Steroid Receptor Coactivator-1 Interacts with the p50 Subunit and Coactivates Nuclear Factor κB-mediated Transactivations*

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Steroid receptor coactivator-1 (SRC-1) specifically bound to the transcription factor NFκB subunit p50 but not to p65 as demonstrated by the yeast two hybrid tests and glutathione S-transferase pull down assays. The p50-binding site was localized to a subregion of SRC-1 (amino acids 759–1141) that encompasses the previously described CBP-p300-binding domain. In mammalian cells, SRC-1 potentiated the NFκB-mediated transactivations in a dose-dependent manner. Coexpression of p300 further enhanced this SRC-1-potentiated level of transactivations, consistent with the recent findings in which CBP and p300 were shown to be transcription coactivators of the p65 subunit (Perkins, N. D., Felzien, L. K., Betts, J. C., Leung, K., Beach, D. H., and Nabel, G. J. (1997) Science 275, 523–527; Gerritsen, M. E., Williams, A. J., Neish, A. S., Moore, S., Shi, Y., and Collins, T. (1997) Proc. Acad. Natl. Sci. U. S. A. 94, 2927–2932). These results suggest that at least two distinct coactivator molecules may cooperate to regulate the NFκB-dependent transactivations in vivo and SRC-1, originally identified as a coactivator for the nuclear receptors, may constitute a more widely used coactivation complex.

The transcription factor nuclear factor κB (NFκB) is important for the inducible expression of a wide variety of cellular and viral genes (reviewed in Refs. 1 and 2). NFκB is composed of homo- and heterodimeric complexes of members of the Rel (NFκB) family of polypeptides. In vertebrates, this family comprises p50, p65 (RelA), c-Rel, p52, and RelB. These proteins share a 300-amino-acid region, known as the Rel homology domain, which binds to DNA and mediates homo- and heterodimerization. This domain also is target of the IkB inhibitors, which include IkBα, IkBβ, IkBγ, Bcl-3, p105, and p100 (3). In the majority of cells, NFκB exists in an inactive form in the cytoplasm, bound to the inhibitory IkB proteins. Treatment of cells with various inducers results in the degradation of IkB proteins. The bound NFκB is released and translocates to the nucleus, where it activates appropriate target genes. IkBα is degraded in response to all of the known inducers of NFκB, whereas IkBβ is degraded only when cells are stimulated with inducers such as lipopolysaccharide (LPS) and interleukin-1, which cause persistent activation of NFκB (4). Following degradation of the initial pool of IkBα in response to LPS or interleukin-1, newly synthesized IkBβ accumulates in the nucleus as an unphosphorylated protein that forms a stable complex with NFκB and prevents it from binding to newly synthesized IkBα (5, 6). Bcl-3 is an unusual IkB protein in that it can not only inhibit nuclear NFκB complexes but can bind to p50 and p52 dimers on DNA and provide the complexes with transactivating activity (7, 8).

Transcription coactivators bridge transcription factors and the components of the basal transcriptional apparatus (reviewed in Ref. 9). Functionally conserved proteins 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 (10–13), CREB (14–16), AP-1 (17, 18), bHLH factors (19), STATs (20, 21), and, most recently, the NFκB component p65 (22, 23). 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. 24). 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 (24). Consistent with this, CBP and p300 have been found to interact directly with nuclear receptors in a ligand- and AF-2-dependent manner (10–13). 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) (12, 25), AIB1 (26), TIF2 (27), RAC3 (28), ACTR (29), TRAM-1 (30), p/CIP (31), and XICO (32). Interestingly, SRC-1 (33) and its homologue ACTR (29), along with CBP and p300 (34, 35), were recently shown to contain potent histone acetyltransferase activities themselves and associate with yet another histone acetyltransferase protein p/CAF (36). In contrast, it was shown that SMRT (37) and N-CoR (38), nuclear receptor corepressors, form complexes with Sin3 and histone deacetylase proteins (39, 40). 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 the NFκB component p65 (22, 23), we tested whether SRC-1 itself participates in the NFκB-mediated transactivations as well. Herein, we show that 1) SRC-1 specifically binds to the NFκB component

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† The abbreviations used are: NFκB, nuclear factor κB; LPS, lipopolysaccharide; β-gal, β-galactosidase; IL, interleukin; CBP, CREB-binding protein.
p50 but not to p65, 2) SRC-1 coactivates the NFkB-mediated transactivations, and 3) p300 synergized with SRC-1 in this coactivation. These results suggest that at least two distinct transcription coactivator molecules with histone acetyltransferase activities (i.e. SRC-1 and CBP-p300) may regulate the NFkB-mediated transactivations in vivo, and SRC-1, originally identified as a coactivator for the nuclear receptors, may regulate many different transcription factors.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—LexA, B42, T7, or GST vectors to express p50 and p65 were as described previously (41). Polymerase chain reaction-amplified fragments of SRC-1 (A–E as depicted in Fig. 1) were subcloned into EcoRI-StuI restriction sites of the LexA fusion vector pEC202PL (42), EcoRI-XhoI restriction sites of the B42 fusion vector pJG4–5 (42), or EcoRI-XhoI restriction sites of the T7 vector pBS-SK (Stratagene, San Diego, CA). The GST fusion vectors encoding CBP-N (amino acids 1–450) and CBP-C (amino acids 1891–2441) (kind gifts from Dr. Chris Glass, University of California at San Diego) were as described (12). The expression vectors for p500 (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 recently identified histone acetyltransferase domain, are as indicated. The amino acid numbers for each construct are as described (15, 33, 43).

**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, SH18–34 (42). Plate and liquid assays of β-gal activity were carried out as described (42, 44–46). Similar results were obtained in more than two similar experiments.

**GST Pull Down Assays**—The GST fusions or GST alone was expressed in Esherichia 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 (47).

**Cell Culture and Transfections**—L929 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 pSV-β-gal and 100 ng of a reporter gene (xβ)-IL-2-Luc (43), along with increasing amounts of expression vectors for SRC-1 or p300. Total amounts of expression vectors were kept constant by adding decreasing amounts of pDNA to transfected cells 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 (48), 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 p50**—We have recently found that XICO (32), a Xenopus homologue of the nuclear receptor coactivator SRC-1 (12, 25), interacts with p50 but not with p65. The smallest clone among the original isolates of XICO that readily interacted with p50 consisted of only the central nuclear receptor-binding and CBP-p300-binding domains, suggesting that the interaction interface should be included within these two domains. These interactions were further characterized in the yeast two-hybrid tests (42), in which formations of p50-p50 homodimer and p50-p65 heterodimer were readily detected as previously reported (41) (Table I). A series of SRC-1 fragments were subcloned into B42 and LexA vectors as depicted in Fig. 1. Among the LexA fusions, only SRC-D (SRC-1 amino acids 759–1141) encompassing the previously described CBP-p300-binding domain (12, 25–32) was found to confer autonomous transactivation function to a LacZ reporter construct controlled by upstream LexA-binding sites (42) (Table I). This is consistent with the previous findings in which the CBP-p300-binding domain was shown to be essential for autonomous transactivation functions (12, 25–32). Consistent with an idea that p50 interacts with SRC-D, coexpression of a B42 fusion to the full-length p50 further stimulated the LexA/SRC-D-mediated LacZ expression, whereas coexpression of a B42 fusion to the full-length p65 was without any effects (Table I). In contrast, the LacZ expressions mediated by LexA fusions to SRC-A, -B, -C, or -E were not stimulated by coexpression of B42/p50 or B42/p65. Similar results were also obtained with B42/fusion to SRC-1 fragments and LexA fusions to p50 and p65, in which coexpression of the B42/SRC-D and LexA/p50 pair efficiently stimulated the LacZ reporter expression (data not shown).

**Coexpression of SRC-1 Stimulates the NFkB-mediated Transactivations**—To assess the functional consequences of these interactions, SRC-1 was cotransfected into L929 cells

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**Table I**

| Receptor | SRC-A | SRC-B | SRC-C | SRC-D | SRC-E |
|----------|-------|-------|-------|-------|-------|
| p50      | 361   | 568   | 779   | 579   | 1141  |
| SRC-1    | 1101  | 1441  | 1441  | 1441  | 1441  |

Fig. 1. Schematic representation of the SRC-1 constructs. The full-length human SRC-1 (12, 25) and a series of five SRC-1 fragments are as depicted. The nuclear receptor-interacting, CBP-p300-interacting, bHLH/PAS, serine/threonine-rich, and glutamine-rich domains, along with the recently identified histone acetyltransferase domain (33), are as indicated. The amino acid numbers for each construct are shown.
Coactivation of the NFκB Component p50 by SRC-1

along with the reporter construct (κB)2-IL-2-Luc. This reporter construct, previously characterized to efficiently mediate the NFκB-dependent transactivations in various cell types, consists of a minimal promoter from the interleukin-2 gene and four upstream κB sites from the IL-6 gene (43). Increasing amounts of cotransfected SRC-1 enhanced the reporter gene expressions in an SRC-1 dose-dependent manner, with cotransfection of 800 ng of SRC-1 increasing the fold activation approximately 3-fold (Fig. 3). Consistent with the reports that CBP and p300 are transcription coactivators of the NFκB component p65 (22, 23), increasing amounts of cotransfected p300 had stimulatory effects on the reporter gene expressions, with cotransfection of 100 ng of p300 increasing the fold activation approximately 2-fold. Consistent with an idea that SRC-1 and p300 synergize to coactivate the NFκB-mediated transactivations, coexpression of p300 and SRC-1 further increased the reporter gene expressions above the levels observed with SRC-1 or p300 alone (Fig. 3). In various cells, SRC-1 also coactivated the LPS- or tumor necrosis factor receptor- and NFκB-mediated transactivations, coexpression of p300 increasing the fold activation approximately 2-fold. Consistent with the reports that CBP and p300 are transcription coactivators of the NFκB component p65 (22, 23), increasing amounts of cotransfected p300 had stimulatory effects on the reporter gene expressions, with cotransfection of 100 ng of p300 increasing the fold activation approximately 2-fold. Consistent with an idea that SRC-1 and p300 synergize to coactivate the NFκB-mediated transactivations, coexpression of p300 and SRC-1 further increased the reporter gene expressions above the levels observed with SRC-1 or p300 alone (Fig. 3). In various cells, SRC-1 also coactivated the LPS- or tumor necrosis factor α-induced level of transactivations (data not shown). 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 the absence of LPS or tumor necrosis factor α (data not shown).

In summary, we have shown that SRC-1 interacts with the NFκB subunit p50 and coactivates the NFκB-mediated transactivations in synergy with p300, which was recently shown to be a coactivator of the NFκB subunit p65 (22, 23). This synergy is believed to reflect a cooperative recruitment of two different coactivator molecules (i.e. SRC-1 and CBP-p300) by p50 and p65, respectively. It is possible that these two distinct histone acetyltransferases (29, 33–35) either modify selective sites on the histone tails or act in a concerted fashion to control different aspects of transcriptional activation. In particular, p50 contains a single consensus acetylation site KXXK (49) (where K is lysine and X is any amino acid) in the Rel domain (p50 amino acids 74–77). It will be interesting to examine whether this site can be acetylated by SRC-1 or CBP-p300 and serves as yet another regulatory target, as recently attested with p53, in which acetylation of the C-terminal regulatory site enhanced its DNA binding activities (49). It is also notable that SRC-1 was originally identified as a coactivator molecule for the nuclear receptor superfamily (12, 25). However, the results presented in this report suggest that SRC-1 may regulate many different transcription factors, joining the class of proteins that were termed integrators (i.e. CBP and p300) (10–13). 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 NFκB-mediated transactivations (41, 50–52).

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Coactivation of the NFκB Component p50 by SRC-1

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