CBP Alleviates the Intramolecular Inhibition of ATF-2 Function*

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Yuji Sano‡‡§, Fumino Tokitou‡‡§, Ping Dai‡, Toshio Maekawa‡, Tadashi Yamamoto§, and Shunsuke Ishii‡¶

From the ¶Laboratory of Molecular Genetics, Tsukuba Life Science Center, RIKEN, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, the §Department of Oncology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-0071, and the ¶Institute of Medical Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-0066, Japan

The transcription factor ATF-2 (also called CRE-BP1), whose DNA-binding domain consists of a basic amino acid cluster and a leucine zipper (b-ZIP) region, binds to the cAMP response element as a homodimer or as a heterodimer with c-Jun. The amino-terminal region of ATF-2 containing the transcriptional activation domain is phosphorylated by stress-activated kinases, which leads to activation of ATF-2. We report here that CBP, which was originally identified as a co-activator of CREB, directly binds to the b-ZIP region of ATF-2 via a Cys/His-rich region termed C/H2, and potentiates trans-activation by ATF-2. The b-ZIP region of ATF-2 was previously shown to interact with the amino-terminal region intramolecularly and to inhibit trans-activating capacity. The binding of CBP to the b-ZIP region abrogates this intramolecular interaction. The adenovirus 13S E1A protein which binds to the b-ZIP region of ATF-2 also inhibited this intramolecular interaction, suggesting that both CBP and 13S E1A share a similar function as positive regulators of ATF-2. We found that the b-ZIP regions of c-Jun and CREB also interact with the C/H2 domain of CBP, suggesting that CBP acts as a regulator for a group of b-ZIP-containing proteins. These results shed light on a novel aspect of CBP function as a regulator for a group of b-ZIP-containing proteins.

So far, a number of transcription factors of the ATF/CREB family have been identified. All members of this family contain a DNA-binding domain consisting of a cluster of basic amino acids and a leucine zipper region, the so-called b-ZIP. They form homodimers or heterodimers through the leucine zipper and bind to the cAMP response element (CRE). Among many of the transcription factors of the ATF/CREB family, three factors, ATF-2 (also called CRE-BP1), ATF-a, and CRE-BPa, form a subgroup (2–5). A common characteristic of this group of factors is the presence of a transcriptional activation domain containing the metal finger structure located in the amino-terminal region (5, 6). These factors are capable of forming homodimers or heterodimers with c-Jun and bind to CRE (1, 5, 6). Among these three factors, ATF-2 has been more extensively studied and shown to be ubiquitously expressed with the highest level of expression being observed in the brain (7). The mutant mice generated by gene targeting exhibited decreased postnatal viability and growth with a defect in endochondrial ossification and a decreased number of cerebellar Purkinje cells (8). The stress-activated protein kinases (SAPK) such as Jun amino-terminal kinase and p38 phosphorylate this group of factors at sites close to the amino-terminal transcriptional activation domain, and stimulate their trans-activating capacity (9–11). Since a group of factors of the ATF/CREB family including CREB are activated via direct phosphorylation by cAMP-dependent protein kinase (PKA) (12), these two groups of factors, CREB and ATF-2, are linked to the distinct signaling cascades involving the PKA and SAPK pathways. The adenovirus 13S E1A activates CRE-dependent transcription, and this transcriptional activation is mediated by ATF-2 (13, 14). E1A binds to the b-ZIP region of ATF-2 (15). Recently, it was reported that the b-ZIP region of ATF-2 interacts with the amino-terminal region intramolecularly (16), and this interaction appears to inhibit the trans-activating capacity of ATF-2. However, the co-activator that binds to the amino-terminal activation domain remains unidentified, and the mechanism of transcriptional activation by ATF-2 needs to be clarified.

The transcriptional co-activator CBP was originally identified as a protein that binds to the PKA-phosphorylated form of CREB (17). CBP also binds to multiple components of the basal transcriptional machinery, including TFIIB (18) and the RNA polymerase II holoenzyme complex (19), suggesting that CBP serves as a molecular bridge between CREB and the basal transcriptional machinery. In addition to CREB, many other transcription factors including c-Jun (20), c-Fos (21), c-Myb (22), nuclear hormone receptors (23, 24), Stat2 (25), and MyoD (26) were recently demonstrated to bind to CBP (for review, see Ref. 27). CBP contributes to the transcriptional activation mediated by each of these factors. Although multiple transcription factors bind to CBP, there is a striking difference in the role of CBP depending on the transcriptional activator. For instance, CBP binds to the transcriptional activation domains of CREB and c-Myb (17, 22). In the case of nuclear hormone receptors, however, other co-activators bind to the transcriptional activation domain, and CBP binds to a different domain, indicating that CBP functions as an integral part of nuclear hormone receptors (23, 24).

The amount of CBP in mammalian cells appears to be limiting, as a 50% reduction in the amount of CBP causes abnormal pattern formation in human (known as Rubinstein-Taybi syndrome) (28) and mouse (29). Recent genetic analyses using Drosophila CBP mutants indicated that CBP is required for

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‡ To whom correspondence should be addressed: Laboratory of Molecular Genetics, Tsukuba Life Science Center, RIKEN, 3-1-1 Koyadai, Tsukuba, Ibaraki 305, Japan. Tel.: 81-298-36-9031; Fax: 81-298-36-9030; E-mail: sishii@rtc.riken.go.jp.

§ The abbreviations used are: CRE, cAMP response element; HAT, histone acetyltransferase; PKA, cAMP-dependent protein kinase; SAPK, stress-activated kinase; PBS, phosphate-buffered saline; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; CAT, chloramphenicol acetyltransferase.
transcriptional activation by Cubitus interruptus (Ci) and Dorsal (Dl), which are homologs of mammalian factors gisbiasmota (GLI) and NF-кB, respectively (30, 31). These results suggest that the decreased expression level of target genes of these transcription factors such as Bmp and Twist lead to the deficiency in pattern formation.

In addition to the finding that CBP itself has histone acetyltransferase (HAT) activity (32, 33), CBP forms a complex with multiple HATs such as P/CAF, ACTR, and SRC-1 (34–36), suggesting that the CBP complex contributes to transcriptional activation by disrupting the repressive chromatin structure. The cbp gene family contains at least one other member, p300, that was originally identified through its ability to bind to the adenovirus E1A protein (37), and E1A binds to both CBP and p300 (38, 39). Binding of E1A to CBP inhibits transcriptional activation mediated by CBP. Since E1A and HAT P/CAB bind to the same region of CBP, the mechanism of inhibition of CBP activity by E1A was postulated to be due to blocking of P/CAB binding to CBP (34).

To investigate the possibility that CBP is also involved in transcriptional activation by ATF-2, we have examined for a direct interaction between CBP and ATF-2. Our results indicate that CBP functions as a regulator of ATF-2 by binding to its b-ZIP region.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—To express various forms of GST-CBP fusion proteins in *Escherichia coli*, the plasmids pGEX-KIX, pGEX-Bromo, pGEX-C/H2, and pGEX-C/H3 were made by inserting the appropriate fragment encoding the 265- (amino acids 454–718), 104- (amino acids 1087–1190), 437- (amino acids 1190–1626), and 257-amino acid (amino acids 1621–1877) regions of mouse CBP, respectively, into the appropriate site of the pGEX vector (see Ref. 40; Amersham Pharmacia Biotech). The plasmid to express the GST fusion protein containing the amine-terminal 255 amino acids of ATF-2 was constructed using the pGEX-2TK vector. The modified pSP65 vector pSPUTK (Stratagene) was used for in vitro transcription/translation of various forms of ATF-2. A series of mutants of ATF-2 was described previously (6). The plasmid to express CREB, c-Jun, or c-Fos, or E1A was replaced by the DNA-binding domain of c-Myb (amino acids 291–505), the plasmid to express the GST fusion protein containing the carboxyl-terminal region of ATF-2 (amino acids 1182–1500) was fused to the DNA-binding domain of Gal4 (amino acids 1–147) were constructed by the polymerase chain reaction-based method using the cytomegalovirus promoter-containing vector, pSTCX556 (41). The plasmid encoding the VP16 fusion protein containing the carboxyl-terminal region of ATF-2 and the plasmid to express CREB, c-Jun, or c-Fos were synthesized with [35S]methionine using an in vitro transcription/translation system was also made using pGEM (Promega), pBluescript, and a phDTP vector (Invitrogen), respectively. The plasmids to express Gal4-CBPC/H2 in which the C/H2 domain of CBP (amino acids 1182–1500) was fused to the DNA-binding domain of Gal4 (amino acids 1–147) were constructed by the polymerase chain reaction-based method using the cytomegalovirus promoter-containing vector, pSTCX556 (41). The plasmid encoding the VP16 fusion protein containing the amine-terminal 255 amino acids of ATF-2 was constructed using the pGEX-2TK vector. The modified pSP65 vector pSPUTK (Stratagene) was used for in vitro transcription/translation of various forms of ATF-2. A series of mutants of ATF-2 was described previously (6). The plasmid to express CREB, c-Jun, or c-Fos, or E1A was replaced by the DNA-binding domain of c-Myb (amino acids 291–505), the plasmid to express CREB, c-Jun, or c-Fos were synthesized with [35S]methionine using an in vitro transcription/translation system according to the procedures described by the supplier (Promega). Then, a sample from the reaction was mixed with 750 µl of binding buffer and the GST or GST-CBP affinity resin. After 2 h at 4°C, the resin was washed with 1 ml of binding buffer five times and mixed with SDS-sample buffer, and the bound proteins were released by boiling. The proteins were analyzed by SDS-PAGE followed by autoradiography. In the experiments to examine the effect of phosphorylation by PKA, 25 µl of lysate containing the in vitro translated CREB was mixed with 175 µl of the kinase buffer to give final concentrations of 20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 12 mM MgCl₂, and 2.5 mM ATP, and the mixture was incubated with or without 100 units of the catalytic subunit of bovine PKA (Sigma).
loration, cells were washed with PBS 8 h before preparation of cell lysates and irradiated (45 J/m²) for 15 s. All co-transfection experiments were repeated at least two times, and the difference between each set of experiments was no more than 20%. Typical results are shown in Fig. 4.

RESULTS

In Vitro Binding of CBP to ATF-2—To examine whether CBP directly binds to ATF-2, we first used the GST pull-down assay. The full-length form of mouse CBP synthesized using an *in vitro* transcription/translation system bound to the GST fusion protein containing full-length ATF-2 (data not shown). To narrow down the specific region in CBP responsible for interaction with ATF-2, a series of GST fusion proteins containing various parts of CBP were made and used for the GST pull-down assays (Fig. 1, A and B). The [35S]-ATF-2 protein was synthesized using an *in vitro* transcription/translation system and mixed with various GST-CBP resins. Approximately 51% of input ATF-2 bound to the GST fusion protein containing the Cys/His-rich region of CBP, termed the C/H2 domain, whereas other GST fusion proteins containing other regions of CBP and GST alone as a control did not interact with ATF-2 (less than 2% of input) (Fig. 1C).

To examine further which region of ATF-2 interacts with CBP, we used deletion mutants of ATF-2 for the binding assay (Fig. 2, A and B). We previously identified the amino-terminal region between amino acids 19 and 50, which contains a metal finger, as a transcriptional activation domain (6). The NT50 mutant lacking this activation domain still retained almost full capacity to bind to CBP. About 27 and 30% of the input wild type ATF-2 protein and NT50 bound to the GST-CBP resin, respectively. The two amino-truncated mutants, NT253 and NT341, lacking the amino-terminal 253 and 341 amino acids, respectively, also bound to CBP as efficiently as the wild type. These results indicate that CBP binds to the carboxy-terminal 164-amino acid region containing the b-ZIP region. The two mutants lacking the cluster of basic amino acids of the b-ZIP region, ΔBR and NT253ΔBR, failed to interact with CBP. In addition, the two mutants in which the third and fourth leucine in the b-ZIP region were changed to valine, L34V and NT253L34V, did not bind to CBP. The CT91 mutant lacking the carboxy-terminal 91 amino acid region bound to the GST-CBP resin, but its binding efficiency was significantly lower than that of wild type. These results indicate that CBP binds to the b-ZIP region of ATF-2 (amino acids 338–407) and that the region downstream from the b-ZIP region enhances the interaction with CBP.

In Vivo Interaction between CBP and ATF-2—To confirm the *in vivo* interaction between ATF-2 and CBP in mammalian cells, co-immunoprecipitation was performed (Fig. 3A). The two plasmids to express ATF-2 and CBP were transfected into 293T cells, and the cell lysates were immunoprecipitated with anti-CBP antibody or control antibody against anti-β-galactosidase. ATF-2 was co-immunoprecipitated with anti-CBP antibody but not with the anti-β-galactosidase antibody.

To confirm further the *in vivo* interaction between ATF-2 and CBP, we performed *in vivo* two-hybrid assays in mammalian cells (Fig. 3B). Two chimeric proteins were created by fusing the CBP fragment containing the C/H2 region in frame to the DNA-binding domain of Gal4 and by fusing the carboxy-terminal region of ATF-2 containing the b-ZIP to the transcriptional activation domain of VP16. Transcriptional activation was then examined in HepG2 cells transfected with a combination of these constructs. The basal activity is the luciferase activity obtained by a combination of Gal4 DNA-binding domain and VP-16. The VP16 protein fused to the carboxy-terminal region of ATF-2 stimulated Gal4-CBP activity 7.3-fold, whereas VP16 alone stimulated only 2.2-fold. Furthermore, VP16-ATF-2 enhanced the Gal4 activity only 1.7-fold. These results indicated that the C/H2 domain of CBP interact with the carboxy-terminal region of ATF-2 containing the b-ZIP structure.

Potentiation of ATF-2-dependent trans-Activation by CBP—To investigate the role of CBP in transcriptional activation by ATF-2, we performed some CAT co-transfection experiments using Chinese hamster ovary cells (Fig. 4). Since ATF-2 was expressed in all of the cell lines examined, it was difficult to analyze the transcriptional activation resulting from the exogenous ATF-2 expressed from the transfected DNA. Therefore, we used the fusion protein consisting of the c-myb gene product (c-Myb) and ATF-2 (14). c-Myb...
is a sequence-specific DNA-binding protein, which is predominantly expressed in immature hematopoietic cells but not in many other cells. Therefore, the transcriptional activation by the fusion protein consisting of the DNA-binding domain of c-Myb and full-length ATF-2 (MybDBD-ATF-2) can be analyzed without any interference from the endogenous protein. The plasmid pMFcolCAT6MBS-I, in which the CAT gene is linked to the mouse a2(I)-collagen promoter and six tandem repeats of the Myb-binding site MBS-I, was used as a reporter. Under the conditions used, CAT expression from this reporter plasmid was activated 3.5-fold by the MybDBD-ATF-2 fusion protein (compare lanes 1 and 4). Co-transfection of the CBP expression plasmid with the MybDBD-ATF-2 expression plasmids enhanced the level of CAT activity about 2.7-fold (cf. lanes 4 and 5). The E1A13S product also potentiated by 2.6-fold the trans-activation by MybDBD-ATF-2 as reported previously (cf. lanes 4 and 6), but additional enhancement of the trans-activating capacity of MybDBD-ATF-2 was not observed by co-expression of both CBP and E1A (lane 7). Thus, both E1A and CBP enhance the trans-activating capacity of ATF-2, but they cannot additionally stimulate ATF-2 activity.

To confirm further that CBP and E1A stimulate the ATF-2 activity via binding to the b-ZIP region of ATF-2, we constructed the fusion protein MybDBD-ATF-2-Gal4, in which the b-ZIP region was replaced by the DNA-binding domain of Gal4. This fusion protein enhanced the CAT expression from the pMFcolCAT6MBS-I reporter 3.1-fold. As expected, neither CBP nor E1A enhanced the trans-activating capacity of this fusion protein (Fig. 4, lanes 8–10). Although we expected that this fusion protein would have the higher trans-activating capacity...
than that of MybDBD-ATF-2 due to a lack of intramolecular association, the trans-activating capacity of this fusion was almost the same as that of MybDBD-ATF-2. This could be due to the lower protein stability of this fusion protein.

UV irradiation is known to lead to activation of SAPK which then phosphorylates ATF-2 at Thr-69, Thr-71, and Ser-90. This phosphorylation enhances the trans-activating capacity of ATF-2, possibly by enhancing the binding to a putative co-activator. By treating the transfected cells with UV, we performed similar co-transfection experiments to those described above (lanes 11–15). CBP and E1A stimulated the trans-activation by MybDBD-ATF-2 5.4- and 9.3-fold, respectively, after treatment of the cells with UV light (cf. Lanes 12–14). These results indicated that SAPK can enhance the ATF-2 activity additionally in the presence of CBP or E1A.

Inhibition of the Intramolecular Interaction of ATF-2 by CBP—The b-ZIP region of ATF-2 interacts with the amino-terminal region intramolecularly, and this interaction appears to inhibit the trans-activating capacity of ATF-2. Therefore, we speculated that CBP might block this intramolecular interaction by directly binding to the b-ZIP region of ATF-2. To examine this possibility, we investigated the effect of CBP on the intramolecular interaction between the amino- and carboxyl-terminal regions of ATF-2 (Fig. 5A). The carboxyl-terminal 165-amino acid region of ATF-2 (amino acids 341–505) synthesized in the in vitro translation system bound to the GST fusion protein containing the amino-terminal 253-amino acids region of ATF-2, and the in vitro translated [35S]ATF-2 mutant, which has the carboxyl-terminal 165-amino acid region containing the b-ZIP domain. To examine the effect of the CBP C/H2 domain on the intramolecular interaction of ATF-2, increasing amounts of in vitro translated CBP C/H2 were added. As a control, the in vitro translation reaction for CBP C/H2 was performed without T7 RNA polymerase. The relative amounts of carboxyl-terminal [35S]-ATF-2 bound to the GST-ATF-2 resin are plotted below.

CBP Binds to the b-ZIP of ATF-2

![Diagram](image1.png)

**Fig. 4.** Potentiation of ATF-2-activated gene expression by CBP. To examine the trans-activating capacity of ATF-2, the fusion protein consisting of the DNA-binding domain of c-Myb and the full length of ATF-2 protein was used. Many endogenous CRE-binding proteins affect the assay using the CRE-containing reporter but not using the Myb site containing reporter, because the level of c-Myb in the Chinese hamster ovary cells is very low. The structures of the MybDBD-ATF-2 and MybDND-ATF-2-Gal4 fusion proteins used are indicated. The structure of the pMFColCAT6MBS-I reporter plasmid is also shown. Chinese hamster ovary cells were transfected with a mixture of the CAT reporter plasmid containing Myb-binding sites pMFColCAT6MBS-I, the MybDBD-ATF-2 or MybDND-ATF-2-Gal4 expression plasmid, the CBP expression plasmid, the 13S E1A expression plasmid, or the control plasmid and the internal control plasmid p intact b-gal. CAT assays were performed, and the degree of trans-activation (compared with samples without any effector plasmid) was measured. Experiments were repeated three times, and the average degree of trans-activation is indicated by a bar graph with S.E. In lanes 11–15, the transfected cells were irradiated by UV to induce the phosphorylation of ATF-2 by SAPK. Enhancement of the trans-activating capacity of ATF-2 by CBP or E1A is shown by a solid bar graph.
PKA treatment enhanced 3-fold the binding of CREB-CBP interaction. c-Jun, but not with VP16 alone. To confirm further that CREB fusion was co-immunoprecipitated with VP16-CREB or VP16-immunoprecipitated with anti-VP16 antibody. The Gal4-C/H2 fusion with the C/H2 domain of CBP, and the cell lysates were immunoprecipitated with anti-Gal4 antibody. The Gal4-C/H2 fusion was co-immunoprecipitated with VP16-CREB or VP16-c-Jun, but not with VP16 alone. To confirm further that CREB binds to CBP via the amino-terminal region of ATF-2. To examine this possibility, we examined whether SAPK-phosphorylated ATF-2 preferentially binds to CBP (Fig. 7). The HepG2 cells transfected with the ATF-2 expression plasmid were treated by sorbinol which leads to activation of SAPK, and whole cell lysates were prepared. Lysates were incubated with the GST-CBP resins containing the KIX, bromo, or C/H2 domain of CBP, and the bound ATF-2 proteins were detected by anti-ATF-2 antibodies. ATP-2 bound to the C/H2 domain, and the binding efficiency for C/H2 was not affected by phosphorylation by SAPK. In addition, neither the unphosphorylated form nor the phosphorylated forms bound to the bromo or KIX domain. These results suggest that an uncharacterized co-activator other than CBP binds to the SAPK-phosphorylated transcriptional activation domain of ATF-2.

**Discussion**

Our results indicate that CBP stimulates transcriptional activation by ATF-2 via binding to the b-ZIP region of ATF-2. CBP abrogates the intramolecular interaction between the CBP C/H2 domain and the b-ZIP region of ATF-2 in a dose-dependent manner. Addition of 15 μl of CBP C/H2 inhibited by 66% the interaction between the amino- and carboxyl-terminal regions of ATF-2. As a control, the *in vitro* transcription/translation reaction for CBP C/H2 protein was performed without T7 RNA polymerase, and lysates were used as a competitor. These lysates did not affect the intramolecular interaction of ATF-2.

We also examined whether the E1A13S product could also inhibit the intramolecular interaction of ATF-2, like in the case of CBP (Fig. 5B). The *in vitro* translated E1A inhibited the binding of the carboxyl-terminal ATF-2 to the GST fusion containing the amino-terminal ATF-2 in a dose-dependent manner, whereas the control lysates synthesized without T7 RNA polymerase did not. Thus, both CBP and E1A block the intramolecular interaction between the amino- and carboxy-terminal regions of ATF-2 by interacting with the b-ZIP region.

**Interaction between CBP and Other b-ZIP-containing Proteins**—Our results described above indicated that CBP directly binds to the b-ZIP region of ATF-2 via the C/H2 domain of CBP. The b-ZIP domain is a common structure shared by members of the ATF/CREB and Jun/Fos family. Therefore, we examined whether the C/H2 domain of CBP could also interact with the b-ZIP region of other members of the ATF/CREB and Jun/Fos family. The *in vitro* translated CREB protein bound efficiently to the GST fusion containing the C/H2 domain (46% of the input), whereas *in vitro* translated c-Jun bound with low efficiency but still significantly (5% of the input) (Fig. 6A). In contrast, c-Fos failed to bind to the C/H2 domain of CBP. The smaller fragment containing the b-ZIP region of CREB or c-Jun efficiently bound to the GST-C/H2 fusion, but the c-Fos b-ZIP region did not. These results indicate that the b-ZIP region of some transcription factors binds to the C/H2 domain of CBP (Fig. 6B). To confirm the *in vivo* interaction between the C/H2 domain and the b-ZIP region of CREB or c-Jun, co-immunoprecipitation was performed (Fig. 6C). The plasmid to express VP16 fused to the b-ZIP region of CREB or c-Jun was co-transfected into 293 cells with the plasmid to express the Gal4-C/H2 domain and the b-ZIP region of CREB. The full-length CREB, c-Jun, and c-Fos were translated, and their binding to GST-CBP fusion protein containing the KIX domain or C/H2 domain was examined. These results suggest that CBP might also bind to the SAPK-phosphorylated transcriptional activation domain in the amino-terminal region of ATF-2. To investigate this possibility, we examined whether SAPK-phosphorylated ATF-2 preferentially binds to CBP (Fig. 7). The HepG2 cells transfected with the ATF-2 expression plasmid were treated by sorbinol which leads to activation of SAPK, and whole cell lysates were prepared. Lysates were incubated with the GST-CBP resins containing the KIX, bromo, or C/H2 domain of CBP, and the bound ATF-2 proteins were detected by anti-ATF-2 antibodies. ATP-2 bound to the C/H2 domain, and the binding efficiency for C/H2 was not affected by phosphorylation by SAPK. In addition, neither the unphosphorylated form nor the phosphorylated forms bound to the bromo or KIX domain. These results suggest that an uncharacterized co-activator other than CBP binds to the SAPK-phosphorylated transcriptional activation domain of ATF-2.
CBP Binds to the b-ZIP of ATF-2

The HepG2 cells transfected with the ATF-2 expression plasmid were treated with sorbitol (+), which leads to activation of SAPK, or PBS (−) as a control. Whole-cell lysates were prepared and mixed with the GST-CBP resin containing various portions of CBP as indicated above each lane. The bound proteins were eluted, separated on 10% SDS-PAGE, and analyzed by Western blotting using the anti-ATF-2 antibody (upper panel). In the lower panel, whole cell lysates were directly analyzed by Western blotting using the anti-ATF-2 antibody.

FIG. 7. Effect of SAPK-dependent phosphorylation of ATF-2 on binding to CBP. The HepG2 cells transfected with the ATF-2 expression plasmid were treated with sorbitol (+), which leads to activation of SAPK, or PBS(−) as a control. Whole-cell lysates were prepared and mixed with the GST-CBP resin containing various portions of CBP as indicated above each lane. The bound proteins were eluted, separated on 10% SDS-PAGE, and analyzed by Western blotting using the anti-ATF-2 antibody (upper panel). In the lower panel, whole cell lysates were directly analyzed by Western blotting using the anti-ATF-2 antibody.

FIG. 8. Schematic model for the role of CBP. In the absence of CBP or 13S E1A, the amino-terminal region of ATF-2 containing the activation domain intramolecularly interacts with the carboxyl-terminal b-ZIP region. When ATF-2 is bound to CBP or 13S E1A, the amino-terminal activation domain is exposed and able to interact with a co-activator.

Our results indicate that the b-ZIP region of ATF-2, c-Jun, and CREB functions not alone as a DNA-binding domain but also as an interaction domain for CBP. Probably the protein surface of the b-ZIP region which is exposed to the solvent serves for interaction with CBP. This is not surprising as there already exist a number of examples of DNA-binding domains that interact with specific proteins. For instance, E1A interacts with the DNA-binding domain of a number of transcription factors such as the b-ZIP of ATF-2, the metal fingers of Sp1, and the basic helix-loop-helix of upstream stimulatory factor (15). In addition, the DNA-binding domain of c-Myb is known to interact with HSF3 and Cyp40 (43, 50). Interestingly, the glucocorticoid receptor directly binds to the leucine zipper region of the c-Jun/c-Fos heterodimer and inhibits AP-1 activity (51, 52). The direct binding of glucocorticoid receptors to the b-ZIP region of c-Jun may inhibit the interaction between CBP and c-Jun. This mechanism may partly contribute to the inhibition of AP-1 activity by the glucocorticoid receptor.

E1A 13S protein stimulates CRE-dependent transcription through binding to the b-ZIP region of ATF-2. Since both the amino-terminal metal finger and the carboxyl-terminal b-ZIP region of ATF-2 are required for E1A-induced trans-activation (53), E1A cooperatively functions with an unidentified co-activator that binds to the amino-terminal transcriptional activation domain of ATF-2. The results of our co-transfection experiments indicate that E1A and CBP do not additionally stimulate transcriptional activation by ATF-2. This suggests that E1A and CBP cannot bind simultaneously to the b-ZIP region of ATF-2. E1A may drive out CBP from the b-ZIP region of ATF-2 and may replace its function as a regulator.

Recently, Liu and Hai (54) reported an interaction between ATF-4 and CBP via multiple domains. Both the amino-terminal and carboxyl-terminal regions of ATF-4 bind to the four regions of CBP as follows: the KIX domain, C/H3 domain, carboxyl-terminal Q-rich region, and the HAT domain. The carboxyl-terminal region of ATF-4 contains the b-ZIP domain and the HAT domain comprising the C/H2 region. Therefore, the interaction between the b-ZIP region and the C/H2 domain of CBP may also occur in the case of ATF-4. During the preparation of this manuscript, a report by Kawasaki et al. (55) showed that the trans-acting complex on the c-jun promoter contains p300 and ATF-2. The relatively broad region between the amino-terminal transcriptional activation domain and the b-ZIP region of ATF-2 (amino acids 112–350) was demonstrated to interact with the region containing both the bromo and C/H2 domains of CBP. However, our results indicate that the N-truncated mutant lacking the amino-terminal 341 amino acid region binds to CBP and that the two mutants of the b-ZIP region do not bind to CBP. In addition, not only ATF-2 but also the b-ZIP regions of other transcription factors including CREB and c-Jun were confirmed to bind to CBP. Furthermore, our
data indicate that the C/H2 domain of CBP is sufficient for interaction with ATF-2, and that the bromo domain is not required.

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