CREB-binding Protein Activates Transcription through Multiple Domains*

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CREB-binding protein (CBP) functions as a coactivator molecule for a number of transcription factors including CREB, c-Fos, c-Jun, c-Myb, and several nuclear receptors. Although binding sites for these factors within CBP have been identified, the regions of CBP responsible for transcriptional activation are unknown. In this report, we show that the N-terminal half of CBP is sufficient for activation of CREB-mediated transcription and that this region contains a strong transcriptional activation domain (TAD). Both deletion of this TAD or sequestering of factors that the TAD binds using a squelching assay were found to greatly decrease the ability of CBP to activate CREB-mediated transcription. In vivo studies by others have shown that p300/CBP associates with TBP; using an in vitro approach, we show the N-terminal TAD binds TBP. We also examined the ability of the C terminus of CBP to activate transcription using GAL-CBP chimeras. With this approach, we identified two C-terminal TADs located adjacent to the c-Fos binding site. In previous studies, cAMP-dependent protein kinase A (PKA) increased the transcriptional activity of a GAL full-length CBP chimera in F9 cells, and of the C terminus in PC-12 cells. Here, we demonstrate that PKA also increased the ability of the N-terminal TADs of CBP to activate transcription in PC-12 but not F9 or COS-7 cells, suggesting that this PKA-responsiveness is cell type-specific.

Cyclic AMP induces transcription of a number of genes through activation of the members of the cAMP response element-binding protein (CREB)1 family of transcription factors (1, 2), which interact with a conserved promoter element termed the CRE (cAMP response element) (3–6). CREB activates basal transcription through several domains that bind basal transcription factors (TFIIB, TATA-binding protein (TBP), and TAFII110) (7, 8) and activates cAMP-responsive transcription through a separate region termed the kinase-inducible domain (9). Serine 133 within the kinase-inducible domain is phosphorylated by a number of kinases including cyclic AMP-dependent protein kinase (PKA), and this phosphorylation promotes interaction of CREB with a second nuclear factor, CREB-binding protein (CBP) (10). This interaction is critical for activation of transcription, since antibodies that block formation of a CBP-CREB complex prevent CAMP-responsive transcription (11). In contrast, increasing the amount of CBP available to interact with CREB enhances PKA-activated CREB-mediated transcription by up to 6-fold (12). CBP fused to a heterologous DNA binding domain can directly activate transcription, suggesting that PKA-phosphorylated CREB provides a scaffold for recruitment of CBP that activates transcription (10).

While PKA phosphorylation of CREB is required for association of CREB with CBP, several studies indicate it is not sufficient for activation of transcription (13). This requires additional PKA-mediated events, which may include modification of the activity of CBP, since PKA enhances the ability of a GAL4-CBP chimera to activate transcription (10). The ability of CREB-CBP complexes to activate transcription is also regulated by phosphorylation of CREB. Phosphorylation of CREB on serine 142 by calcium/calmodulin kinase II inhibits the ability of CREB to activate transcription. Mutations that mimic this phosphorylation block the ability of CREB to activate transcription without affecting binding to CBP (13).

CBP binds to several different transcription factors in addition to CREB. CBP activates c-Fos-mediated transcription using the c-Fos binding site located within the C terminus of CBP (amino acids 1621–1877) (14). The adenoviral protein EIA also binds to the C-terminal end of CBP, acting to repress both CREB- and c-Fos-mediated transcription (15, 16). Several studies have indicated CBP interacts with basal transcription molecules. Co-precipitation studies indicated that TBP forms complexes with CBP/p300; however, no conclusion could be drawn about the nature of this interaction, e.g. direct or through additional associated proteins (17). Co-precipitation studies indicate that CBP associates with RNA polymerase II through an indirect mechanism (18), and in vitro studies indicate that TFIIB binds directly to the C-terminal end of CBP (12).

Previous structural studies have shown that both full-length CBP and a C-terminal form (amino acids 1667–2441) can activate transcription when fused to the DNA binding domain of GAL4. However, these studies have not precisely localized regions of CBP that are involved in the activation of transcription, nor have they localized regions of CBP that might be specifically involved in either CREB-mediated or c-Fos-mediated transcription.

In the study presented, we asked which regions of CBP are required specifically for CREB-mediated transcription. Plasmids expressing CBP deletion mutants were transfected into undifferentiated F9 cells, and the loss of function was used to identify regions required for CREB-mediated transcription. By this approach, we demonstrated the N-terminal half of CBP is sufficient to activate CREB-mediated transcription. To more precisely localize regions within CBP that mediate this activity and that function as transcriptional activation domains...
(TADs), we constructed a series of GAL-CBP chimeras spanning the length of CBP and tested them for the ability to directly activate transcription from a GAL-CAT reporter plasmid. By this approach, we showed that CBP contains several TADs: one strong and one weak TAD in the N terminus, adjacent to the CREB binding domain; and two weak TADs in the C terminus, adjacent to the C-Fos binding domain. Deletion of the strong N-terminal TAD was found to have greatly reduced the ability of CBP to activate CREB-mediated transcription. Consistent with these studies, co-expression of mutant CBP protein encoding only the N-terminal 460 amino acids of CBP resulted in a complete squelching of the ability of full-length CBP to activate transcription. PKA treatment was found to enhance the transcriptional activity of the N terminus of CBP 17-fold, suggesting PKA activation of CBP occurs in part through modulation of the activity of this domain. TBP has been previously demonstrated to interact with CBP/p900 in vivo. We demonstrated here that the strong N-terminal TAD binds to TBP in vitro, suggesting a means by which CBP can activate transcription.

MATERIALS AND METHODS

Construction of Plasmids Encoding GAL Chimeras and CBP Deletion Mutants—The pGAL-(1–147) plasmid encoding the DNA binding domain of the yeast protein GAL4 (19) was digested with HindIII and XbaI, and the DNA fragment encoding GAL4 amino acids 1–147 subcloned into the plasmid Rc/RSV (Invitrogen). This plasmid, Rc/RSV GAL-(1–147), was used as a basis to construct the remaining chimeric plasmids. GAL-(1–147) was digested with an EcoRI fragment (nucleotides 5032–7326) of the CBP cDNA into the same site in pGem7. This fragment was excised as XhoI-BamHI fragments and subcloned into the same sites within Rc/RSV GAL-(1–147). The pGAL-CBP-(721–1679) chimera was made by ligating linkers (5'-GCACTAGTCAG-3', 5'-AATTTCGACTGTCG-3') to the EcoRI fragment of the CBP cDNA (nucleotides 2161–5037). Following addition of the linkers, the fragment was digested with SpeI and subcloned into the XbaI site of Rs/RV GAL-(1–147). The pGAL plasmids pGAL-CBP-(1–460) (nucleotides 1–1380), pGAL-CBP-(1–232) (nucleotides 1–696), pGAL-CBP-(227–460) (nucleotides 679–1380), pGAL-CBP-(721–1332) (nucleotides 2161–3996), pGAL-CBP-(1333–1679) (nucleotides 3997–5037), pGAL-CBP-(1548–1679) (nucleotides 4642–5037), pGAL-CBP-(1678–2441) (nucleotides 5530–5868), pGAL-CBP-(1844–1956) (nucleotides 5530–5868), pGAL-CBP-(1961–2039) (nucleotides 5881–6117); pGAL-CBP-(2060–2179) (nucleotides 6178–6537); pGAL-CBP-(2173–2288) (nucleotides 6517–6864); and pGAL-CBP-(2282–2441) (nucleotide 6844–7326) were made by PCR using Vent™ polymerase (New England Biolabs) to generate CBP DNA fragments containing 5' and 3' XhoI sites and subcloned into this same site within Rc/RSV GAL-(1–147). The pGAL-CAT reporter was the pGAL/E1B TATA plasmid described in Ref. 20. The pGAL-VP16 chimera was a gift of R. Maurer, Oregon Health Sciences University, Portland, OR.

The plasmid pCBP-HA (16) was either digested with XhoI or HindIII and KpnI and the DNA fragment encoding amino acids 1099–2441 or 1–826 were gel purified. To generate plasmids encoding the CBP-CBP-(1–1332), CBP-(1–1956), and CBP-(1–2179) deletion mutants, DNA fragments encoding the additional amino acids were generated by PCR and subcloned into the XhoI-digested pCBP-HA. To generate pCBP-(227–460), PCR was used both to generate a DNA fragment encoding CBP amino acids 1–271 which contained a 5' HindIII site and a 3' KpnI site, and to generate a DNA fragment encoding CBP amino acids 461–826 with a 5' and 3' KpnI sites. To generate pCBP-(227–460), PCR was used to generate a DNA fragment encoding a 5'-methionine and CBP amino acids 461–826 which contained a HindIII site and a 3' KpnI site. Following restriction digestion, these DNA fragments were subcloned into HindIII/KpnI-digested pCBP-HA. To generate pCBP-(1–460), PCR was used to generate a DNA fragment encoding amino acids 1–460 of CBP which contained a HindIII site and a 3' XhoI site. This fragment was subcloned into the same site within the plasmid pDNA3 (Invitrogen).

Cell Culture—COS-7 cells and undifferentiated murine F9 teratocarcinoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 100 mg/ml penicillin and 100 mg/ml streptomycin. PC-12 cells were maintained in DMEM supplemented with 10% horse serum, 5% fetal bovine serum, and penicillin/streptomycin as above.

RESULTS

CBP Transcriptional Activation Domains

The N-terminal Half of CBP Is Sufficient to Activate CREB-mediated Transcription—In order to identify domains within CBP involved in activating CREB-mediated transcription, we constructed a series of mutants of CBP in which various portions of the C-terminal end have been removed (Fig. 1A) and tested these for loss of function in the F9 cell assay (Fig. 1B) described (12). As previously reported, co-translation of plasmids expressing exogenous CREB and PKA stimulated transcription from the SS-CAT reporter gene 15-fold over the basal level;
co-transfection of plasmids encoding CREB, PKA, and CBP stimulated transcription 80–90 fold over basal level (Fig. 1B). When we substituted the CBP deletion mutants for full-length CBP, none of the ability of CBP to activate CREB-mediated transcription was lost even when the entire C-terminal half of CBP was deleted. This indicates that the first 1098 amino acids of CBP contain sufficient information to fully enhance PKA-activated CREB-mediated transcription.

Characterization of an N-terminal Transcriptional Activation Domain of CBP—To localize regions in the N-terminal end of CBP that participate in enhancement of CREB-mediated transcription, we constructed two GAL4 DNA-binding domain-CBP chimeras: GAL-CBP-(1–460) and GAL-CBP-(721–1679) (Fig. 2A). Neither of these chimeras overlap the CREB-binding domain within CBP (460–661, Ref. 10), eliminating the possibility that they could recruit CREB to the promoter and activate transcription in that manner. Prior to determining the relative ability of the chimeras to activate transcription, the level of expression of each chimeric protein was measured by immunoprecipitation using an antibody raised against pGAL-(1–147) (Fig. 2B). Compared to the expression of GAL-CBP-(1–460), the other chimeras were expressed at the following levels: GAL-CBP-(721–1679) (0.01-fold) and GAL-(1–147) (4.7-fold). The relative transcription activity, as discussed below, was determined by dividing the observe CAT enzymatic activity by the relative level of protein expressed. The high level of expression observed with the smaller GAL-CBP chimeras (e.g. GAL-(1–147) and GAL-CBP-(1–460)) suggested these proteins may be expressed at levels beyond which the transcriptional machinery is saturated. Thus, the observed transcriptional activity may not be directly proportional to the amount of chimeric protein present. Normalizing the observed transcriptional activity to the level of chimeric protein will result, therefore, in

FIG. 1. A, schematic of CBP deletion mutants. CREB and c-Fos binding domains, as well as N-terminal and C-terminal activation domains, are identified as indicated. B, F9 cells were transiently transfected with plasmids encoding CREB, the catalytic subunit of PKA, reporter gene somatostatin (SS-CAT), and the plasmids encoding CBP or the indicated CBP mutants containing either C-terminal or internal deletions (marked by Δ). The CAT enzymatic activity resulting from transcription of the reporter gene SS-CAT is reported relative to activity seen with full-length CBP. Data from at least three independent experiments are shown and the standard error (S.E.) indicated by the bars.

FIG. 2. A, schematic of various chimeric molecules made by fusing the DNA binding domain of GAL4 to CBP peptides. The CREB (Ⅲ) and c-Fos (Ⅳ) binding domains within CBP are indicated. B, plasmids encoding GAL-CBP chimeras GAL-CBP-(1–460), GAL-CBP-(721–1679), and GAL-CBP-(1678–2441) spanning the length of CBP were transfected into COS-7 cells. Following transfection, protein expression levels were determined by metabolically labeling the cells with [35S]cysteine/methionine, followed by immunoprecipitation using anti-GAL antibodies, and analysis of precipitated proteins by SDS-PAGE and autoradiography. The position of the arrows indicates the position of each chimera. In the right panel, extracts from cells transfected with pGAL-(1–147) and extracts from control non-transfected cells were analyzed on a 12% gel. In the left panel, extracts from cells transfected with pGAL-CBP-(1–460), pGAL-CBP-(721–1679), and pGAL-CBP-(1678–2441) were analyzed on a 6% gel. The relative level of protein expression is indicated below each lane (as n-fold compared to GAL-CBP-(1–460)) and was determined by PhosphorImager analysis, then used to normalize the transfection data in C. C, plasmids encoding the indicated GAL-CBP chimeras were transfected into COS-7 cells. Transcription from the reporter gene pGAL-CAT was analyzed by measuring CAT enzymatic activity. The CAT enzymatic activity is reported as activity relative to expression seen with pGAL-CBP-(1–460) and to the relative amount of chimeric protein expressed. Transfectional efficiency was normalized to activity of an internal control plasmid (RSV-βGAL) and the S.E. indicated by the bars.
underestimation of the ability of a chimera to activate transcription. In contrast, the larger chimera (Gal-CBP-(721–1679)) was poorly expressed and thus not likely to be at saturation levels. In this case, normalization of transcription activity to chimeric protein levels will give a better indication of the chimera’s ability to activate transcription. Titration experiments in COS-7 cells (to test whether the transcriptional activity is proportional to protein expression level) are difficult to interpret since these cells amplify transfected DNAs to high copy number per cell; therefore, increased protein levels are more likely to reflect increased transfection efficiency rather than changes in the amount of protein expressed per cell.

Because of these difficulties, we have presented the data in both as relative activity corrected to the amount of each chimeric protein expressed and as uncorrected data (see Table I).

As shown in Fig. 2C, we compared the ability of the GAL-CBP-(1–460) and GAL-CBP-(721–1679) chimeras to activate transcription to that of a C-terminal chimera, GAL-CBP-(1678–2441), whose activity we have previously characterized (12) and which was expressed 0.08-fold the level of the GAL-CBP-(1–460) chimera (Fig. 2B). The GAL-CBP-(1–460) chimera was found to be a strong transcriptional activator approximately equivalent to a GAL-VP16 chimera, and about 8-fold stronger than the GAL-CBP-(1678–2441) chimera as a transcriptional activator (Figs. 2C and 3B). The DNA binding domain of GAL4 (amino acids 1–147) has been shown previously to be inactive in vivo, and this has been confirmed in this study, indicating that the transcriptional activity observed with the GAL-CBP chimeras is due to the CBP portions. The GAL-CBP-(721–1679) chimera was also found to be transcriptionally inactive. To confirm that this inactivity was due to the absence of a transcriptional activation domain, rather than due to the low level of protein expression observed, several additional chimeras (GAL-CBP-(721–1332), GAL-CBP-(1548–1679), and GAL-CBP-(1333–1679)) within this same region were tested. These chimeras were also found to be transcriptionally inactive (data not shown).

To more precisely localize the TADs within the N-terminal 460 amino acids of CBP, plasmids encoding two additional chimeras were constructed, GAL-CBP-(1–232) and GAL-CBP-(227–460). Plasmids encoding these chimeras, GAL-(1–147) and GAL-VP16, were transfected into COS cells, the relative level of protein expression was determined (compared to GAL-CBP-(1–460)), and this number was used to normalize the transfection data in B (see Fig. 2). The position of the arrows indicates the position of each chimera. B, the ability of the indicated N-terminal chimeras to activate transcription from pGAL-CAT was tested. The relative CAT enzymatic activity is reported as activity relative to expression seen with GAL-CBP-(1–460) and transfection efficiency was normalized as described in Fig. 2.

### Table I

| Construct          | Uncorrected activity | Relative activity |
|--------------------|----------------------|-------------------|
| GAL-(1–147)        | 0                    | 0                 |
| GAL-CBP-(1–460)    | 1.0 (0.05)           | 1.0 (0.05)        |
| GAL-CBP-(1–232)    | 0.031 (0.004)        | 0.029 (0.004)     |
| GAL-CBP-(227–460)  | 1.08 (0.05)          | 2.24 (0.17)       |
| GAL-CBP-(1961–2039)| 0.13 (0.05)          | 0.01 (0.004)      |
| GAL-CBP-(2060–2179)| 0.59 (0.07)          | 0.067 (0.009)     |
| GAL-CBP-(1961–2441)| 0.001 (0.0001)      | 0.12 (0.018)      |
| GAL-CBP-(721–1679)| 0                    | 0                 |
| GAL-VP16           | 1.1 (0.17)           |                   |

### FIG. 3

A. To define motifs within the N-terminal end of CBP that directly transcriptional activation, two additional chimeras were constructed, GAL-CBP-(1–232) and GAL-CBP-(227–460). Plasmids encoding these chimeras, GAL-(1–147) and GAL-VP16, were transfected into COS cells, the relative level of protein expression was determined (compared to GAL-CBP-(1–460)), and this number was used to normalize the transfection data in B (see Fig. 2). The position of the arrows indicates the position of each chimera. B, the ability of the indicated N-terminal chimeras to activate transcription from pGAL-CAT was tested. The relative CAT enzymatic activity is reported as activity relative to expression seen with GAL-CBP-(1–460) and transfection efficiency was normalized as described in Fig. 2.

3B and Table I) when compared to the GAL-CBP-(227–460) or GAL-CBP-(1–460) chimeras. The presence of a TAD within amino acids 1–1098 of CBP, the region of CBP indicated by our deletion studies to be sufficient for CREB-mediated transcription, suggested these TADs might be important for activation of CREB-mediated transcription by CBP. To test this hypothesis, an expression plasmid encoding the deletion mutant CBP-(Δ2–460), which lacks both N-terminal TADs, and an expression plasmid encoding the deletion mutant CBP-(Δ272–460), which lacks portions of the stronger N-terminal TAD (amino acids 227–460), were generated. Relative expression of the CBP-(Δ2–460) and CBP-(Δ272–460) proteins was tested by Western blot analysis using antibody raised against amino acids 1736–2179 of CBP (Upstate Biotechnology, Inc.) and found to be equivalent in expression level to that of full-length CBP protein (data not shown). As shown in Fig. 1B, both CBP-(Δ2–460) and CBP-(Δ272–460) lost about 75% of the activity displayed by full-length CBP. These results suggest that, although amino acids 272–460 (and the TAD located within amino acids 227–460) are important for CBP activation of CREB-mediated transcription, other regions of CBP can contribute to this activity as well.

Overexpression of Amino Acids 1–460 of CBP Squelches CREB-mediated Transcription—The previous studies indicate
that amino acids 1–460 of CBP contributed 75% of the ability of CBP to activate CREB-mediated transcription. If this occurs by interaction of amino acids 1–460 of CBP with additional proteins, then blocking this interaction should squelch the ability of CBP to activate CREB-mediated transcription. To test this hypothesis, we constructed a plasmid encoding CBP-(1–460) and co-transfected this into F-9 cells along with the plasmids encoding PKA, CREB, and full-length CBP. As shown in Fig. 4, increasing the amounts of the CBP-(1–460) plasmid transfected squelched in a dose-dependent manner the ability of the reporter gene SS-CAT is reported relative to activity seen with full-length CBP. Data from at least two to three independent experiments are shown, and the standard error (S.E.) indicated by the bars.

Identification of C-terminal TADs within CBP—Although the C-terminal end of CBP appears to be dispensable for full activation of CREB-mediated transcription, our studies have indicated that the C-terminal end of CBP can activate transcription as a GAL-CBP chimera. This suggests that it has TADs that may be utilized by other transcription factors such as c-Fos to activate transcription. To precisely localize the regions within the C-terminal end of CBP capable of functioning as TADs, we constructed and tested a series of smaller GAL-CBP chimeras spanning amino acids 1678–2441 (Fig. 5A). By co-transfecting these chimeras with the GAL-CAT reporter gene, two smaller C-terminal domains able to activate transcription were identified: GAL-CBP-(1961–2039) and GAL-CBP-(2060–2179) (Fig. 5C). Again, GAL-CBP chimeric proteins were immunoprecipitated to eliminate the possibility that some were not expressed, and the transcriptional activity was normalized using the level at which each protein was expressed (Fig. 5B). Comparison of the relative ability of C-terminal and N-terminal TADs to activate transcription is shown in Table I. The strong N-terminal TAD was found to be a 30-fold stronger activator than the weak N-terminal TAD, 15-fold stronger than the more distal C-terminal TAD (amino acids 2060–2179), and 100-fold stronger than the more proximal C-terminal TAD (amino acids 1961–2060).

PKA Enhances the Ability of the N-terminal TAD of CBP to Activate Transcription—The mechanism by which PKA enhances the ability of CBP to activate transcription is unknown. Because our data indicate that the N terminus of CBP is important for CREB-mediated transcription, we asked whether PKA might directly enhance the transcriptional activity of the GAL-CBP-(1–460) chimera. Data from these experiments are summarized in Table II. PKA treatment did not specifically increase the activity of GAL-CBP-(1–460) in either F9 or COS-7 cells. Since previous studies indicated that the GAL-CBP-(1678–2441) chimera was activated by PKA treatment in PC-12 cells, we tested whether the ability of the pGAL-CBP-(1–460) chimera would also be enhanced by PKA in PC-12 cells. As shown in Table II, PKA treatment enhanced the activity of the N-terminal TAD by 17-fold in PC-12 cells. To ensure this was not due to nonspecific effects on general transcription, PKA effects on the reporter gene RSV-CAT were also tested, and activation was found to be enhanced by 2.8-fold.

The N-terminal Activation Domain of CBP Binds TBP—Previous co-precipitation studies demonstrated that CBP/p300 could co-precipitate with anti-TBP antibodies, suggesting that CBP might directly bind TBP. Since transcriptional activation domains within other transcription factors, including CREB, have been shown to bind TBP, we asked whether the TADs of CBP could bind TBP. For these studies, we generated GST-CBP-(227–460) and GST-TFIIB fusion proteins. TFIIB has been shown previously to bind TBP (25), and was used as a positive control for binding. Bacterially expressed GST fusion proteins were incubated with glutathione-agarose resin to generate affinity columns. When bacterially expressed human TBP was incubated with these affinity columns, we observed that TBP bound specifically to GST-TFIIB and to GST-CBP-(227–460) but did not bind to GST alone or to either GST-CBP-(1961–2039) or GST-CBP-(2060–2179) affinity columns (Fig. 6). These observations suggest that TBP binds directly to the N-terminal activation domain of CBP, and points to a mechanism by which this domain of CBP might enhance CREB-mediated transcription.

DISCUSSION

Phosphorylation/dephosphorylation of several DNA binding transcription factors is triggered by activation of second messenger pathways and often results in modulation of their transcriptional activity. A multitude of evidence indicates that this occurs by the action of kinases and phosphatases, whose activity may be specific for a class of transcription factors (26, 27). Phosphorylation/dephosphorylation has been documented to affect transcriptional activity directly altering DNA binding affinity and by increasing transactivation potential. Although many transcription factor domains involved with transactivation have been defined, precisely how they function is not clear. In the case of CREB, PKA-mediated phosphorylation of the kinase-inducible domain permits interaction of CREB with the co-activator molecule CBP. Previous work has shown that CBP is an example of a transcriptional coactivator, able to enhance transcription mediated by phospho-CREB bound to a CRE. CBP is recruited to the CRE by phospho-CREB; once situated at the promoter, CBP may act as a “bridge” between CREB and the transcriptional machinery. However, from previous studies it is unclear which regions of CBP interact with the basal transcriptional machinery, or which components of the basal transcriptional machinery contact CBP.

In this work we have presented data showing that CBP contains four separate domains that function to activate transcription. Multiple transcriptionally active domains have also been identified in several transcription factors (c-Jun, CREB) (9, 28–31), as well as in the TATA-associated proteins (32) and other adapter molecules (33). The redundant activation domains within these transcription factors or within CBP may allow precise control of the rate of transcription depending on the availability of factors through which these TADs activate transcription. Redundant activation domains may also permit synergistic activation of transcription. For example, the Dro-
sophila transcription factor Bicoid binds both TAF\(_{160}\) and TAF\(_{110}\), resulting in synergistic activation of transcription (34).

The N-terminal 1098 amino acids of CBP were found to contain sufficient information to enhance PKA-activated CREB-mediated transcription to the same extent seen with full-length CBP. Earlier studies have indicated this region also contains the CREB binding domain (amino acids 461–661, Ref. 10), and our studies using GAL-CBP chimeras show that this region also contains a strong TAD located within amino acids 227–460. Comparison of the amino acid sequence found within amino acids 227–460 of CBP to the same region of the CBP related protein p300, which like CBP activates CREB-mediated transcription, shows that these proteins share 68% identity in this domain (16, 35), and thus this same region of p300 is also likely to participate in activation of CREB-mediated transcription. We have also demonstrated that amino acids 1–232 of CBP can weakly activate transcription when expressed as a GAL-CBP chimera; recent reports indicate that this region of CBP interacts with the retinoic acid receptor and other nuclear receptors (44). Because of this finding, we cannot rule out the possibility that the GAL-CBP-(1–232) chimera activates tran-

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**FIG. 5.** A, schematic of the structure of chimeric molecules made by fusing the DNA binding domain of GAL4 to CBP C-terminal peptides. The CREB and c-Fos binding domains are indicated. B, to define motifs within the C-terminal end of CBP that directly transcribe into DNA, a number of GAL-CBP chimeras were constructed: GAL-CBP-(1678–1843), GAL-CBP-(1844–1956), GAL-CBP-(1961–2039), GAL-CBP-(2060–2179), GAL-CBP-(2173–2288), and GAL-CBP-(2282–2441). Plasmids encoding these chimeras were transfected into COS-7 cells, the relative level of protein expression determined (n-fold compared to GAL-CBP-(1–460)) as described in Fig. 2B, and this number was used to normalize the transfection data in C. The position of the arrows indicates the position of each chimera. C, the ability of the indicated C-terminal GAL-CBP chimeras to activate transcription from pGAL-CAT was tested. The CAT enzymatic activity is reported as activity relative to expression seen with pGAL-CBP-(1–460) and to the relative amount of chimeric protein expressed. Transfection efficiency was normalized as described in Fig. 2.
TABLE II
PKA treatment enhances the transcriptional activity of N terminus of CBP

The plasmid encoding the catalytic subunit of PKA was co-transfected with the GAL-CBP-(1–460) chimera or the GAL-VP16 chimera into the indicated cell types. Fold activation induced by PKA is shown and is relative to the ability of the GAL-CBP-(1–460) chimera alone to activate transcription of pGAL-CAT. In PC-12 cells, the ability of PKA to modulate the activity of pRSV-CAT reporter gene was used as a control for PKA affects on general transcription. Each number represents the average fold activation induced by PKA in three or more independent experiments. The standard error is given in parentheses. NT, not tested.

| Constructs       | Cell lines | PC-12 | COS-7 | F9   |
|------------------|------------|-------|-------|------|
| GAL-CBP-(1–460)  | 17 (4.9)   | 0.96 (0.07) | 2.3 (0.3) |
| RSV-CAT          | 2.8 (0.7) | NT    | NT    |      |
| GAL-VP16         | NT         | 1.6 (0.05) | 2.62 (0.01) |

Fig. 6. TBP binds the N-terminal CBP. A, crude extracts from bacteria expressing human TBP were incubated with glutathione agarose beads loaded with GST protein, a GST-CBP-(227–460) chimera, or a GST-TFIIB chimera. Following washing, specifically bound proteins were eluted in SDS-PAGE buffer and analyzed by Western blotting using anti-TBP antisera. B, to ensure equal loading of the glutathione beads by each GST protein, control beads were stripped of protein by boiling in SDS-PAGE buffer, size-fractionated by SDS-PAGE and detected by staining with Coomassie Blue dye.

CBP Transcriptional Activation Domains

CBP that contacts TBP greatly diminished the ability of CBP to activate CREB-mediated transcription. To further examine the role of CBP TADs in transcriptional activation, we tested the ability of CBP-(1–460) to activate CREB-mediated transcription in several cell lines. As shown in Fig. 6A, CBP-(1–460) activated CREB-mediated transcription in PC-12 cells, but not in COS-7 or F9 cells. This result suggests that the ability of CBP to activate CREB-mediated transcription is dependent on the presence of TBP.

In support of this notion, as discussed below, our deletion experiments suggest that amino acids 1–232 of CBP are not required for CREB-mediated transcription. Consistent with this result, we have found that complete deletion of amino acids 1–232 results in a near complete loss of CREB-mediated transcription. Similar findings have been reported for the interaction of Sp1 with TBP, and E1A with TFIID (37, 38). Our studies have also indicated that, like CREB, CBP binds directly with TBP. Deletion of the region of CBP that contacts TBP greatly diminished the ability of CBP to enhance the PKA-activated CREB-mediated transcription, suggesting that CBP may activate transcription at least in part by contact with TBP.

One interesting aspect of these studies is the finding that CBP contains C-terminal TADs not required for CREB-mediated transcription. Since these TADs are located adjacent to the c-Fos binding site within CBP, one possibility is that they are involved in c-Fos-mediated transcription. These studies

C. Mueller and J. Chirivia, unpublished data.
have also indicated that deletion of the E1A binding site within CBP does not abolish CREB-mediated transcription (15). This indicates that E1A does not repress the ability of CBP to activate CREB-mediated transcription by simple competitive inhibition of binding of basal transcription factors.

Recent studies have indicated that several PKA-mediated events are required for activation of CREB-mediated transcription. Treatment of T-cells with forskolin has been reported to result in phosphorylation of CBP on serine 133 and in the activation of transcription of a CRE-CAT reporter gene. In contrast, treatment with okt3 (a monoclonal antibody to the T cell receptor) stimulated phosphorylation of CBP on serine 133 to the same extent as forskolin, but did not activate transcription from the CRE-CAT reporter gene (39). These studies suggest that although phosphorylation of CREB is required for formation of CREB-CBP complexes, other PKA-mediated events are also required for activation of CREB-mediated transcription. This hypothesis prompted us to test whether the N-terminal region of CBP (amino acids 1–460) had its ability to activate transcription enhanced by PKA. The results of these experiments indicated that PKA strongly enhanced the ability of the N terminus of CBP to activate transcription in PC-12 cells. Since this region of CBP does not contain a consensus phosphorylation site for PKA, PKA-mediated activation of CBP must occur through indirect means. One possible mechanism is that PKA phosphorylates other kinases that phosphorylate CBP. Sequential kinase phosphorylations appear to be responsible for the direct activation of other transcription factors, most notably, c-Jun (40, 41). Since phosphorylation has been shown to modulate the activity of general transcription factors, PKA or subsequently activated kinases may also phosphorylate general transcription factors allowing interaction with CBP. We noted that the ability of PKA to enhance transcriptional activity of the N-terminal of CBP was cell type-dependent. This suggests that events initiated by PKA, and which lead to enhancement of the ability of CBP to activate transcription, may be activated in F9 and COS-7 cells as compared to PC-12 cells. One consequence of such activation may be a predisposition of certain types of cells to activate cAMP-responsive transcription at lower levels of cAMP and activated PKA.

Previous studies have indicated that CBP has binding sites for several transcription factors including the nuclear receptors c-Jun, c-Fos, c-Myb, YY-1, and CREB (14, 41–45). The studies presented here indicate that although CBP also has multiple domains that can activate transcription, CREB predominantly utilizes one of these domains located within CBP amino acids 227–460 to activate transcription. The presence of other domains within CBP that function as TADs suggests that these may be used by other transcription factors to activate transcription via CBP. Our finding that CBP binds to TBP suggests that CBP may function as a TAF utilized by multiple signal transduction systems.

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