Identification of the Functional Domains of the Transcriptional Regulator CRE-BP1*

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We present evidence that CRE-BP1 binding to the cyclic AMP (cAMP) response element (CRE) is a transcriptional activator. Transcriptional activation was assayed by cotransfection into CV-1 cells of a CRE-BP1 expression plasmid together with a reporter plasmid in which the thymidine kinase promoter and four tandem repeats of CRE were linked to the chloramphenicol acetyltransferase (CAT) gene. Cotransfection with the CRE-BP1 expression plasmid caused an 8-fold stimulation of CAT activity, while cotransfection with the plasmids to express CRE-BP1 and c-Jun induced a 32-fold stimulation of CAT activity, suggesting that a heterodimer of CRE-BP1 with c-Jun is a stronger trans-activator than a homodimer of CRE-BP1. By using a series of deletion and point mutants of CRE-BP1 in this cotransfection assay, two functional domains of CRE-BP1 were identified: the putative metal finger structure in the amino-terminal region and the leucine zipper motif linked to a cluster of basic amino acids in the carboxyl-terminal region. The former was a transcriptional activation domain in the absence of c-Jun. The latter was a DNA-binding domain, and was essential in both the presence and absence of c-Jun.

In mammalian cells, increases in cyclic AMP (cAMP) inhibit or stimulate the growth of cells depending on the kind of cell (reviewed in Refs. 1 and 2). The cAMP response element (CRE) (TGACGTCA) was first identified as being an inducible enhancer of genes such as those of preproenkephalin and somatostatin, which can be transcribed in response to increased cAMP levels (3, 4). Some growth control genes such as the c-fos protooncogene also have CRE in their regulatory region and its expression is induced by increases in the intracellular cAMP levels. Our study, which examined the levels of transcription from different promoters with or without CRE in various cells, indicated that CRE had two kinds of enhancer activity, one of which is an inducible enhancer activity in response to increased cAMP levels, and the other is a constitutive enhancer activity, depending on cell type and promoter (5). These results suggested that mammalian cells have multiple transcriptional regulators bound to CRE.

In fact, multiple CRE-binding proteins were identified by cDNA cloning: CREB (6, 7), CRE-BP1 (8), ATF5 (9, 10), and TREBs (11). All of these proteins have a basic amino acid cluster linked to the leucine zipper as a DNA binding domain, indicating that these proteins bind to CRE as a homodimer or heterodimer as indicated in the case of c-Jun and c-Fos. Among multiple CRE-binding proteins, only the function of CREB was well analyzed. Phosphorylation of CREB occurs at Ser323 in response to an increase in the intracellular cAMP levels, and phosphorylation of Ser323 is critical to the transcription of the somatostatin gene by cAMP (12). Little is known about the function of other CRE-binding proteins.

The activating transcription factor (ATF) site, which is similar to CRE, in the adenovirus early gene promoter is required for activity by the E1A gene product of adenovirus. Since E1A does not appear to bind to a specific DNA sequence, some cellular protein(s) bound to CRE is suspected to mediate activation by E1A. Recently, ATF2/CRE-BP1 (the cDNAs of both proteins were independently isolated, and the sequences of the two proteins are identical except for two amino acid differences) was shown to mediate the E1A-induced trans-activation (13, 14). CRE-BP1 can bind to CRE as a homodimer or heterodimer with c-Jun (15, 16), and the level of its mRNA is fairly abundant in brain, especially hippocampus, and is induced in the regenerating liver (17). These results suggest that CRE-BP1 is important for both cellular proliferation and signal transduction in the brain. However, little is known about the function of CRE-BP1 in transcription regulation through CRE.

Here we report the transcriptional activation by CRE-BP1 and the identification of functional domains of CRE-BP1. Our results indicated that CRE-BP1 activated transcription through CRE as a homodimer or heterodimer with c-Jun. The DNA binding domain, consisting of a basic amino acid cluster linked to the leucine zipper, was essential for transcription by both a homodimer and a heterodimer, but the putative metal finger domain in the amino-terminal region appeared to be required only for the homodimer.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—The reporter plasmid pTKCAT4CRE contains four tandem repeats of CRE in the BamHI site of the plasmid pTKCAT, in which the bacterial chloramphenicol acetyltransferase (CAT) gene was linked to the thymidine kinase promoter of herpes simplex virus (see Fig. 1). The control reporter plasmid pTKCAT4mCRE contains four tandem repeats of mutated CRE. The sequences of the wild-type and mutant CRE, which was derived from CRE of the human somatostatin gene, were described previously (8). The effector plasmids pACT-CRE-BP1, in which the 5'-regulatory region of the chicken cytoplasmic β-actin gene was linked to the human
CRE-BP1 cDNA, and pactl, which contains the chicken β-actin promoter alone, have been described (14).

All plasmids designed to express mutant CRE-BP1 proteins (see Figs. 3B and 4A) in cultured cells were generated from the plasmid pact-CRE-BP1. The nucleic acid number +1 corresponds to the A of the initiation methionine codon and the amino acid number +1 corresponds to the amino-terminal methionine of the CRE-BP1 protein.

To construct the NT18 and NT50 mutants, an NcoI site was introduced at nucleotides 53 and 149, respectively, of the CRE-BP1 gene by the polymerase chain reaction (18). The introduction of this site caused a one-base mutation in the protein-coding region of each gene and the threonine at amino acid 51 into an alanine. The NcoI site generated those four tandem repeats of CRE linked to the thymidine kinase promoter of herpes simplex virus. In the effector plasmid pact-CRE-BP1, the CRE-BP1 cDNA was linked to the chicken β-actin promoter.

To construct the NT18 and NT50 mutants, an NcoI site was introduced at nucleotide 283, of the generated plasmid was replaced by the CRE-BP1 cDNA, and pactl, which contains the chicken β-actin promoter. This resulted in an in-frame deletion of the entire region of interest. To construct the ΔAR, Δ155/212, and CRE-BP2 mutants, the oligonucleotides containing sequences on either side of the amino acids to be deleted were synthesized and used. This resulted in an in-frame deletion of the entire region of interest. To construct the Δ156/270, Δ155/338, Δ271/358, and ΔBR mutants, two HpaI sites were generated by oligonucleotide-directed mutagenesis at both ends of the region to be deleted. Deletion of the HpaI fragment between these two HpaI site generated those four mutants. To make a Δ196/270 mutant, a HpaI site was generated at nucleotide 808 and the ScaI-HpaI fragment (nucleotides 586-808) was deleted. To generate CT91, CT48, and CT27 mutants, termination codons were introduced at nucleotides 1243, 1370, and 1433, respectively, by oligonucleotide-directed mutagenesis. To construct L34V, C27S, C32S, and C418S mutants, one or two bases at nucleotides 1180/1201, 95/96, and 1253 were changed, respectively, by oligonucleotide-directed mutagenesis. After the oligonucleotide-directed mutagenesis was done using single strand DNA; each mutation was transferred to the plasmid pact-CRE-BP1 by exchanging the appropriate DNA fragments.

**CAT Cotransfection Analysis**—A mixture of 2 μg of the reporter plasmid DNA pTKCAT4CRE or pTKCAT4mCRE, 5 μg of effector plasmid DNA, 1 μg of the internal control plasmid pact-β-gal (14), and 0.2 μg of pRL-CMV or pRSV-CMV (20) was transfected into the African green monkey kidney cells (CV-1) or Chinese hamster ovary cells (CHO-K1) as described (14). The plasmid RSV-c-Jun, in which the mouse c-Jun cDNA was linked to the long-term repeat of Rous sarcoma virus, was provided by Dr. S. Hirai (20). The control plasmid pRSV-MG was generated by joining the mouse β-globin gene with the long-terminal repeat of Rous sarcoma virus. 48 h after transfection, CAT assays were done for 1 h by the procedure of Gorman et al. (21). In some experiments, the concentration of serum was reduced to 0.2% after transfection until the CAT assay was done. The amounts of cell extract used for the CAT assays were normalized with β-galactosidase activity. The degree of conversion was measured by the density of the spot corresponding to either unreacted [14C]chloramphenicol or its acetylated forms in an autoradiogram using a Bioimage analyzer (Fuji Photo Film Co., Ltd.).

**RESULTS**

**Trans-activation by CRE-BP1-To examine the trans-activation capacity of CRE-BP1, cotransfection experiments were done. The plasmid pTKCAT4CRE, in which four tandem repeats of CRE and the thymidine kinase promoter of herpes simplex virus were linked to the CAT gene, was used as a reporter plasmid (Fig. 1). This was cotransfected into CV-1 cells with the effector plasmid to express CRE-BP1 (pact-CRE-BP1) or no protein (pactl). The level of CAT activity obtained with the CRE-BP1 expression plasmid was 8-fold higher than that with the control effector plasmid (Fig. 2, lanes 1 and 2).

The c-Jun protein can bind to the CRE sequence used here as a heterodimer with CRE-BP1, but the heterodimer has little affinity with the CRE-BP1 sequence.**

**FIG. 1. Structure of the reporter and effector plasmid.** The CAT reporter plasmid pTKCAT4CRE contains four tandem repeats of CRE linked to the thymidine kinase promoter of herpes simplex virus. In the effector plasmid pact-CRE-BP1, the CRE-BP1 cDNA was linked to the chicken β-actin promoter.

**FIG. 2. Trans-activation by CRE-BP1 and c-Jun through CRE.** Mixtures of the reporter plasmid indicated above, effector plasmid to express CRE-BP1 (+), c-Jun (+), or no protein (−), and the internal control plasmid pact-β-gal DNA were transfected into CV-1 cells, and CAT activation was assayed. On the right, CAT activity is indicated by the bar graph.

**RESULTS**

**Trans-activation by CRE-BP1-To examine the trans-activation capacity of CRE-BP1, cotransfection experiments were done. The plasmid pTKCAT4CRE, in which four tandem repeats of CRE and the thymidine kinase promoter of herpes simplex virus were linked to the CAT gene, was used as a reporter plasmid (Fig. 1). This was cotransfected into CV-1 cells with the effector plasmid to express CRE-BP1 (pact-CRE-BP1) or no protein (pactl). The level of CAT activity obtained with the CRE-BP1 expression plasmid was 8-fold higher than that with the control effector plasmid (Fig. 2, lanes 1 and 2).

The c-Jun protein can bind to the CRE sequence used here as a heterodimer with CRE-BP1, but the heterodimer has little affinity with the 12-O-tetradecanoylphorbol-13-acetate response element derived from that of the collagenase gene (15, 16). To examine the effect of c-Jun on the CRE-BP1-dependent trans-activation, we used a c-Jun expression plasmid in a cotransfection experiment. Cotransfection with the c-Jun expression plasmid stimulated CAT activity expressed from the reporter plasmid pTKCAT4CRE about 3-fold (Fig. 2, lanes 1 and 3), suggesting a heterodimer of the endogeneous CRE-BP1 with c-Jun protein expressed from the transfected DNA stimulated the CAT expression. Furthermore, cotransfection with two effector plasmids to express CRE-BP1 and c-Jun stimulated CAT activity 32-fold (Fig. 2, lanes 1 and 4). When the control reporter plasmid pTKCAT4 mCRE containing four tandem repeats of mutant CRE was used, the...
degree of stimulation of CAT activity by c-Jun or c-Jun and CRE-BP1 was much less than that with PTKCAT4CRE (Fig. 2, lanes 5, 7, and 8). These results suggested that CRE-BP1 is a trans-activator, and a heterodimer of CRE-BP1 with c-Jun has a stronger trans-activation capacity than a homodimer of CRE-BP1. Essentially the same results were obtained by using other cell lines such as CHO cells (data not shown).

Trans-activation by CRE-BP1 Deletion Mutants—To identify the functional domains of CRE-BP1, a series of deletion mutants were made and used for the cotransfection assay (Fig. 3B). The mutant cDNAs were linked to the chicken β-actin promoter to be expressed in cultured cells, and the trans-activation capacity of these mutants were examined using a cotransfection assay with the reporter plasmid pTKCAT4CRE as described above. In this assay, three different conditions were used: first, the CRE-BP1 expression plasmid alone was used as an effector plasmid; the second condition was the same as the first one except that the serum concentration was reduced to 0.2%; third, two plasmids to express CRE-BP1 or c-Jun were used as effector plasmids. We examined the trans-activation by CRE-BP1 under the second condition, since the level of endogenous trans-acting factor, which interacts with CRE-BP1, might be changed by reducing the serum concentration as reported in the case of c-Fos (22).

When we examined the trans-activation by a set of deletion mutants of CRE-BP1 under the first and second conditions, the results were the same and not affected by the serum concentration. Results obtained with four amino-terminal truncated mutants (NT18, NT50, NT107, and NT253) indicated that the region between amino acids 19 and 50 was required, because the trans-activation capacity of NT18 was almost the same as the wild type but the activity of NT50 was completely lost.

To examine any regions downstream of the acidic region (amino acids 124–148) is required for trans-activation, six internal deletion mutants (ΔAR, Δ155/212, Δ155/270, Δ155/338, Δ271/338, and Δ196/270) were made and used for cotransfection assays. The ΔAR mutant lacking the acidic region had almost the same stimulation activity as the wild-type CRE-BP1, indicating that this acidic region is not a transcriptional activation domain. The other four internal deletion mutants (Δ155/212, Δ155/270, Δ155/338, and Δ271/338) also had the same stimulation activity as the wild-type protein. The Δ196/270 mutant had a reduced stimulation activity, but the Δ155/270 mutant, which lacks the region covering the deleted portion in the Δ196/270 mutant, had the same stimulation activity as the wild-type CRE-BP1. Therefore, the change of conformation or protein stability induced by deletion of amino acids 196–270 may cause a small decrease of activity. Recently, the CRE-BP2 protein, which was produced from the CRE-BP1 gene by an alternative splicing, was identified by cDNA cloning (15). CRE-BP2 lacks the region between amino acids 151 and 246 of CRE-BP1. We made a plasmid to express CRE-BP2 and examined the trans-activation by CRE-BP2. CRE-BP2 stimulated CAT activity in our assay, but its stimulatory activity was lower than the wild-type protein, consistent with the data from the six internal deletion mutants described above.

To analyze the functional domain(s) in the carboxyl-terminal region, four deletion mutants (ΔBR, CT91, CT48, and CT27) were constructed and used for cotransfection assays. The ΔBR mutant lacking the cluster of basic amino acids, which is linked to the leucine zipper motif and is thought to be a DNA binding domain, had no transcriptional stimulatory activity. Among the three carboxyl-terminal truncated mutants, two mutants, CT27 and CT48, had the almost same stimulatory activity as the wild-type CRE-BP1, and CT91 was less active. These results indicated that two regions in CRE-BP1, the amino-terminal portion (amino acids 19–50) and the basic region in the DNA binding domain, are essential for transcriptional activation in the cotransfection assay using the CRE-BP1 expression plasmid alone as a effector plasmid.

We also examined the trans-activation by a series of deletion mutants of CRE-BP1 under the third condition in which two plasmids to express CRE-BP1 or c-Jun were used as effector plasmids. The results obtained under this condition were similar to those described above, except for the case of the NT50 and NT107 mutants. These two mutants had obvious transcriptional stimulatory activity in the presence of the c-Jun expression plasmid, although they had no activity.
without the c-Jun expression plasmid.

**Trans-activation by CRE-BP1 Point Mutants**—The cotransfection assay using deletion mutants of CRE-BP1 indicated that the amino-terminal region between amino acids 19 and 50 was essential for trans-activation. This region contains the putative metal finger structure consisting of 2 cysteine and 2 histidine residues. To examine whether this putative metal finger structure is required for trans-activation, a single amino acid mutation was introduced into the cysteine residue, and the generated point mutant was used for cotransfection assay. The C27S and C32S, in which the cysteine residues at amino acids 27 and 32 were replaced by serine residues, respectively, had no transcriptional stimulatory activity in a cotransfection assay without the c-Jun expression plasmid (Fig. 4). In contrast, transcriptional activation with C32S was almost the same as that with normal CRE-BP1 in a cotransfection assay in the presence of the c-Jun expression plasmid. These results indicate that the putative metal finger structure in CRE-BP1 is essential for transcriptional activation, but not in the presence of an excess of c-Jun.

CRE-BP1 forms a homodimer or a heterodimer with c-Jun through its leucine zipper domain (15, 16). To investigate whether this leucine zipper structure is required for trans-activation, the mutation was introduced into the leucine residues of this region by site-directed mutagenesis. The L34V mutant, in which the third and fourth leucine residues were replaced by valine residues, had no transcriptional stimulatory activity in a cotransfection assay without the c-Jun expression plasmid (Fig. 4). However, L34V had a low but significant activity in a cotransfection assay with the c-Jun expression plasmid.

CT91 had a lower transcriptional stimulatory activity than normal CRE-BP1 and CT48 in a cotransfection assay without the c-Jun expression plasmid (Fig. 3), suggesting that the region between the carboxyl-terminal ends of CT91 and CT48 (amino acids 415-458) affects on the activity of CRE-BP1. Based on the idea that the cysteine residues in this region might be important to maintain the conformation of this region, we introduced a mutation into the cysteine residue at amino acid 418. The generated mutant C418S, in which the cysteine residue at amino acid 418 was replaced by a serine residue, was used for a cotransfection assay. When the serum concentration was 10%, C418S had the same level of transcriptional stimulatory activity as normal CRE-BP1 in cotransfection with or without the c-Jun expression plasmid (Fig. 4). However, C418 had no activity when the serum concentration was decreased to 0.2% after transfection. Thus, the region immediately downstream of the leucine zipper structure appears to be important for trans-activation under specific conditions such as a low concentration of serum.

**Immunodetection of CRE-BP1 Mutant Proteins**—To confirm that the results of the CAT assays described above reflect differences in the activity of CRE-BP1 mutants, and not variations in the level of each mutant protein, the levels of some mutant proteins after transfection were examined. To transfet the plasmid DNA to express mutants, we used CHO cells, since the efficiency of transfection into CHO cells was high and the results of CAT assay obtained using CHO cells were the same as those with CV-1 cells. The deletion mutant proteins, which had no or lower transcriptional stimulatory activity than normal CRE-BP1, were expressed in CHO cells, and separated by 10% SDS-polyacrylamide gel electrophoresis. After a transfer of proteins to a nitrocellulose filter, the proteins were detected by the rabbit anti-CRE-BP1 polyclonal antibodies (Fig. 5). When the control plasmid to express no protein was transfected, only endogenous CRE-BP1 was detected (Fig. 5, lane 1, for pAct1). The density of the band corresponding to the wild-type CRE-BP1 protein was increased about 2-3-fold when the plasmid to express normal CRE-BP1 was transfected (Fig. 5, lanes 2 for wild type), indicating that significant amount of normal CRE-BP1 was expressed after transfection. When the plasmid to express NT50, NT107, or ΔBR was transfected, those mutant proteins were expressed at steady-state levels equal to or greater than that of the wild-type CRE-BP1 and CT48 in a cotransfection assay without the c-Jun expression plasmid (Fig. 3), suggesting that the region between the carboxyl-terminal ends of CT91 and CT48 (amino acids 415-458) affects on the activity of CRE-BP1. Based on the idea that the cysteine residues in this region might be important to maintain the conformation of this region, we introduced a mutation into the cysteine residue at amino acid 418. The generated mutant C418S, in which the cysteine residue at amino acid 418 was replaced by a serine residue, was used for a cotransfection assay. When the serum concentration was decreased to 0.2% after transfection. Thus, the region immediately downstream of the leucine zipper structure appears to be important for trans-activation under specific conditions such as a low concentration of serum.

**Immunodetection of CRE-BP1 mutant protein in transfected cells.** A mixture of the effector DNA to express the CRE-BP1 proteins and the internal control plasmid pact-β-gal DNA was transfected, and the cells were harvested and lysed in SDS-sample buffer. The serum concentration was 10% or 0.2% as shown above. The proteins were separated by 5% SDS-polyacrylamide gel electrophoresis and then transferred to a nitrocellulose filter. The proteins were detected by anti-CRE-BP1 polyclonal antibodies. The arrow indicates the endogenous CRE-BP1.

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**Fig. 4. Trans-activation by CRE-BP1 point mutants.** A, a summary of CAT cotransfection analysis. CRE-BP1 mutations were schematically represented. CRE-BP1 amino acids 27 to 49, 380 to 408, and 415 to 432 are listed in the single letter amino acid code. The leucines of the zipper are numbered 1-5 in the amino-to-carboxyl-terminal direction. The results of trans-activation shown in B are indicated on the right as in Fig. 3B. B, transient expression of CAT activity. CAT cotransfection assays were done as described in the legend to Fig. 3. The results shown in the column indicated by −serum was obtained when the serum concentration was decreased to 0.2%.

**Fig. 5. Immunodetection of CRE-BP1 mutant protein in transfected cells.** A mixture of the effector DNA to express the CRE-BP1 proteins and the internal control plasmid pact-β-gal DNA was transfected, and the cells were harvested and lysed in SDS-sample buffer. The serum concentration was 10% or 0.2% as shown above. The proteins were separated by 5% SDS-polyacrylamide gel electrophoresis and then transferred to a nitrocellulose filter. The proteins were detected by anti-CRE-BP1 polyclonal antibodies. The arrow indicates the endogenous CRE-BP1.
type protein. The CT91 mutant had a lower transcriptional stimulatory activity than wild-type when the serum concentration was reduced. However, the CT91 mutant protein was expressed at the level greater than that of wild-type protein under this condition. These results indicate that the failure or decrease of mutant NT50, NT107, ßBR, and CT91 to trans-activate was not due to a lack of protein production or stability.

**DISCUSSION**

We demonstrated the trans-activation by CRE-BP1 in a cotransfection assay using the reporter plasmid pTKCAT4CRE. The level of CAT activity from this reporter plasmid was also activated slightly by cotransfection with the c-Jun expression plasmid. We observed that a heterodimer of c-Jun with CRE-BP1 bound to the sequence of CRE used here, but neither a homodimer of c-Jun nor a heterodimer of c-Jun with c-Fos bound (data not shown). Therefore, the c-Jun-induced trans-activation may be caused by a heterodimer of c-Jun with endogenous CRE-BP1. Interestingly, activation by both activators, CRE-BP1 and c-Jun, was greater than the sum of the effects of each working alone. This synergy between CRE-BP1 and c-Jun suggests that a heterodimer of CRE-BP1 with c-Jun is a stronger transcriptional activator than a homodimer of c-Jun. Although CRE-BP1 stimulated CAT activity from the reporter plasmid containing four tandem repeats of CRE, the trans-activation by CRE-BP1 was not observed when the reporter plasmid containing only one CRE was used (data not shown). This is consistent with the observation by other groups that CRE-BP1 had no transcriptional stimulatory activity (13). These results suggest to us that the target gene whose expression is modulated by CRE-BP1 has multiple CREs, not a single CRE. When we used the natural promoter containing CRE as a reporter in a cotransfection assay, CRE-BP1 stimulated the human somatostatin and adenovirus E4 promoter activity significantly. However, the degree of activation of those promoters were not so high, i.e. about 3-fold (data not shown).

Among multiple CRE-binding proteins identified so far, only CREB was demonstrated to be regulated by a cAMP-dependent kinase (12) and possibly a calcium/calmodulin-dependent kinase (23). Phosphorylation of CREB at Ser'33 was proposed to alter the tertiary structure of CREB and thereby render other regions accessible for the interaction with proteins in the RNA polymerase II complex (24, 25). In contrast, the transcriptional stimulatory activity of CRE-BP1 was not affected by an increase of the intracellular cAMP levels under the condition in which the activity of CREB was induced by forskolin (data not shown). In spite of that, we observed the efficient phosphorylation of CRE-BP1 by a cAMP-dependent kinase and protein kinase C in vitro (data not shown). Therefore, it is the next important point to examine whether the function of CRE-BP1 is not modulated by these kinases or modulated under some conditions such as in a specific type of cells.

As a domain required for the trans-activation, we identified the putative metal finger structure in addition to the DNA binding domain comprising a cluster of basic amino acids and the leucine zipper structure in CRE-BP1. Neither the deletion (NT50 and NT107) nor point mutants (C27S and C32S) of the putative metal finger structure had a transcriptional stimulatory activity. The level of the deletion mutant proteins was equal to or greater than that of the wild-type protein. Furthermore, we confirmed that the introduction of a point mutation at Cys'27 or Cys'22 did not affect its stability by checking that the level of fusion protein between the c-Myc DNA binding domain and C27S or C32S was almost the same as that of fusion protein with normal CRE-BP1 (data not shown). Thus, the metal finger structure is essential for the trans-activation and appeared to be a transcriptional activation domain. However, the fusion protein consisting of this metal finger domain, and the DNA binding domain of other proteins such as c-Myb had no transcriptional stimulatory activity (14). Therefore, a coactivator, which is supposed to connect CRE-BP1 with the RNA polymerase II complex, may interact with two metal finger domains of a CRE-BP1 homodimer. The metal finger domain of CRE-BP1 was not essential in the presence of an excess of c-Jun, suggesting that a transcriptional activation domain in Jun acts for the trans-activation by a heterodimer of CRE-BP1 with c-Jun.

CRE-BP1 mediates the E1A-induced trans-activation (13, 14). To mediate the E1A inducibility, both the putative metal finger and leucine zipper structure in CRE-BP1 are essential, suggesting that E1A interacts with the metal finger structure of CRE-BP1. Thus, the putative metal finger structure is required for both the trans-activation by a CRE-BP1 homodimer and the E1A-induced trans-activation. Therefore, the trans-activation by a CRE-BP1 homodimer could be caused by a cellular E1A-like protein.

The L34V mutant, in which the third and fourth leucine residues in the leucine zipper domain of CRE-BP1 were replaced by valine residues, had no transcriptional stimulatory activity without the c-Jun expression plasmid, but had a low but significant activity in the presence of the c-Jun expression plasmid. A similar observation was also reported: the replacement of the fourth and fifth leucine residues of c-Jun by valine residues reduced complex formation with Fos, but not completely, and a significant complex formation was observed (26). In fact, a gel shift analysis by using L34V and c-Jun indicated that a L34V homodimer had no DNA binding activity, but a L34V/c-Jun heterodimer still had a low but detectable DNA-binding activity (data not shown). These results suggest that CRE-BP1 homodimer and CREBP-1/c-Jun heterodimer are active forms for trans-activation in the absence and presence of the c-Jun expression plasmid, respectively.

**REFERENCES**
