos SRE: ELK-1 (10), SAP-1 (11), and SAP-2/NET/ERF (12, 13). In addition, the SRE is recognized by oncogenic fusion proteins such as EWS-FL1 (14). Both SRF and TCF contain a transactivation domain (15, 16). SRF is a direct target for a poorly defined signal transduction pathway (17), whereas transactivation by TCF is induced by mitogens (18, 19). Transactivation domains of TCFs are direct targets for the Ras/MAP kinase transduction pathway and are substrates for ERK-1 and ERK-2 (20–22), suggesting that phosphorylation by MAP kinases activates these domains.

Transcription coactivators bridge transcription factors and the components of the basal transcriptional apparatus (23). 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 (24–27), CREB (28–30), NFκB (31, 32), bHLH factors (33), STATs (34, 35), AP-1 (36, 37), and SRF/TCF (38, 39). 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. 40). Transcriptional regulation by nuclear receptors depends primarily upon a ligand-dependent activation function, AF2, located in the C terminus and predicted to undergo an allosteric change upon ligand binding (40). Consistent with this, CBP and p300 have been found to interact directly with nuclear receptors in a ligand- and AF2-dependent manner (24–27). In addition, a series of factors that exhibit ligand- and AF2-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) (26, 41), xSRC-3 (42), AIB1 (43), TIF2 (44), RASC (45), ACTR (46), TRAM-1 (47), and p/CIP (48). Interestingly, SRC-1 (49) and its homologue ACTR (46), along with CBP and p300 (50, 51), were recently shown to contain potent histone acetyltransferase activities themselves and associate with yet another histone acetyltransferase protein pCAF (52). In contrast, it was shown that SMRT (53) and N-CoR (54), nuclear receptor corepressors, form complexes with Sin3 and histone deacetylase proteins (55, 56). 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 coactivates the c-fos SRE through TCF (38) and SRF (39), we tested whether SRC-1 itself participates in the SRE-mediated transactivations as well. Herein, we show that 1) SRC-1 specifically binds to SRF, 2) SRC-1 coactivates the SRE-mediated transactivations, and 3) p300 synergizes with SRC-1 in this coactivation. These results suggest that at least two distinct classes of coactivator molecules (i.e. SRC-1 and CBP/p300) may cooperate to regulate SRF-dependent transactivations in vivo.

EXPERIMENTAL PROCEDURES

Plasmids—T7 and B42 vectors to express SRC-1 and fragments of SRC-1 (SRC-A to SRC-E as depicted in Fig. 3) were as described previously (57, 58). An XhoI-BamHI fragment encoding a full-length SRF was filled in by Klenow fragment and dNTPs and subcloned into a Smal restriction site of pGEX-4T (Amersham Pharmacia Biotech) to express GST/SRF. A polymerase chain reaction-amplified fragment encoding a full-length SRF was cloned into EcoRI and XhoI restriction sites of pG4–5 to express B42/SRF. The VP16/SRC-1 expression vector was a

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The abbreviations used are: SRE, serum response element; SRF, serum response factor; TCF, ternary complex factor; CBP, CREB-binding protein; CREB, cAMP response element-binding protein; SRC-1, steroid receptor coactivator-1; GST, glutathione S-transferase; MAP, mitogen-activated protein.
gift of Dr. Ming Tsai (Baylor College of Medicine, Houston, TX). The mammalian expression vectors for p300, SRC-1, SRF, and Gal4-SRF along with the transfection indicator construct pRSV-β-gal, a reporter construct Gal4-TK-LUC, and the serum-responsive reporter construct SRE-c-fos-LUC, were as described previously (57–62).

**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 (61).

**Cell Culture and Transfections**—HeLa 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 SRE-c-fos-LUC or Gal4-TK-LUC, along with an increasing amount of expression vectors for SRC-1, p300, or VP16/SRC-1. Total amounts of expression vectors were kept constant by adding decreasing amounts of pcDNA3 to transfections containing increasing amounts of the SRC-1, p300, or VP16/SRC-1 vector. These cells were incubated with medium containing 0.5% fetal calf serum for 20 h. Cells were washed and re-fed with Dulbecco's modified Eagle's medium containing 20% fetal calf serum. Cells were harvested 4 h later, luciferase activity was assayed as described (61), 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 and SRF**—We and others have recently found that SRC-1 (26, 41) or its homologue p/CIP (48), originally identified as a coactivator of nuclear receptors, also functions as a coactivator of NFκB (57), AP-1 (58), CREB, and STAT1 (63). These transcription factors were previously shown to require CBP and p300 (28–32, 34–37) that are capable of forming a complex with SRC-1. Because CBP and p300 were also found to coactivate the c-fos SRE through interactions with TCF (38) and SRF (39), we tested whether SRC-1 itself functionally interacts with SRF by using the *in vitro* glutathione S-transferase (GST) pull down assays. GST alone and GST-

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**TABLE I**

| LexA fusions | SRC-A | SRC-B | SRC-C | SRC-D | SRC-E |
|--------------|-------|-------|-------|-------|-------|
| B42/−        | −     | −     | −     | +     | +     |
| B42/SRF      | −     | −     | −     | +++   | +++   |

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**Fig. 1. Interactions of SRF with SRC-1.** Luciferase, SRC-1 (A), and a series of five SRC-1 fragments (B) were labeled with [35S]methionine by *in vitro* translation and incubated with glutathione beads containing GST alone or GST/SRF as indicated. Beads were washed, and specifically bound material was eluted with reduced glutathione and resolved by SDS-polyacrylamide gel electrophoresis. Approximately 10–20% of total input was typically retained.

**Fig. 2. Effects of SRC-1 and p300 cotransfection on the SRE-mediated transactivations.** HeLa cells were transfected with LacZ expression vector, p300 expression vector, and increasing amounts of SRC-1 expression vector along with a reporter gene SRE-c-fos-LUC (60), as indicated. Cells were shocked with 20% fetal calf serum before harvest, as described (59, 60). Normalized luciferase expressions from triplicate samples are presented relative to the LacZ expressions, and the standard deviations are less than 5%.

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Coactivation of SRF by SRC-1

Fig. 3. SRC-1 as an integrator. The full-length human SRC-1 and a series of five SRC-1 fragments (57, 58) are as depicted. The nuclear receptor-interacting (receptor), CBP-p300-interacting (p300), basic helix-loop-helix/PAS (bHLH/PAS), serine-threonine-rich (S/T-rich), and glutamine-rich (Q) domains, along with the recently identified histone acetyltransferase domain (HAT) (49), the AP-1 components c-Jun and c-Fos binding domain (c-Jun/c-Fos) (58), and the NFκB component p50-binding domain (p50) (57), are as indicated. The amino acid numbers for each construct are shown. The SRC-binding domains among various SRC-1 fragments are shaded, as + indicates interactions with SRC and − means no interaction.

To independently prove these interactions in vivo, we exploited the mammalian two-hybrid tests (62). Consistent with the existence of a strong transcriptional repressive domain at the N-terminal sequences (16), Gal4 fusion protein to a full-length SRF (Gal4-SRF) significantly repressed the basal level of transcription directed by Gal4 alone (data not shown). The Gal4-SRF-mediated transactivation, however, was stimulated by coexpression of VP16-SRC-1, but not VP16 alone, in a dose-dependent manner with cotransfection of 100 ng of VP16/SRC-1 increasing the fold activation approximately 3-fold, indicating that SRC-1 specifically interacts with SRF in vitro (Fig. 1C). In addition, these interactions were also confirmed in the yeast two-hybrid tests (57, 58) (Table I). Coexpression of a B42 fusion to the full-length SRF further stimulated the LexA/SRC-D- and LexA/SRC-E-mediated LacZ expressions, whereas coexpression of a B42 alone was without any effects (Table I). Consistent with the in vitro results, the LacZ expressions mediated by LexA fusions to SRC-A or -C were not stimulated by coexpression of B42-SRF. However, the interaction of SRC-1 with SRC-B, which was readily detected in the GST-pull downs (Fig. 1B), was not observed in yeast. This discrepancy is not currently understood but may reflect the fundamental differences between two different assay systems. These results, along with the mammalian two-hybrid and GST pull-down results, suggest that SRC-1 directly associates with SRF through multiple subregions of SRC-1.

Cotransfection of SRC-1 Stimulates SRE-mediated Transactivations—To assess the functional consequences of these interactions, SRC-1 was cotransfected into HeLa cells along with a reporter construct SRE-c-fos-TK-LUC. This reporter construct, previously characterized to efficiently mediate the SRE-mediated transactivations in various cell types, consists of a minimal promoter from the c-fos gene and a single upstream consensus SRE (60). Serum shock with 20% fetal calf serum resulted in an approximately 10-fold increase in transactivation of this reporter construct, relative to the level with non-shocked cells (data not shown). Increasing amounts of cotransfected SRC-1 enhanced the reporter gene expressions in an SRC-1 dose-dependent manner, with cotransfection of 200 ng of SRC-1 increasing the fold activation approximately 8-fold (Fig. 2). Consistent with the reports that CBP and p300 are transcription coactivators of SRF and TCF (38, 39), increasing amounts of cotransfected p300 also had stimulatory effects on the reporter gene expressions, with cotransfection of 50 ng of p300 increasing the fold activation approximately 2-fold. Consistent with an idea that SRC-1 and p300 synergize to coactivate the SRE-mediated transactivations, coexpression of p300 and SRC-1 dramatically increased the reporter gene expressions above the levels observed with SRC-1 or p300 alone, with cotransfection of 50 ng of p300 and 200 ng of SRC-1 increasing the fold activation approximately 80-fold (Fig. 2B). In contrast, cotransfection of SRC-1 did not affect the LacZ reporter expression of the transfection indicator construct pRSV-β-gal either in the presence or absence of serum shock (data not shown).

In summary, we have shown that SRC-1 interacts with SRF and coactivates SRE-mediated transactivations in synergy with p300, which was also shown to be a coactivator of SRF and TCF (38, 39). This synergy is believed to reflect a cooperative recruitment of two different coactivator molecules (i.e. SRC-1 and CBP-p300) by SRF and TCF. It’s possible that these two distinct histone acetyltransferases (46, 49–51) either modify selective sites on the histone tails or act in a concerted fashion to control different aspects of transcriptional activation. These results, along with the recent reports in which SRC-1 and its homologue pCIP were shown to be coactivators for NFκB (57), AP-1 (58), CREB, and STAT-1 (63), clearly demonstrate that SRC-1 is an integrator molecule like CBP and p300. Further characterization of these integrator molecules should provide important insights into the multifactorial control of biological processes under regulation of multiple signal transduction pathways in vivo.

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