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The CREB-binding Protein (CBP) Cooperates with the Serum Response Factor for Transactivation of the c-*fos* Serum Response Element*

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The serum response element is one of the major promoter elements of the immediate early response to extracellular signals. The serum response element includes two main binding sites for proteins: the Ets box, which binds p62^{TCF}, and the CArG box, which binds p67^{SRF}. These two proteins are direct targets for signal transduction pathways; p62^{TCF} is a nuclear end point of the Ras/mitogen-activated protein kinase pathway, and p67^{SRF} is targeted by the Rho/Rac small G-proteins. The mechanism by which the signal is further transduced from the transcription factors to the basal transcriptional machinery is poorly understood. Recent data have suggested that the cAMP-responsive element-binding protein (CREB)-binding protein, a transcriptional adaptor involved in the transactivation through a wide variety of enhancer elements, participates in p62^{TCF} activity. We here show that the CREB-binding protein also cooperates in the process of transactivation by p67^{SRF}. Cotransfections of expression vectors for the CREB-binding protein increased the expression, in response to serum, of reporters under the control of the c-*fos* serum response element. Interestingly, the C-terminal moiety of the CREB-binding protein was not necessary to observe this effect. The cooperation did not require the Ets box in the serum response element, and the CArG box was sufficient, indicating that the CREB-binding protein is able to cooperate with p67^{SRF} in the absence of an Ets protein. Co-immunoprecipitation experiments using cell extracts showed that p67^{SRF} could be retained with antibodies directed against the CREB-binding protein, suggesting that the two proteins form a multimolecular complex in live cells. The physical interaction between p67^{SRF} and the CREB-binding protein was further confirmed by two-hybrid assays in mammalian cells. Our results indicate that the CREB-binding protein cooperates with p67^{SRF} and, thus, suggest that the serum response element is regulated by a multimolecular complex, which includes the CREB-binding protein, p67^{SRF}, and p62^{TCF}, with multiple interactions between the components of the complex.

The serum response element (SRE)[1] enhancer (1) is present in the upstream regulatory sequence of a number of immediate early genes such as c-*fos* (2, 3). The SRE is constitutively occupied by a complex of two proteins, p67^{SRF} (4) and p62^{TCF} (5). p67^{SRF} belongs to the MADS box family of proteins (6) and recognizes a CArG box in the SRE (7). p62^{TCF} does not bind autonomously to the element, but requires the assistance of p67^{SRF} to efficiently contact the DNA (8, 9). The sequence recognized by p62^{TCF}, located upstream of the CArG box, is in the form 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/ERP (12, 13). The SRE is also recognized by oncogenic fusion proteins such as EWS-FLI (14). TCFs can be distinguished by their pattern of expression (15, 16), by their affinity for the c-*fos* SRE Ets box (6, 17), or on a functional basis (13).

Both p67^{SRF} and p62^{TCF} contain a transactivation domain (TAD) (18, 19). Transactivation by TCF TADs is induced by mitogens (20, 21). TCF-TADs are direct targets for the Ras/MAP kinase transduction pathway and are substrates for ERK-1 and ERK-2 (22–24), suggesting that phosphorylation by MAP kinases activates these domains. p67^{SRF} is a direct target for a poorly defined signal transduction pathway (25).

The mechanism by which the activating signal, transmitted through the SRE, is further transduced to the transcriptional machinery and the minimal promoter is unknown. Recent data suggest that activation through TCFs could be mediated by a coactivator or adaptor protein, the CREB-binding protein or CBP (26, 27). The CBP adaptor protein was first characterized as a co-activator for CREB, a cAMP-responsive transcription factor (28, 29), but was rapidly shown to be involved in a large variety of responses. CBP is highly homologous to p300, a transcriptional co-activator (30) that is a target for viral transforming proteins such as E1A (31); CBP itself is complexed by E1A (31, 32). CBP and p300 (p300/CBP) are involved in the activation of a large variety of transcriptional enhancer elements through various transcription factors (33), including c-Jun (34, 35), c-Fos (36), c-Myb (37, 38), E2F (39, 40), the STAT proteins (41, 42), MyoD (43, 44), and the nuclear receptor superfamily (45–47).

Co-activators function, at least in part, as bridges between sequence-specific transcriptional activators and general transcription factors of the basal transcription machinery. CBP directly contacts sequence-specific transactivators via one of two interaction domains located, respectively, in the N-termi-

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[1] The abbreviations used are: SRE, serum response element; CBP, CREB-binding protein; CREB, cAMP-responsive element-binding protein; CMV, cytomegalovirus; FCS, fetal calf serum; HA, hemagglutinin; MAP, mitogen-activated protein; SRF, serum response factor; TAD, transactivation domain; TCF, ternary complex factor.

nal or C-terminal part of the molecule (48). Once recruited, CBP can modulate the transcription rate through various mechanisms. First, CBP includes two TADs located in the N-terminal and C-terminal parts of the molecule (33) that contact two general transcription factors: TATA-binding protein for the N-terminal TAD (26, 44, 49), and TFIIB for the C-terminal TAD (29). In addition, CBP recruits a protein that displays a histone acetyltransferase activity (50). Histone acetyltransferases destabilize the nucleosomal structure by acetylation of the N-terminal histone tails, which protrude from the nucleosome (51). CBP not only recruits a histone acetyltransferase, but also displays a histone acetyltransferase enzymatic activity (52, 53). Thus, CBP may use several mechanisms to activate transcription, either by recruiting proteins of the transcripional machinery or by inducing a nucleosomal remodeling process.

CBP has been implicated in the transactivation of the c-*fos* SRE through the p62^{TCF} protein (26, 27). We here show that CBP enhances transcriptional activation of the SRE even in the absence of the Ets-binding site, and thus in the absence of p62^{TCF} recruitment. This result indicates that CBP can also cooperate with p67^{SRF}. Furthermore, we demonstrate the formation of a physical complex between p67^{SRF} and CBP in live cells. In addition, we show that, whereas the transactivation through p62^{TCF} seems to involve the C-terminal TAD, the N-terminal moiety of CBP is sufficient for transactivation through p67^{SRF}. Taken together, our results indicate that CBP participates in c-*fos* SRE activation both through the p62^{TCF} and the p67^{SRF} proteins, and that this transactivation is mediated through two distinct TADs in the CBP molecule.

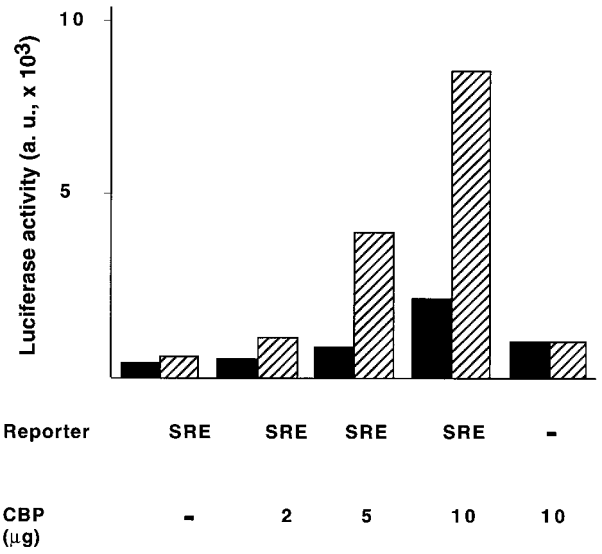

FIG. 1. **CBP stimulates SRE activity.** Reporter constructs (1 μg) containing the luciferase gene under the control of the c-*fos* SRE element (*SRE*) or the c-*fos* minimal promoter (−) were transfected into F9 cells together with increasing doses of a CBP expression vector, as indicated (to keep the amount of promoter constant, the controls received 10 μg of an equal mixture of the backbone vector, pRcRSV, and pBluescript, and all samples were completed to 10 μg with the same mixture). Cells were deprived of serum for 18 h, and then either serum-treated (*striped bars*) or not (*solid bars*) for 4 h, before protein extraction. Luciferase activity is expressed in arbitrary units standardized on the sample's protein contents. All transfection experiments were run in duplicate. Shown is the result of a typical experiment; similar results were obtained in three independent experiments.

EXPERIMENTAL PROCEDURES

Cells and Culture—F9 and NIH 3T3 cells were maintained in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (penicillin-streptomycin; Life Technologies, Inc.), and grown at 37 °C in 5% CO_2. U2OS cells were maintained in McCoy's medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated FCS and antibiotics.

Plasmids—All reporter plasmids contained the luciferase gene under the control of the c-*fos* minimal promoter (−43 to +42 with reference to the transcription start site) and were derived from the *Fos*-40luc plasmid described by Masutani *et al.* (17). SRE-luc reporters were constructed by inserting three tandem repeats of the synthetic oligonucleotides described in Fig. 3*A* into the *Xho*I site of the *Fos*-40luc plasmid.

Two sets of CBP expression vectors were used in this study. pRc-RSV CBP and pRc-RSV CBP 1097 were based on the pRc-RSV vector from Invitrogen and were kind gifts of Dr. T Kouzarides (54). The second set of expression vectors was based on pCMV$_2$N-$_3$T (a kind gift of Dr. T. Kouzarides), which includes two nuclear localization signals and three HA-epitope tags. An *Xba*I-*Xba*I insert from pRc-RSV CBP was inserted into the *Xba*I site of pCMV$_2$N-$_3$T to obtain pCMV$_2$N-$_3$T-CBP 1890. pCMV$_2$N-$_3$T-CBP 1890 was digested with *Xba*I, and an *Xba*I-*Xba*I fragment of pRc/RSV CBP was inserted, resulting in pCMV$_2$N-$_3$T CBP. pCMV$_2$N-$_3$T CBP 1097 was constructed from the pCMV$_2$N-$_3$T CBP by digestion with *Xba*I and religation. *Nhe*I restriction sites were introduced into the sequence at positions corresponding to amino acids 865 and 966 by site-directed mutagenesis using polymerase chain reaction (55). These sites were used to create the deletion mutants pCMV$_2$N-$_3$T CBP 865 and pCMV$_2$N-$_3$T CBP 966, by digestion with *Nhe*I and *Xba*I and religation.

The plasmid pGAL4 contains the GAL4-(1–147) DNA binding domain under the control of the human cytomegalovirus (CMV) promoter-enhancer. The GAL4 chimera expression vector GAL4-CBP-(1–1097) was constructed by insertion of an *Xba*I-*Bam*HI fragment from pCMV$_2$N-$_3$T-CBP 1890 into pGAL4. The correct reading frame was restored using T4 polymerase to fill in the ends generated by *Bam*HI.

The GAL4-CBP-(1–282) chimera expression vector was constructed from pGAL4-CBP-(1–1097) by deletion of a *Kpn*I-*Xba*II fragment. The GAL4-CBP-(1–468) was constructed from pGAL4-CBP-(1–1097) by deletion of an *Afl*II-*Xba*II fragment.

The GAL4-CBP-(271–826) chimera expression vector was constructed by insertion of a *Kpn*I-*Kpn*I fragment from GAL4-CBP-(1–1097) into pGAL4. The GAL4-CBP-(826–1097) chimera expression vector was constructed by insertion of a *Kpn*I-*Xba*II fragment from GAL4-CBP-(1–1097) into pGAL4. For these two last constructs, the correct reading frame was restored using T4 polymerase to fill in the ends generated by *Bam*HI.

The VP16 chimera expression vector pSRF-VP16 was constructed by insertion of a *Eco*RI (filled in)-*Bam*HI fragment from pGEX-SRF (described in Ref. 56) into the pVP16 plasmid (CLONTECH) digested by *Bam*HI (the protruding ends were filled in using T4 polymerase) and *Hin*dIII.

The pGAL4-luc reporter gene contains the luciferase gene under the control of five GAL4 sites and the minimal promoter of the ML adenovirus.

CMV-βGAL, used as a control for transfection efficiency (250 ng) in some experiments, was purchased from Cayla (France).

All constructs were controlled by direct sequence analysis.

Transfection and Luciferase Assays—Transfection experiments were performed using polyethylenimine (57) or calcium phosphate precipitation. The day before transfection, cells were seeded at 1.3×10^5 cells/well in 24-well dishes. After transfection, FCS was added to 0.5%. 20 h later, cultures were treated, except for the controls, with 20% FCS for 4 h, and cells were lysed using a lysis buffer from Promega. When indicated, a CMV-βGAL vector (250 ng) was used as a standard for transfection efficiency. In the other experiments, transfection efficiency was measured by direct estimation of the intracellular plasmid using a Southern blot procedure as described by McIntyre and Stark (58).

Luciferase activity was measured using a kit from Promega and β-galactosidase activity using a kit from Tropix, both on a Lumat B9501 luminometer (Berthold).

In all experiments, each transfection was performed in duplicate.

Coimmunoprecipitation—U2OS Cells were transfected by calcium phosphate co-precipitation with 18 μg of the pCMV$_2$N-$_3$T or pCMV$_2$N-$_3$T-CBP 1097. After 48 h of culture, cells were incubated at 4 °C in 5 volumes of lysis buffer (10 mM Tris, 10 mM NaCl, 2 mM $MgCl_2$ supplemented with a mixture of protease inhibitors: 1 mM PMSF supplemented with 1 mg/ml, 1 mg/ml pepstatin, and 1 mg/ml aprotinin) for 10 min. Nonidet P-40 was added to 0.5%, and incubation was allowed to continue for another 10 min. Cells were centrifuged, resuspended in 500 μl of buffer C (20 mM Hepes, pH 7.9, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1 mM DTT, 10% Glycerol supplemented with the mixture of protease inhibitors), and incubated at 4 °C with slow rotation for 30

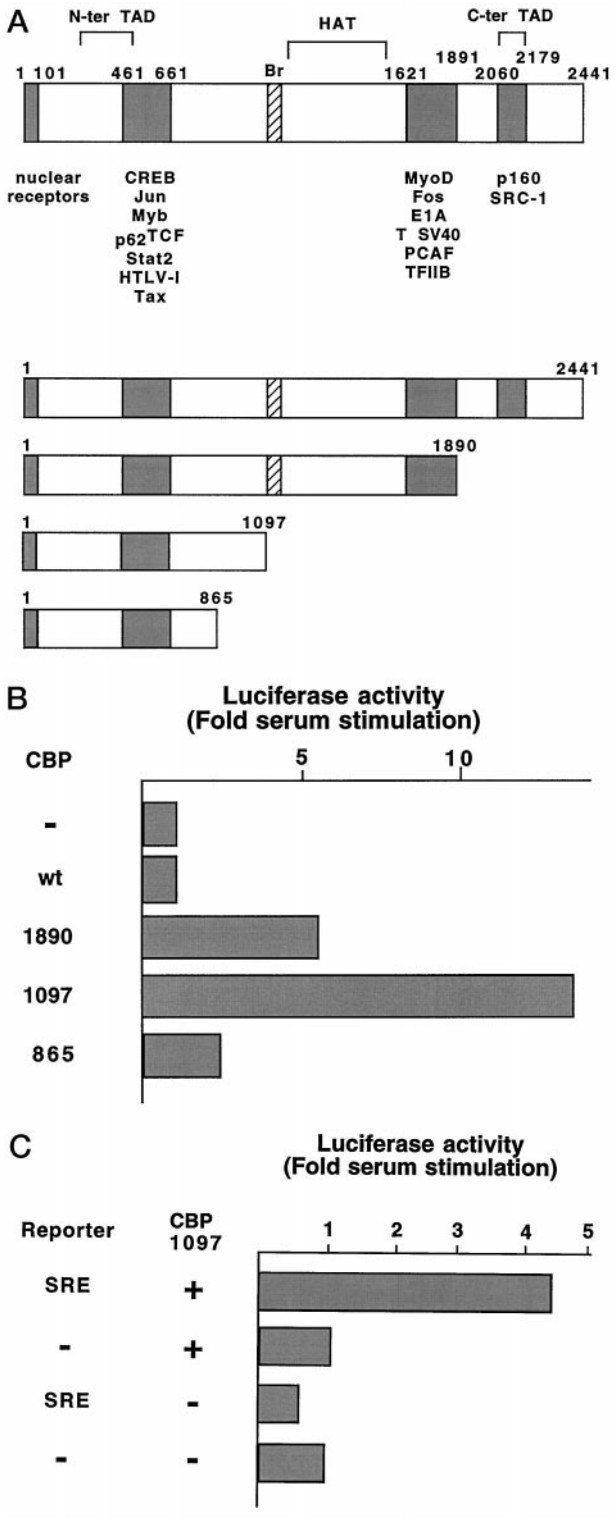

FIG. 2. **The N-terminal moiety of CBP is sufficient for SRE stimulation.** *A*, mutants of CBP used in this study; *shaded boxes* represent sites of interaction with the indicated proteins. *Br*, bromodomain; *N-ter*, N-terminal; *C-ter*, C-terminal. *B*, stimulation of SRE activity by the various mutants of CBP. Transfections were performed as indicated in Fig. 1, using the SRE-luciferase reporter construct (0.4 μg), and expression vectors (0.8 μg) for the various mutants tagged with the HA epitope (as indicated) or a mixture of the control backbone vector, pCMV$_2$N-$_3$T, and pBluescript (−). Shown is the relative activity (the ratio between samples treated with serum and the corresponding control, untreated samples). Similar results were obtained in two independent experiments. *wt*, wild type. *C*, transactivation by the N-terminal moiety of CBP is dependent on the SRE. Cells were transfected as described in Fig. 1, with reporter constructs under the control of the SRE (*SRE*) or of the c-*fos* minimal promoter (−), as indicated, together with an expression vector for the N-terminal moiety of CBP (*CBP 1097*) or the backbone vector as a control (−). Similar results were obtained in three independent experiments.

min. Samples were centrifuged, and the supernatant was diluted 1:1 with 50 mM Tris, pH 8, 0.2% Nonidet P-40. Anti-HA antibodies (12AC5) or irrelevant antibodies (anti-myogenin: F5D) were added, and samples were incubated under rotation at 4 °C for 2 h. Protein A-agarose beads (Sigma) and protein G-Sepharose beads (Pharmacia) were added, and incubation was allowed to continue for 1 h. The beads were washed three times with 1 ml of 50 mM Tris, pH 8, NaCl, 1 mM EDTA, and 0.5% Nonidet P-40; immunoprecipitates were then eluted using 50 μg of an HA-tag peptide. The eluates were mixed with SDS loading buffer and analyzed by SDS-polyacrylamide gel electrophoresis followed by Western blotting. The upper part of the gel, containing the high molecular weight proteins, was probed with the anti-HA antibody; the lower part of the gel was probed with anti p67^{SRF} antibodies (a polyclonal antiserum raised against glutathione *S*-transferase-SRF, prepared as described previously (15).

RESULTS

CBP Stimulates SRE Activity—We have used a co-transfection assay to test the hypothesis that CBP participates in the process of transactivation through the c-*fos* SRE element. An SRE-luc reporter construct (or the minimal promoter as a negative control) was transfected into F9 cells, together with a vector allowing expression of the CBP molecule. Transfected cells were starved by cultivation at low serum concentration and then treated, except for the controls, with high serum. Results from a typical experiment are shown in Fig. 1. In the absence of CBP expression, the SRE-luc reporter vector was expressed at low levels and poorly activated (2.2 ± 1.1-fold , average of 14 experiments). Concomitant expression of CBP increased the basal level of expression (about 5 ± 3.2-fold using 5 μg of plasmid, mean of five experiments) and boosted the response to serum (to 15 ± 5.5-fold with 5 μg of plasmid, mean of five experiments). The response was dependent on the dose of the CBP expression vector. CBP did not stimulate transcription in general, since no increase in the level of luciferase was observed with an enhancerless reporter vector containing only the c-*fos* minimal promoter (from −40 to +42). This result suggests that CBP participates in transactivation through the c-*fos* SRE.

The N-terminal Part of the CBP Molecule Is Sufficient for SRE Stimulation—CBP includes several functional domains located in the N-terminal or C-terminal part of the molecule (Fig. 2*A*). To test which region of CBP was involved in SRE stimulation, we constructed deletion mutants of the molecule (Fig. 2*A*). Direct analysis of the transgene expression demonstrated that these mutants were expressed in transfected cells (data not shown). Interestingly, deletion of the C-terminal moiety of CBP, up to amino acid 1097, did not abolish SRE stimulation (Fig. 2*B* shows the results of a typical experiment): addition of serum resulted in an induction of 9.9 ± 4 (mean of five experiments) of the reporter expression. Note that this deletion did not result in any increase of the activity of CBP, since the stimulation with the mutants was comparable to that obtained with the full-length molecule at optimal doses of expression vectors. Furthermore, similar levels of stimulation were observed in experiments such as that shown in Fig. 2*B* when the data were standardized on the level of expression of the transgenes (as estimated by Western bloting, data not shown). Further deletion, up to amino acid 865, abolished the response, suggesting that the region between 865 and 1097 is required for this activity of CBP. The specificity of the response was confirmed through the use of an SRE-less reporter construct, which did not show any transactivation in the presence

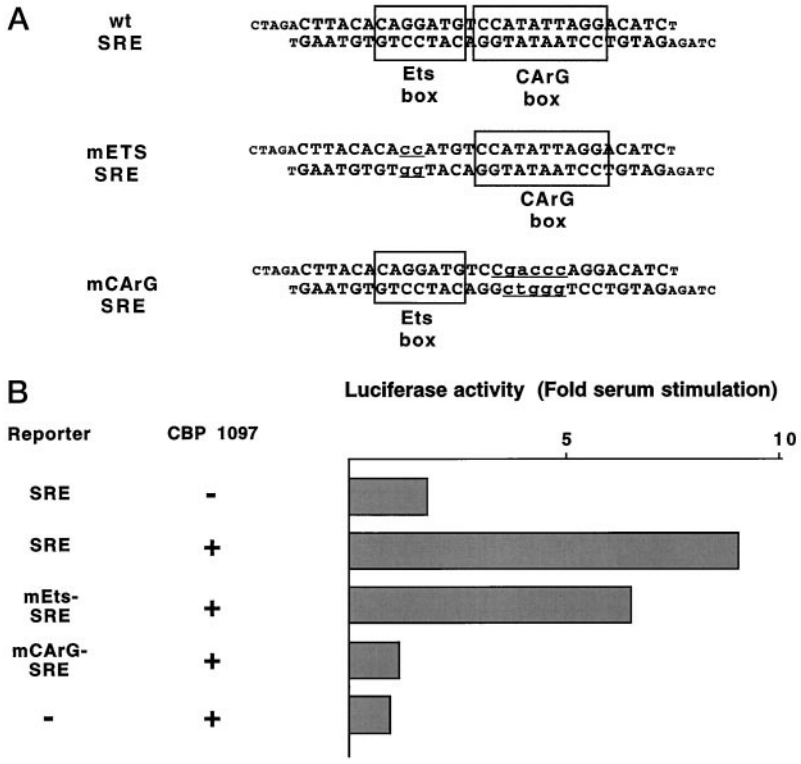

FIG. 3. **CBP cooperates with p67^{SRF} for SRE transactivation. A**: Sequences of the SRE element and mutants inserted in front of the c-*fos* minimal promoter in the reporter constructs. The Ets box and the CArG box are indicated. Nucleotides in mutated mEts-SRE or mCArG-SRE are *underlined*. Restriction sites used for cloning are indicated by *small capital letters*. *wt*, wild type. *B*, cells were transfected as described in Fig. 2*B*, using reporter constructs under the control of the various mutants of the SRE shown in *A* and the expression vector for the N-terminal moiety of CBP (CBP 1097). Similar results were obtained in four independent experiments.

of the CBP N-terminal moiety (Fig. 2*C*).

Stimulation of the SRE Occurs in the Absence of an Ets-binding Site—Two proteins are involved in SRE transactivation: p67^{SRF}, which recognizes a CArG box; and p62^{TCF}, which recognizes an Ets box (Fig. 3*A*). To assess with which of these proteins CBP could collaborate in the process of SRE transactivation, various mutations of the SRE were used to promote the transcription of the luciferase reporter construct. As shown in Fig. 3*B*, mutation of the Ets box had hardly any effect on the SRE response in the presence of CBP (reporter mEts-SRE). In contrast, the CArG box could not be mutated without drastically impairing the SRE response (reporter mCArG-SRE). These results indicate that the Ets box is not necessary for SRE transactivation in the presence of CBP, and strongly suggest that CBP is able to cooperate with p67^{SRF} in the absence of p62^{TCF}.

The CBP N-terminal Moiety Forms a Complex with p67^{SRF} in Live Cells—Our results suggest that CBP acts as a co-activator for p67^{SRF} in the SRE transactivation process. If this is the case, then CBP might be able to interact physically with p67^{SRF}. To test this hypothesis, we have performed co-immunoprecipitation assays. Cells were transfected with a vector allowing the expression of a tagged version of CBP. The tagged CBP protein was immunoprecipitated under mild conditions, and the proteins that were co-precipitated with CBP were analyzed for the presence of p67^{SRF}. As shown in Fig. 4*A*, p67^{SRF} could be detected in samples in which CBP was immunoprecipitated from the extracts (*lane 4*, *upper* and *lower part*). The specificity of the immunoprecipitation was assessed using irrelevant antibodies, which failed to retain CBP (*upper part*, *lane 3*), and with which no p67^{SRF} was detected (*lower part*, *lane 3*). Furthermore, p67^{SRF} was not detected in anti-HA immunoprecipitates from cells that had been transfected with the backbone vector (*lower part*, *lane 2*).

To confirm this result, we used a two-hybrid assay in mammalian cells. Cells were transfected with a reporter construct under the control of GAL4-binding sites, together with expression vectors for fusion proteins between the GAL4 DNA binding domain and different regions of the CBP molecule (shown in Fig. 4*B*), plus an expression vector for a fusion protein between p67^{SRF} and the VP16 viral strong transactivation domain. Results are shown in Fig. 4*C*. The N-terminal moiety of CBP was able to recruit the SRF VP16 protein, resulting in a strong transactivation of the reporter. When various regions of the N-terminal moiety were analyzed in the same assay, none scored clearly positive in the test, suggesting that the two domains which have been described as interacting with transcription factors in this part of CBP are not sufficient to observe the interaction. These results confirm that CBP and p67^{SRF} are able to interact in live cells and suggest that CBP and p67^{SRF} are members of a multimolecular complex in live cells.

DISCUSSION

The SRE, which is a central element of the cell's immediate early response, binds several transcription factors and is targeted by several transduction pathways. In particular, p62^{TCF} is a direct target for the Ras/MAP kinase pathway. p67^{SRF} is required to assist p62^{TCF} binding and is also independently a target for signal transduction pathways involving small G-proteins from the Rho/Rac family. Little is known about the mechanism used by these proteins to further transmit the activation signal to the minimal promoters of the genes controlled by the SRE element. Recently, Janknecht and collaborators (26) have shown that CBP, a versatile adaptor protein, cooperates with p62^{TCF} for transactivation through the SRE. We here show that, indeed, CBP participates in the transactivation through the SRE, since expression of CBP stimulated a response to serum through this element in a dose-dependent manner. Interestingly, in our study, the effect of CBP was observed in the absence of a functional Ets-binding site, and thus in the absence of TCF binding to the element. In many cell systems, the Ets-binding site is not necessary to observe a significant response to serum (22, 25, 59, 60). Moreover, although F9 cells, which were used in this study, express normal amounts of p62^{TCF}, the ternary complex between p67^{SRF} and p62^{TCF} seems to be inactive in these cells (61). Our result

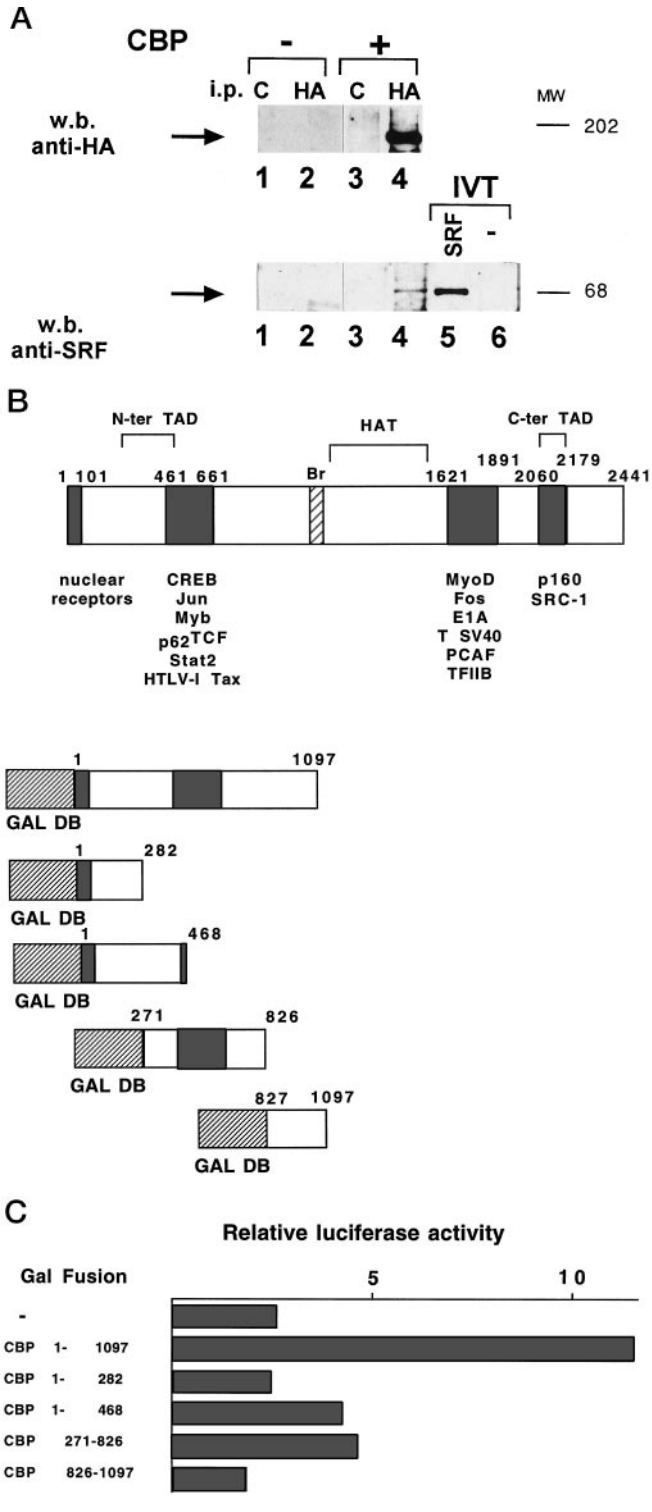

FIG. 4. **The N-terminal moiety of CBP forms a complex with p67^{SRF} in live cells.** *A*, coimmunoprecipitation. U2OS cells were transfected with the expression vector for the HA-tagged N-terminal moiety of CBP (*CBP 1097*), or the backbone vector as indicated. Cell extracts were immunoprecipitated intraperitoneally using an anti-HA monoclonal antibody (*HA*; *lanes 2* and *4*) or an irrelevant control antibody (*C*; *lanes 1* and *3*). Immunoprecipitates were analyzed by Western blotting (*w.b.*), using the anti-HA antibody to detect CBP (*upper part* of the *gel*) or else the anti-p67^{SRF} antibody (*lower part* of the *gel*). *In vitro* translated p67^{SRF} (*SRF*) and non-programmed lysates (−) (*IVT*, *lanes 5* and *6*), were run on the same gel and used as a reference for p67^{SRF}. The migration of the molecular weight markers is shown on the *right*. Similar results were obtained in two independent experiments. *B* and *C*, two-hybrid assay in mammalian cells. *B*, GAL4 fusion proteins used in transfection experiments. The GAL4 DNA binding domain (*GAL DB*) is indicated by a *striped box*. *C*, CBP N-terminal recruits p67^{SRF}. 3T3 cells were transfected with the indicated GAL4-CBP chimera expression plasmids (3 μg) and a luciferase reporter construct (1 μg) under the control of GAL4-binding sites, together with an expression vector for VP16 or SRF VP16 (3 μg). Shown are the relative luciferase activities (ratios between the activities in the presence of SRFVP16 and the corresponding control activities, in the presence of VP16 alone, both of which were first standardized for transfection efficiency using β-galactosidase).

indicates that the participation of CBP in SRE transactivation does not absolutely require the Ets protein p62^{TCF}. In contrast, a functional CArG box was indispensable to observe the stimulation of the SRE response, suggesting that the target protein of CBP in this function was p67^{SRF}. Thus, CBP cooperates both with p62^{TCF} (26, 27) and with p67^{SRF} (this study).

CBP is a large molecule that includes several sites of interaction with various sequence-specific transcription factors and has two TADs. To determine which of these functional domains was involved in SRE transactivation, we have used deletion mutants of the protein. Interestingly, the N-terminal moiety of CBP was sufficient to stimulate the SRE response to serum. A C-terminal transactivation domain of CBP seems to be involved in the cooperation with p62^{TCF} (26). Thus, CBP uses two TADs for transactivation through the SRE, the N-terminal and the C-terminal TAD. In addition, these data demonstrate that CBP can transactivate the SRE in the absence of the domain bearing the histone acetyltransferase activity, which is located in the C-terminal part of the molecule, indicating that this intramolecular activity is not absolutely required in this system. A similar result has been obtained in the CREB model system (49).

In addition, we also show that CBP and p67^{SRF} form a complex in live cells, since the two proteins can be co-immunoprecipitated from cell extracts. The interaction between p67^{SRF} and CBP does not require that the transcription factor be bound to its target DNA sequence, contrary to what has been observed with MyoD (43). The physical interaction between p67^{SRF} and CBP is detected by co-immunoprecipitation assays, which require a high affinity between the proteins, suggesting that this interaction is strong. The interaction between the N-terminal part of CBP and p67^{SRF} was confirmed in a two-hybrid assay in mammalian cells. However, analysis of various subregions of the N-terminal CBP did not allow us to determine more precisely the site of interaction in CBP. This suggests that CBP and p67^{SRF} do not interact through the previously characterized domains of interaction (amino acids 1–101 for the glucocorticoid receptor; amino acid 461–661 for various transcription factors). A possibility is that two physically separate sequences are required for this interaction. p67^{SRF} is not the only member of the MADS box family which is able to interact with CBP. Indeed, CBP is also able to contact, through an undetermined region, MEF-2, a member of this family of proteins that is involved in muscle cell differentiation (43, 62).

For SRE transactivation, p67^{SRF} cooperates with p62^{TCF}. Both p67^{SRF} (this study) and p62^{TCF} (26) physically interact with CBP. Thus, transactivation through the SRE might involve a multimolecular complex including CBP, p67^{SRF}, and p62^{TCF}, stabilized by multiple interactions between the partners in the complex. Interestingly, p67^{SRF} is also involved in other processes such as muscle-cell terminal differentiation (63). Indeed, p67^{SRF} cooperates with the myogenic differentiation factors MyoD and myogenin for some muscle promoters' transactivation (64) and is able to interact physically with these myogenic bHLHs (65). CBP interacts both with MyoD (43, 62) and p67^{SRF} (this study). Thus, the cooperation between MyoD and p67^{SRF} on muscle cell differentiation also might involve the cooperative recruitment of CBP resulting in the formation, on muscle promoters, of a multimolecular complex

including CBP, p67^{SRF} and MyoD, and which would be stabilized by multiple interactions between the various partners of the complex.

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The CREB-binding Protein (CBP) Cooperates with the Serum Response Factor for Transactivation of the c-*fos* Serum Response Element

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Dr. Ait-Si-Ali's name was printed incorrectly. The correct version is shown above.