Differential Interactions of the CREB/ATF Family of Transcription Factors with p300 and Adenovirus E1A*

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The adenovirus E1A-associated protein p300 is a transcriptional cofactor that interacts with YY1 and mediates the relief of YY1 transcriptional repression by E1A. These observations raise the possibility that p300 may function as a bridging factor between E1A and cellular transcription factors. Here we show that p300, but not a mutant defective for binding to E1A, activated cAMP-responsive element-binding protein/activating transcription factor (CREB/ATF) binding site-mediated transcription in the presence of E1A. Among proteins that can recognize the CREB/ATF site, CREB appeared to be modulated by E1A in a p300 binding-dependent manner. This effect of E1A was correlated with a specific physical interaction between CREB and p300. These results suggest that p300 plays a crucial role in mediating the functional interplay between E1A and certain members of the CREB/ATF family. Two separate domains within p300 were identified that are capable of activating transcription. One of the domains interacted with the basal factor TFII B, suggesting that p300 may function as a coactivator by making contacts with both sequence-specific transcription factors and the basal transcription machinery. This pivotal role of p300 may make it a prime target for viral proteins such as E1A in programming the cellular transcription machinery.

The E1A region of human adenoviruses gives rise to two major alternatively spliced products, 12S and 13S mRNAs (1–3). The corresponding protein products are nuclear phosphoproteins of 243 and 289 amino acids, respectively (3–6). Both polypeptides have identical amino- and carboxyl-terminal ends, the only difference being a region of 46 internal amino acids unique to the 13S product (3). These proteins are the first viral polypeptides synthesized after adenovirus infection (7, 8). In addition to activating transcription of other adenoviral genes, E1A affects a whole array of host cell functions such as DNA synthesis and cell cycle progression (9, 10) to facilitate viral replication and propagation. E1A is also capable of immortalizing cells (11, 12), inducing full morphological transformation in cooperation with several oncogenes including the ras, polyoma middle T, and adenovirus E1B (13–15), and inhibiting terminal differentiation (16, 17). The diverse biological activities of E1A are attributable, at least in part, to its ability to modulate the cellular transcriptional machinery, since E1A has been shown to activate and repress a large number of cellular genes important for cell proliferation and differentiation (17–27).

Unlike conventional transcription factors, E1A does not recognize specific DNA sequences (5, 28), and the E1A-responsive promoters do not share common sequence elements (reviewed in Ref. 29). Therefore, it has been proposed that E1A must exert its transcriptional effects via multiple mechanisms that are likely to involve protein/protein interactions (29). Studies in the past several years have provided evidence that supports such an hypothesis. It has been shown that in some cases, a direct interaction between E1A and certain transcription factors targets E1A to the promoters for transcriptional activation (30–34). In other cases, the interaction appears indirect and is mediated by E1A-associated proteins, such as the RB family of proteins. Through its physical interactions with RB, E1A disrupts the RB-E2F complex (35), releasing free, active form of E2F for transcriptional activation (reviewed in Ref. 36).

Another protein that has been implicated in mediating the transcriptional effect of E1A is its associated protein p300 (37, 38). Genetic studies suggested that the ability of E1A to repress viral and cellular enhancers is dependent on the p300-binding domain of E1A (18, 19, 22, 23, 39–41). The cDNA that encodes the p300 protein was cloned, and direct evidence was obtained that demonstrated the involvement of p300 in E1A-mediated repression of the SV40 enhancer (42). P300 shares extensive sequence homology with the transcriptional coactivator CBP1 (CREB-binding protein) (43–45). As predicted from the sequence comparison, p300 functions like CBP as a coactivator of CREB and is capable of mediating the effect of E1A on CREB (46, 47). Recently, by analyzing the ability of E1A and its mutant derivatives to convert the transcription factor YY1 from a repressor to an activator, we identified p300 as a bridging factor that mediates the functional interaction between YY1 and E1A (48).

The observation that p300 mediates the ability of E1A to modulate YY1 activity led us to ask whether p300 is a common cofactor that mediates the transcriptional effects of E1A. Promoter elements that were previously shown to respond to E1A were examined. One of the cis elements through which E1A exerts its transcriptional effects is the recognition sequence for the CREB/ATF family of proteins (49, 50). The consensus sequence of the CREB/ATF binding sites can serve as a recognition site for either homo- or heterodimers between members of the CREB/ATF and the AP-1 family of transcription factors (51, 52).
One of the ATF family members, ATF2, has been shown previously to mediate E1A-induced transcriptional activation via a direct interaction with E1A (32).

In this paper, evidence is presented that p300 is involved in mediating the E1A-induced transcriptional activation via an ATF site (abbreviated as ATFf hereafter) taken from the fibronectin promoter (53, 57). In contrast, p300 failed to activate transcription via an Sp1 site in the presence of E1A. By gel shift/antibody supershift experiments, two CREB/ATF family members, CREB and ATF-1, were found to bind the ATF, site. Using a GAL4 fusion protein-based assay, CREB, but not ATF-1 or Sp1, was shown to respond to E1A in a p300 binding-dependent manner. This suggests that CREB, but not ATF-1, participates in the response of the ATF, site to p300/E1A-induced transcriptional activation. Consistent with this hypothesis, CREB, but not ATF-1 or Sp1, was shown to physically interact with p300 in HeLa cells. Interestingly, E1A activated CREB-mediated transcription in HeLa cells but repressed it in U2OS cells. The activation and repression functions of E1A on CREB-dependent transcription in different cells both required an intact p300 binding domain. To better understand the role of p300 as a cofactor of CREB-mediated transcription, experiments were initiated to analyze how p300 regulates transcription. Two separate domains of p300 were identified that activated transcription when targeted to a promoter via the heterologous GAL4 DNA-binding domain. One of these activation domains was shown to interact with the basal transcription factor TFIIIB. These results suggest that p300 may function as a coactivator by making contacts with both sequence-specific DNA-binding transcription factors and the basal transcription machinery.

**Materials and Methods**

Cell and Transfections—Cells were grown on 10-cm dishes in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated calf serum (HeLa) or fetal calf serum (293 and U2OS). Transfections were performed by the calcium-phosphate precipitation method as described (54). The total amount of DNA was adjusted to be identical for each set of transfections. Cells were harvested 48 h after addition of the precipitates. All transfection assays were carried out with at least two independent DNA preparations and were repeated at least three times.

CAT Assays—Whole cell extracts were prepared from the transfected cells. CAT activity was assayed as described (54) and quantitated with a Beckman LS6500 scintillation counter. To ensure that CAT assays were performed within linear range, the proper amount of cell extracts was used to measure CAT activity. For instance, less extracts from cells transfected with stronger transactivators, such as GAL4-VP16 or p300, were used for CAT reactions. For all the data presented, at least three independent transfections and CAT assays have been performed.

Plasmids—pHT-1CAT, pSp1-TA-CAT, pTA-CAT, and pTATAA-CAT are kind gifts of D. Dean (Washington University School of Medicine). Wild-type p300 expression plasmid and its parental vector were courtesy of R. Eckner and D. Livingston (Dana Farber Cancer Institute). The p300 mutant d10 plasmid was described previously (48). pGAL4-VP16 was cloned into the RC/CMV expression vector (Intronogene) with the activation domain of VP16 inserted into the Nhel site (aa 2377) of p300 cDNA. pGAL4-VP16 (aa 1–744) was expressed in HeLa cells and 2 μg of nuclear extracts in a final volume of 15 μl. The reaction mixture was incubated at room temperature for 20 min and analyzed by nuclease protection assay. The specificity of the protein-DNA complexes was demonstrated by competition using unlabeled competitor oligonucleotides. To identify individual transcription factors involved in the complex formation, various antibodies were incubated with the nuclear extracts for 4 h at 4°C before the addition of the probes. The results were visualized by autoradiography. Dr. M.-E. Lee (Harvard School of Public Health) kindly provided us with antibodies including α-ATF1 (C41-5.1, Santa Cruz, catalog sc-243), α-ATF2 (Upstate Biotechnology Inc., UBI, catalog 06–326), α-CREB (UBI, catalog 06–244), and α-c-Jun (UBI, catalog 06–115). The α-YY1 polyclonal antibodies are affinity-purified.

**Results**

p300 Mediates E1A-induced Transcriptional Activation through an ATF, but Not a Sp1 Binding Site—Previously, we demonstrated that p300 activates YY1 binding site-mediated transcription in 293 cells (which constitutively express E1A proteins) and that this function is dependent on the ability of p300 to interact with both YY1 and E1A (48). Together with several other lines of evidence, we proposed that p300 mediates the modulatory effects of E1A on the transcriptional activity of YY1 (48). We then wished to determine whether p300 is a general mediator of the transcriptional effects of E1A. Since a number of transcription factor binding sites have been demonstrated to mediate E1A responsiveness (reviewed in Ref. 29), we first asked whether, in the presence of E1A, p300 is capable of activating transcription of the reporter constructs previously shown to respond to E1A. The reporter CAT plasmids contain either an ATF site (ATFf) which was taken from the fibronectin promoter (nucleotide 176 to 184 relative to the start site of transcription (Ref. 57, kind gifts of D. Dean). The parental vector pTA-CAT was derived from pTATAA-CAT which contains the fibronectin gene sequence from +8 to –36 (53). pTA-CAT is essentially the same as pTATAA-CAT except that the TATA element extending from –20 to –24 of the fibronectin gene promoter (TATAA) was replaced by the simian virus 40 (SV40) early gene TATA box equivalent TATTAT, which has been shown not to respond to E1A (60). It has been shown that all reporters except pTA-CAT responded to E1A in transfection assays (57).

To determine whether the E1A response of these reporters may be mediated by p300, each reporter plasmid was cotransfected with a CMV-p300 expression plasmid into 293 cells. As shown in Fig. 1A, p300 selectively activated CAT expression from pTATAA-TATCAT but not from the parental vector pTA-CAT (lanes 1–2, and 7–8), suggesting that the ATF, site may be responsible for the p300-induced activation. Importantly, the p300 mutant, p300 d10, which is deleted of the E1A-binding domain (42, 48) was unable to activate the ATF, site-mediated transcription (Fig. 1A, lane 3).

The result indicated that the interaction between p300 and E1A is critical for the observed transcriptional activation. This is consistent with the observa-
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Involved in mediating E1A-induced transcriptional activation through the ATF site. Consistent with this notion, without the ATF site, the parental vector pTA-CAT did not respond to E1A (57) and also failed to respond to p300 in this assay (Fig. 3A, lane 8). Interestingly, p300 also did not activate the other two reporters, pSp1-TA-CAT and pTATAA-CAT (Fig. 1A, lanes 5 and 11), which previously were shown to respond to E1A (57). These results suggest that E1A transactivates these reporters through alternative mechanisms that do not involve the p300 protein. In the case of pTATAA-CAT, it is likely that E1A, in particular the 13 S gene product, may activate the reporter by directly targeting the basal transcription factor TATA-binding protein (30, 31).

How does p300 activate transcription through the ATF site in 293 cells? Previously, it has been shown that p300 has specific DNA binding activity for NF-kB/H2TF1-like sites (61), which bear no resemblance to the YY1 (48) or the ATF site (this study) shown to respond to p300. In fact, accumulating evidence suggests that p300 may function in a more indirect way, i.e. as a transcriptional cofactor (42, 46, 47). If this property of p300 is indeed the mechanism by which p300 activates transcription, the failure of p300 dl10 to activate pATF-TA-CAT could also be due to a defect in its ability to function as a transcriptional coactivator. To address this issue, the ability of the carboxyl-terminal half of p300 (aa 1257–2414) and its mutant derivative containing the same internal deletion (aa 1679–1812) as p300 dl10 to regulate transcription was analyzed. The reporter plasmid pGAL4-E1BCAT contains five GAL4 DNA binding sites immediately upstream of the minimal adenovirus E1B promoter. pGAL4-E1BCAT has been widely used for studies of transcriptional activation, including E1A-induced transcriptional activation. As shown in Fig. 1B, both forms of p300, when fused to the GAL4 DNA-binding domain, activated transcription of the target gene GAL4-E1BCAT, whereas the GAL4 DNA-binding domain alone had virtually no effects (data not shown, Fig. 5). These data suggest that the carboxyl-terminal half of p300 may contain a functional domain for its transcriptional coactivator function, which is unaffected by the dl10 deletion mutation. Thus, the failure of p300 dl10 to activate pATF-TA-CAT in 293 cells is most likely due to its inability to interact with E1A. However, it is unclear at the present time why the p300 dl10 activated transcription better than the wild-type p300. Taken together, these results demonstrated that p300 is capable of mediating E1A-induced transcriptional activation via the ATF, but not the Sp1 site.

CREB and ATF-1 Bind the ATF Site That Responds to p300 in 293 Cells—Since multiple CREB/ATF-related proteins can bind an ATF consensus site, we wished to determine which members of the CREB/ATF family bind the ATF site that responded to p300 in 293 cells (Fig. 1A). An oligonucleotide representing the ATF site was labeled with 32P and used in gel shift assays. As shown in Fig. 2, when the ATF oligonucleotides were incubated with nuclear extracts prepared from 293 cells, two predominant complexes (labeled as A and B) were formed that were competed by molar excess of unlabeled ATF, but not by an unrelated YY1 oligonucleotide (lanes 1–7). Addition of α-ATF1 antibodies supershifted both the A and the B complexes whereas the α-CREB antibody supershifted only the A complex (Fig. 2, lanes 8 and 10). This suggests that the A and B complexes both contain ATF-1 while only the A complex contains CREB. In contrast, addition of α-ATF2, α-c-jun, and αYY1 antibodies had no effect on either the A or the B complexes (Fig. 2, lanes 9, 11, and 12). As a control, only αYY1 antibodies abolished a YY1 complex (Fig. 2, lower panel, lane 12). Taken together, these results suggest that ATF-1 and CREB are the main components of the DNA-
protein complexes formed on the ATFf site in 293 cells. However, these data do not rule out the possibility that other untested ATFs may also bind the ATFf site. In addition, the results do not differentiate whether the A complex is composed of ATF-1/CREB heterodimers or comigrating ATF-1 and CREB homodimers.

The p300-binding Domain of E1A is Critical for E1A to Modulate the Activities of CREB—Since both ATF-1 and CREB, and possibly other untested ATFs may bind the ATFf site, it was important to determine which one (or both) is responsible for the response of the ATFf site to the E1A-induced transcriptional activation via p300 (Fig. 1A). To address this issue, individual ATF family members were fused to the GAL4 DNA-binding domain and assayed for their ability to respond to E1A using pGAL4-E1BCAT as a target plasmid (54). As shown in Fig. 3, GAL4-CREB responded to E1A in a cell type-dependent manner. In U2OS cells, E1A, especially the 12S gene product, repressed GAL4-CREB-mediated transcription (Fig. 3A, lanes 1 to 3). In contrast, in HeLa cells, 12S E1A activated GAL4-CREB-mediated transcription (Fig. 3B, lane 4), albeit to a lesser extent compared with 13 S E1A (Fig. 3B, lanes 1 and 2). The quantitative difference between the effects of the 12 S and 13 S E1A is most likely attributable to the 46 amino acids (CR3) unique to the 13 S E1A, to the E1A-induced transcriptional activation domain (62). With this activation domain, 13 S E1A is often found to be a more potent transactivator than the 12 S E1A (24–27, 35, 63), as also observed in Fig. 3B. Perhaps by the same token, lack of this domain may explain why the 12 S product repressed CREB activity in U2OS cells more efficiently (Fig. 3A, compare lanes 2 and 3). The repressive effect of E1A on CREB-mediated transcription observed in U2OS cells is in line with the recent reports that E1A represses CREB-activated transcription in U2OS cells (46) and in mouse F9 cells (47). In the same assay, GAL4-ATF1-mediated transcription was unaffected by E1A in either cell type, consistent with the previous report (64). These results suggest that CREB, but not ATF-1, contributed to the observed activation of the pATF-TA-CAT by p300 in 293 cells (Fig. 1A).

Mutational analyses of the domains of E1A showed that the ability of E1A to modulate CREB-mediated transcription correlated with its ability to bind p300. As shown in Fig. 3A, while wild-type 12 S E1A repressed the activity of GAL4-CREB in U2OS cells (lane 3), the point mutant RG2 which is impaired for binding to p300 (41, 65) failed to do so (lane 4). As a control, a double point mutant pm47/124 defective for binding to RB/p107 (41) repressed CREB-mediated transcription to the wild-type level (lane 5). These results suggest that the ability of E1A to bind p300 is crucial for its ability to repress CREB-mediated transcription in U2OS cells. Interestingly, the ability of 13 S E1A to activate CREB-mediated transcription in U2OS cells was also dependent on an intact p300-interacting domain as deletion of aa 2–36 virtually abolished this ability (Fig. 3B, compare lanes 2 and 3). These results suggest that the interaction of E1A with p300 is important for E1A to modulate the transcriptional activities of CREB. The implication of the p300 involvement in this process is consistent with the functional assays performed in 293 cells (Fig. 1A). The finding that regulation of CREB activity by E1A is mediated by p300 is consistent with the results published in two recent reports (46, 47). Our results further suggest that the outcome of the CREB/E1A interaction mediated by p300 may vary depending on the cell types.

In Fig. 1A, we showed that p300 did not transactivate Sp1 binding site-mediated transcription in 293 cells (Fig. 1A, lane 5). Here, the effect of E1A on GAL4-Sp1-dependent transcription was examined. As shown in Fig. 3C, GAL4-Sp1-mediated transcription was only slightly repressed by both 12 S and 13 S E1A in both cell types. The repression of Sp1 by E1A was also unaffected by E1A (data not shown) (66). These results attest to the specificity of the functional interaction observed among CREB, p300, and E1A.

To determine whether CREB in its native form can also be modulated by E1A through p300, expression plasmids encoding CREB and p300 were transfected either alone or in combination with E1A and the reporter pATF-TA-CAT into HeLa cells. As shown in Fig. 4, 13 S E1A activated pATF-TACAT in HeLa cells (Fig. 4, compare lane 5 with 1). Importantly, this activation was dependent on the p300-binding domain of E1A as mutations that abrogated p300 binding also abolished the ability of E1A to activate the reporter (Fig. 4, compare lane 5 with lanes 6 and 7). This result suggested the involvement of p300 in
this E1A-induced activation and is consistent with the finding presented in Fig. 3 in which the stimulatory effect of E1A on GAL4-CREB-mediated transcription was shown to be dependent on the p300 binding domain of E1A. Since E1A had no stimulatory effect on pTA-CAT (Ref. 57 and data not shown), the E1A-induced activation of pATF-TA-CAT is mostly likely mediated by the ATF site. As shown in Fig. 4, CREB and p300, when transfected alone (lanes 2 and 3) or together (lane 4), did not result in a significant activation of the reporter under our assay conditions. Since HeLa cells contain both endogenous CREB and p300, one explanation for the lack of activation is that the two proteins are not limiting for transcription of pATF-TA-CAT in HeLa cells. Indeed, when CREB was directed to the promoter of pGAL4E1BCAT via the GAL4 DNA-binding domain fused to its N terminus, the GAL4-CREB fusion protein was shown to activate GAL4-E1BCAT and to respond to E1A activation (Fig. 3).

Taken together, these results suggested that the ability of E1A to interact with p300 is critical for E1A to activate transcription mediated by GAL4-CREB (Fig. 3, A and B) and the CREB/ATF binding site (Fig. 4) in HeLa cells.

Physical Interactions between p300 and the CREB/ATF Family of Proteins—The fact that E1A modulates the activity of a transcription factor (such as CREB) in a p300 binding-dependent manner predicts a physical interaction between p300 and this transcription factor. To test this hypothesis, we analyzed potential protein/protein interactions between p300 and members of the CREB/ATF family in HeLa cells using the "two-hybrid" assay (67). This approach utilizes various CREB/ATF members fused to the DNA-binding domain of GAL4 as
“baits” and p300/VP16 (p300 fused to the herpes simplex virus VP16 activation domain) as a “prey.” As summarized in Table I, p300/VP16 further activated transcription mediated by GAL4-CREB (lane 3) but not by GAL4-ATF1 (lane 4) or GAL4-ATF2 (lane 5). As a positive control, p300/VP16 activated GAL4-YY1-mediated transcription as reported (lane 2, Ref. 48). GAL4-Sp1 failed to respond to p300/VP16 as expected, since p300 was not able to activate Sp1 site-dependent transcription in 293 cells (Fig. 1A). In addition, p300/VP16 failed to activate target gene transcription when the GAL4 DNA-binding domain alone (lane 1) or GAL4-MU4 (lane 7) was used as the bait. These results suggest that p300 physically interacts with CREB in vivo, which is consistent with the in vitro biochemical evidence published previously (47), and fulfilled the prediction from our functional analyses described earlier (Fig. 3). In this assay, p300 did not interact with ATF1 or Sp1. The lack of a physical interaction between p300 and these two proteins is in line with the finding that ATF1- or Sp1-mediated transcription was unaffected by E1A (Ref. 3, Fig. 64). We have also not been able to detect an interaction between p300 and ATF2 with the two-hybrid assay in HEK293 cells (Table I, row 5). Together with the previous observations (32, 64), it appears that the activation of ATF2-mediated transcription by E1A is most likely to be independent of p300. Taken together, these results demonstrated physical interactions between p300 and two transcription factors, CREB and YY1, whose activities were modulated by E1A in a p300-dependent manner (Fig. 3, Ref. 48).

The N- and the C-terminal Portions of p300 Are Capable of Activating Transcription—The evidence provided in this study is consistent with the notion proposed by several recent reports that p300 may function as a transcriptional coactivator (42, 46–48, 66). To understand the mechanisms that underlie the ability of p300 to regulate transcription, we undertook a study to identify domains of p300 that are involved in transcriptional regulation. The p300 protein was divided into three parts, each of which was fused to the GAL4 DNA-binding domain. As shown in Fig. 5, the N- (aa 1–596) and C-terminal (aa 1257–2414) portions of p300 induced significant activation of the reporter plasmid pGAL4-E1BCAT, while the central portion of p300 (aa 744–1571) weakly activated transcription, if at all. We then asked whether the transcriptionally active domains of p300 interacted with basal transcription factors. Individual basal factors were then tested in vitro and tested for their interactions with the N- or C-terminal or the central portions of p300 interacted with basal transcription factors. Individual basal factors were then tested in vitro and tested for their interactions with the N- or C-terminal or the central portions of p300. The results were expressed as the average and standard deviation of three independent transfections and CAT assays. FS, frameshift mutant expressing the first 22 amino acids of E1A.

### DISCUSSION

In this report, we have identified the critical protein components that are involved in the responsiveness of an ATF site (ATF1) to the E1A-dependent transcriptional activation by p300. In 293 cells, two CREB/ATF family of proteins, CREB and ATF-1 bind the ATF1 site (Fig. 2). However, when
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Three regions of p300 contain transcriptional activation domains. These regions of p300 were fused to GAL4 DNA-binding domain. S pmol of expression plasmids encoding each fusion protein (lanes 1-3) or the GAL4 DNA-binding domain alone (lane 4) were cotransfected with 10 pmol of the GAL4-E1B-CAT reporter plasmid. The same amount of labeled TFIIB used in the CAT assay to ensure that the results were within the linear range.

In a separate study, we identified p300 as a coactivator for c-jun and J unB, the major components of the AP-1 transcriptional factor, and demonstrated that E1A modulates the activities of the J un proteins via its interactions with p300 (66). Significantly, in U2OS cells, overexpression of p300 rescues both J un- and CREB-dependent transcription that is repressed by E1A. This lends further support to the contention that, like CBP, p300 may function as a transcriptional cofactor for CREB and that E1A targets CREB via p300 and CBP. These conclusions are consistent with the recent reports demonstrating the functional interactions among CREB, p300, and E1A (46, 47).

Our gel shift/antibody supershift experiments (Fig. 2) clearly demonstrated that both CREB and ATF-1 bind to the AT f site that is responsive to p300/E1A for transcriptional activation. However, E1A only affected CREB- but not ATF-1-mediated transcription. This is intriguing since, among members of the CREB/ATF family, CREB and ATF-1 are closely related with sequence homology extending beyond the leucine zipper region (68, 69). Despite the sequence similarity, the two proteins clearly behaved differently in terms of their interactions with p300 (Table I) and their response to E1A (Fig. 3). It has been noted that CREB and ATF-1 are most divergent in their sequences at the N-terminal region (68, 69). The sequence divergence was proposed to account for the different transcriptional activities of AT f-1 and CREB in the cAMP signaling pathway observed before (69). Perhaps this structural difference also dictates the differential interactions of CREB and AT f-1 with p300, hence the differential response to E1A. Finally, it is also possible that the failure to detect an AT f-1 response to p300/E1A is due to the assay conditions. For instance, the interactions of p300 with AT f-1 may require specific posttranslational modifications of the AT f-1 and/or p300 proteins. Interestingly, it has been noted that, while the recognition sites for cAMP-dependent protein kinase (PKA) and Ca^2+ -calmodulin-dependent protein kinases I and II are conserved between these two proteins (68, 69), calmodulin-dependent protein kinase II phosphorylates an additional site on AT f-1 in vitro (69, 70). Whether this difference between the two proteins contributes to their differential interactions with p300 awaits future study.

In the GAL4 fusion protein-based assays, CREB-mediated transcription was repressed by E1A in U2OS cells (Fig. 3A). In contrast, CREB-dependent transcription was activated by E1A in HeLa cells (Fig. 3B). The apparent opposing effects of E1A are not unprecedented. The ability of E1A to repress and to activate transcription has been amply documented (17, 18, 20–27, 40, 63). However, the precise mechanism underlying this phenomenon is unclear and warrants further studies. Recently, we have analyzed a number of transcription factors for their response to E1A. Some of these transcription factors are up-regulated by E1A, such as YY1 (48, 54, 58) and CREB (this study, in HeLa cells), and others are down-regulated, such as c-jun and J unB (66, 71). Interestingly, in all these cases, the ability of E1A to modulate the activity of these transcription factors is dependent on the ability of E1A to bind p300. When the E1A response of these proteins was further examined,
CREB is the only protein whose activity was found to be both up- and down-regulated by E1A in different cell types, in a manner that is dependent on p300. Thus, it is possible that a better understanding of the CREB-mediated transcription in different cells and the precise role of p300 may provide insight into the mechanism of the dual transcriptional activities of E1A.

Previously, it has been shown that CREB is activated by protein kinase A (PKA) (72–74). To test whether PKA can play a role in the apparent cell-type dependent E1A responses of CREB-mediated transcription, we performed similar experiments as in Fig. 3, A and B, with cotransfection of a PKA expression plasmid (courtesy of R. Goodman, Oregon Health Sciences University). As reported (72–74), cotransfection of 1.5 μg of the PKA expression plasmid enhanced CREB activity by about 10-fold in both cell types. PKA, however, did not change the response of CREB to E1A in these cells, i.e. the PKA-stimulated CREB activity was still activated by E1A in HeLa cells and repressed by E1A in U2OS cells.

As another attempt to understand CREB-mediated transcription, we also analyzed the transcriptional activity of the coactivator protein p300. Our results identified two main activation domains that are located at the N- and C-terminal regions of p300 (Fig. 5). Compared with the C-terminal region, the N-terminal domain of p300 appeared to be more potent in activating transcription. In contrast, the central region of p300 barely activated transcription in the same assay. This is for the apparent difference in the ability of the different parts of p300 to activate transcription is unclear. However, it is interesting to note that the C- but not the N-terminal or the central portion of p300 interacted with the basal transcription factor TFIIIB (Fig. 6). Our finding is consistent with the recent report that demonstrated that the same region of CBP that interacts with the basal transcriptional machinery and the sequence-specific DNA-binding transcription factors. It is worth cautioning, however, that the data demonstrating the interaction between p300 and TFIIB are preliminary. Further experiments are necessary to demonstrate an in vivo p300/TFIIIB interaction and to determine whether this physical interaction is indeed correlated with the ability of p300 to activate transcription.

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