MOLECULAR AND CELLULAR BIOLOGY, Aug. 1996, p. 4312–4326 Vol. 16, No. 8
0270-7306/96/$04.00+0

Adenovirus E1A Downregulates cJun- and JunB-Mediated Transcription by Targeting Their Coactivator p300

JENG-SHIN LEE,[1]† RAYMOND H. SEE,[1] TILIANG DENG,[2] AND YANG SHI[1]*

Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115,[1] and Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida 32610[2]

Received 17 January 1996/Returned for modification 26 February 1996/Accepted 20 May 1996

Transcription factors and cofactors play critical roles in cell growth and differentiation. Alterations of their activities either through genetic mutations or by viral oncoproteins often result in aberrant cell growth and tumorigenesis. The transcriptional cofactor p300 has recently been shown to be complexed with transcription factors YY1 and CREB. Adenovirus E1A oncoproteins target these transcription complexes via physical interactions with p300, resulting in alterations of transcription mediated by these transcription factors. Here we show that p300 is also critical for repression by E1A of the activities of cJun and JunB, two members of the AP-1 transcriptional complexes. This repressive effect of E1A is dependent on the p300-binding domain of E1A and can be relieved by overexpression of p300. These results suggest that p300 serves as a mediator protein for downregulation of AP-1 activity by E1A. This hypothesis was further supported by the following observations: (i) in the absence of E1A, overexpression of p300 stimulated transcription both through an AP-1 site present in the collagenase promoter and through Jun proteins in GAL4 fusion protein-based assays; and (ii) overexpression of a mutant p300 lacking the E1A-interacting domain reduced the responsiveness of Jun-dependent transcription to E1A repression. As predicted from the functional results, p300 physically interacted with the Jun proteins. These findings thus established that p300 is a cofactor for cJun and JunB. We propose that p300 is a common mediator protein through which E1A gains control over multiple transcriptional regulatory pathways in the host cells.

Transcriptional regulation is a control mechanism that is critical for fundamental biological processes such as cell growth and differentiation. Proteins that are involved in transcriptional control can be divided into two classes; those that bind specific DNA sequences and those that do not bind DNA and are brought to the promoters through protein-protein interactions. The latter are collectively known as transcriptional coactivators or adaptors (54). Inactivation and/or alterations of the activities of these proteins have been correlated with developmental abnormalities and tumorigenesis. Because of their crucial biological roles, transcription factors are often targeted by viral oncoproteins during oncogenic transformation of cells. For instance, adenovirus E1A oncoproteins are capable of immortalizing cells (36, 62) and inducing full morphological transformation in cooperation with several other oncoproteins, including Ras, polyomavirus middle T, and adenovirus E1B (57, 63, 64). The ability of E1A to transform cells is closely associated with its ability to interact with pRB and p300 (23, 24, 37, 39, 66, 68), both of which are involved in transcriptional regulation. When complexed with the sequence-specific DNA-binding protein E2F, RB is capable of repressing E2F-dependent transcription (31, 33, 67, 70). Interactions of E1A with the RB-E2F complex results in the disruption of the complex. The free E2F activates rather than represses its target genes (6, 9, 14, 67). The alteration of E2F-dependent transcription by E1A is generally considered an important component in the determination of the transforming properties of E1A.

Initially identified as an E1A-associated cellular protein in coimmunoprecipitation experiments (32, 69), p300 is another regulatory molecule that is a target of E1A. p300 has been demonstrated recently to be a transcriptional adaptor (22) that belongs to a family of proteins that includes CREB-binding protein (CBP), a coactivator of CREB-mediated transcription (3, 4, 41, 49). Recent studies in a number of laboratories have provided experimental evidence that suggests a role for p300 in mediating functional interactions between E1A and cellular DNA-binding transcription factors. p300 has been shown to be complexed with the transcriptional repressor-activator YY1. E1A relieves YY1-mediated transcriptional repression by physically interacting with the YY1-p300 complex, resulting in the alteration of the transcriptional activity of YY1 (43). p300 has also been shown to be complexed with CREB, thus allowing E1A to affect CREB-mediated transcription via the p300-CBP connection (3, 42, 49). These observations raise the possibility that p300 is a general cofactor that mediates the transcriptional effects of E1A.

Another transcription factor that may be targeted by E1A through p300 is AP-1. The transcription factor AP-1 is considered to play a central role in cell differentiation, proliferation, and transformation (for reviews, see references 2 and 65). AP-1 is composed of protein dimers formed between members of two families of transcription factors termed Jun and Fos. The Jun family includes cJun, JunB, and JunD, while cFos, FosB, Fra-1, and Fra-2 belong to the Fos family (12, 16, 26, 50, 55, 56, 71). It has been documented that E1A represses AP-1 activity (27, 52) and that the ability of E1A to downregulate AP-1 activity is dependent on conserved region 1 (CR1), but not CR2 or CR3, of E1A (52). The N-terminal region and CR1 of E1A constitute the interaction domain for p300 (66, 68).

In this paper, we have provided evidence that supports two main conclusions: (i) p300 is a cofactor for cJun and JunB, and (ii) E1A targets the Jun proteins through p300. Using deletion and point mutants of E1A, we demonstrated that the ability of E1A to repress transcription mediated by these proteins was

* Corresponding author. Mailing address: Department of Pathology, Harvard Medical School, 200 Longwood Ave., Boston, MA 02115. Phone: (617) 432-4318. Fax: (617) 432-1313. Electronic mail address: yshi@warren.med.harvard.edu.

† Present address: Children's Hospital, Boston, MA 02115.

closely correlated with its ability to bind p300. The repressive effect of E1A on both cJun and JunB can be relieved by overexpression of p300. Interestingly, although CBP was effective at rescuing CREB-mediated transcription that was repressed by E1A, it had a marginal effect on Jun-mediated transcription repressed by E1A, suggesting that CBP may interact more readily with CREB than with Jun in vivo. In the absence of E1A, overexpression of p300 activated the collagenase promoter in an AP-1 binding site-dependent manner. In addition, p300 also enhanced transcription mediated by the Jun proteins in GAL4 fusion protein-based assays. Significantly, an intact E1A-interacting domain of p300 is required for E1A to repress Jun-mediated transcription, as overexpression of a mutant p300 lacking the E1A-binding domain reduced the responsiveness of Jun to E1A repression. Consistent with the functional results, both Jun proteins were found to physically interact with p300. In vitro binding studies identified a p300-interacting domain of cJun that coincides with one of the transcriptional activation domains of cJun. These studies also revealed at least two separate domains within p300 that are capable of mediating its interactions with cJun. By using proteins purified from bacteria, it was demonstrated that cJun can directly interact with p300 in vitro. Taken together, these results strongly suggest that p300 functions as a cofactor for cJun and JunB and that p300 is a natural target of E1A through which E1A controls the activity of AP-1.

MATERIALS AND METHODS

Cells and transfections. HeLa and U2OS cells were grown in 10-cm-diameter dishes in Dulbecco's modified Eagle's medium supplemented with either 10% heat-inactivated calf serum (for HeLa cells) or 10% fetal calf serum (for U2OS cells). Transfections were performed by the calcium phosphate precipitation method as described previously (59). 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. Chloramphenicol acetyltransferase (CAT) activity was assayed as described previously (59) and quantitated with a Beckman LS6500 scintillation counter. The amount of cell extract used was such that CAT activity was within the linear range.

Plasmids. pColl −517/+63 CAT with and without the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) response element (TRE) (−60 to −73) was kindly provided by Alex J. van der Eb (Sylvius Laboratory, Leiden, The Netherlands). pCMV-12S E1A and its mutant derivatives have been described previously (43). GAL4-cJun (amino acids [aa] 1 to 246), GAL4-JunB (aa 1 to 259), pGAL4-ATF1 (a gift of M. Green, University of Massachusetts Medical Center), and GAL4-VP16 were all subcloned into the Rc/RSV expression vector (Invitrogen). pGAL4-CREB was a kind gift of J. Licht (Mt. Sinai Medical School). Wild-type p300 cloned in the CMVβ expression vector as well as the plasmids with full-length and deletion mutants of p300, which were used here for in vitro transcription-translation, were described previously (22). The p300 *dl*10 mutant is also in the CMVβ expression vector and contains an internal deletion of p300 aa 1679 to 1812 (43). pCMV-CBP was a gift of R. Goodman (Oregon Health Sciences University). pGAL4-E1BCAT and glutathione *S*-transferase (GST)–YY1 were described previously (44, 59). Np300 and Np300/VP16 have been described previously (43). Cp300 used in this study was derived from the plasmid BglII ATG (22) and thus expressed p300 aa 1257 to 2414. Cp300/VP16 was constructed by inserting the VP16 activation domain into the *Nhe*I site and thus expressed p300 aa 1257 to 2378 with VP16 fused to the C terminus. Both Np300 and Cp300, with and without BP16, were subcloned into the Rc/CMV expression vector (Invitrogen). The series of GST-cJun deletional mutants was constructed by inserting each corresponding region of cJun into compatible pGEX vectors (Pharmacia) to create in-frame fusions. The in vitro transcription vectors of cJun and JunB were constructed by inserting cJun aa 1 to 246 and full-length JunB into the pGEM vector. GST-p300 amino-terminal, carboxy-terminal, and central portions have been described previously (43). GST-VP16 was provided by M. Green (University of Massachusetts Medical Center). GST-E1A (12S) was provided by E. Harlow (Massachusetts General Hospital Cancer Center).

In vitro protein interaction assays. GST fusion proteins were induced and purified as described previously (45). p300, cJun, and JunB proteins were ^{35}S labeled and synthesized by in vitro translation reactions with the TNT kit (Promega). In vitro binding assays were performed by incubating 4 μg of GST fusion protein coupled to glutathione-agarose beads (Sigma) with in vitro-translated protein diluted in 200 μl of EBC buffer (50 mM Tris [pH 8.0], 140 mM NaCl, 100 mM NaF, 200 μM Na_3VO_4, 0.5% Nonidet P-40) at room temperature for 2 h. The beads were then washed four times with EBC buffer and subsequently boiled in 2× Laemmli sample buffer. Following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), bound proteins were visualized by autoradiography. Jun-CBP interaction studies were performed with cell lysates prepared from 90% confluent HeLa cells in E1A lysis buffer (25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.0], 250 mM NaCl, 2.5 mM EDTA, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 0.1% Nonidet P-40) containing protease inhibitors (2 mM phenylmethylsulfonyl fluoride, 10 μg of leupeptin per ml, 1 μg of chymostatin per ml, 1 μg of pepstatin per ml, and 10 μg of aprotinin per ml). Cellular debris was removed by centrifugation. Glutathione-agarose beads containing GST fusion proteins were incubated with the cell lysates and washed as described above. Proteins were resolved by SDS-PAGE, and CBP was detected by Western blot (immunoblot) analysis with a CBP-specific polyclonal antibody (C-20; Santa Cruz Biotechnology Inc., Santa Cruz, Calif.).

RESULTS

An intact p300-binding domain on adenovirus E1A is critical for its modulation of cJun and JunB activities. The human collagenase gene promoter is activated by the phorbol ester TPA through the TPA-responsive element (CollTRE) which is recognized by the AP-1 transcription factor. Adenovirus E1A abrogates the TPA response of the collagenase promoter by downregulating the AP-1 activity (27, 52). The AP-1 family is composed of the Jun and Fos subfamilies. Both Fos-Jun heterodimers and Jun-Jun homodimers can bind to AP-1 sites to stimulate transcription. In contrast, Fos proteins alone are incapable of binding DNA. We therefore focused on the Jun proteins and tested the ability of three Jun-related proteins to activate transcription through CollTRE.

As shown in Fig. 1, when cotransfected with the collagenase-CAT construct (pColl −517/+63 CAT), cJun and JunB activated the reporter gene by 13.8- and 4.3-fold, respectively (lanes 2 and 3). On the other hand, JunD failed to activate transcription in the same assay (lane 4), which is consistent with the earlier finding that JunD is a poor transactivator in a similar cotransfection assay (38). The ability of cJun and JunB to activate pColl −517/+63 CAT is dependent on the presence of a functional TRE, located between nucleotides −73 and −60 relative to the transcription start site, since both Jun proteins had little effect (twofold or less) on the reporter lacking the TRE (compare lanes 6 and 7 with lane 5). Therefore, the response of the collagenase promoter to the Jun-induced activation is predominantly mediated by the TRE site.

Experiments were then performed to examine the effects of E1A on Jun-mediated transcription. As shown in Fig. 2A, cJun-induced transcription of the collagenase promoter was repressed by cotransfection of 12S E1A (lane 3) but not by a frameshift mutant expressing the first 22 amino acids of E1A (lane 2). To identify domains of E1A that are responsible for repressing the cJun activity, a series of E1A mutants were analyzed in the same cotransfection assay. These studies revealed that the N-terminal region (lane 4) and CR1 (52) (data not shown) are required for the repressive activity of E1A but CR2 is not (lane 5). Since the N-terminal region and CR1 of E1A together constitute the p300-interacting domain, we further tested the point mutant RG2 (Arg-to-Gly mutation at position 2), which is specifically defective for interactions with p300 (66). As shown in Fig. 2A, the ability of E1A to repress cJun-mediated transcription was severely compromised by the RG2 (lane 6) mutation but not by the double point mutation Pm47/124 (Tyr-to-His mutation at position 47 and Cys-to-Gly mutation at position 124) (lane 7), which compromised the ability of E1A to bind the pRB family of proteins (66). All E1A mutants were driven by CMVβ expression vectors and were expressed in the transfected cells at levels comparable to those of the wild-type proteins (data not shown). These results are

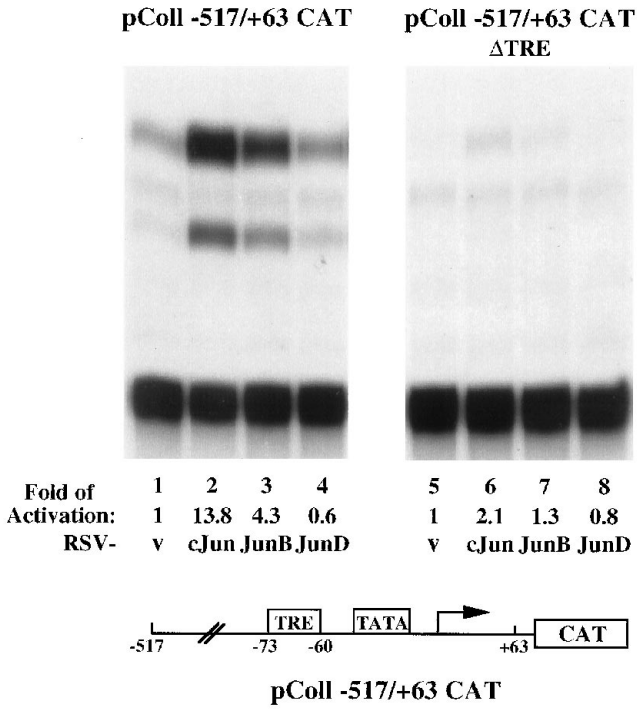


FIG. 1. Jun proteins activate the collagenase promoter. HeLa cells were transfected with the pColl −517/+63 CAT (lanes 1 to 4) or pColl −517/+63 CAT ΔTRE (lanes 5 to 8) reporter gene (5 μg) and Rous sarcoma virus (RSV) promoter-driven plasmids expressing cJun, JunB, and JunD (5 μg). v, vector alone. The fold activation values were calculated by normalizing the CAT activities against the ones obtained from transfection with the reporter genes and the Rc/RSV vector (lane 1 for pColl −517/+63 CAT and lane 5 for pColl −517/+63 CAT ΔTRE). The results represent the averages of three independent transfections and CAT assays. The architecture of pColl −517/+63 CAT is shown at the bottom.

consistent with the recent report that the N-terminal region is critical for negative regulation of cJun activity by E1A (20). Our analyses of the E1A point mutants provided further genetic evidence that strongly implicated the involvement of p300 in mediating the repressive effect of E1A on cJun-mediated transcription. Similar cotransfection assays and mutational analyses were carried out to examine the effects of E1A on JunB-mediated activation of the collagenase promoter. As shown in the Fig. 2B, E1A also repressed JunB-mediated activity in a p300 binding-dependent manner.

Members of the AP-1 family of proteins are known to form homo- or heterodimers with other proteins in that family and with members of the CREB/ATF family (29, 30). It is therefore possible that E1A exerts its effects on the Jun protein indirectly via their partner proteins. To address this issue, the E1A responsiveness of individual Jun proteins was analyzed in a GAL4 fusion protein-based assay. The dimerization domain, located at the C terminus, was deleted from Jun proteins, and the proteins and were fused to the GAL4 DNA-binding domain. As shown in Fig. 3, this GAL4 fusion-based assay fully recapitulated the results obtained with the collagenase promoter and native Jun proteins. Both GAL4-cJun (Fig. 3A, lane 1) and GAL4-JunB (Fig. 3B, lane 1) activated the reporter gene that contains five GAL4 binding sites upstream of the minimal adenovirus E1B promoter (GAL4-E1BCAT). Transcriptional activation mediated by either GAL4-cJun or GAL4-JunB was repressed by cotransfection of 12S E1A (Fig. 3A and B, lanes 2). Analysis of the E1A mutants for their ability to modulate GAL4-Jun-mediated transcription confirmed our earlier observation with the collagenase-CAT reporter, i.e., the ability of E1A to downregulate cJun- and JunB-mediated transcription is dependent on the ability of E1A to bind p300 (Fig. 2). The fact that the truncated cJun and JunB proteins lacking the leucine repeats responded to E1A in a manner similar to the full-length proteins suggested that the E1A response is likely mediated by the Jun proteins themselves. Finally, the specificity of the functional interaction between the Jun proteins and E1A was further demonstrated, as shown in Fig. 3C, by the determination that an acidic activator-mediated transcription was unaffected by cotransfection of E1A (lane 2).

Repression of cJun- and JunB-mediated transcription by E1A is relieved by overexpression of p300. To further explore the role of p300 in the E1A-Jun functional interaction, we

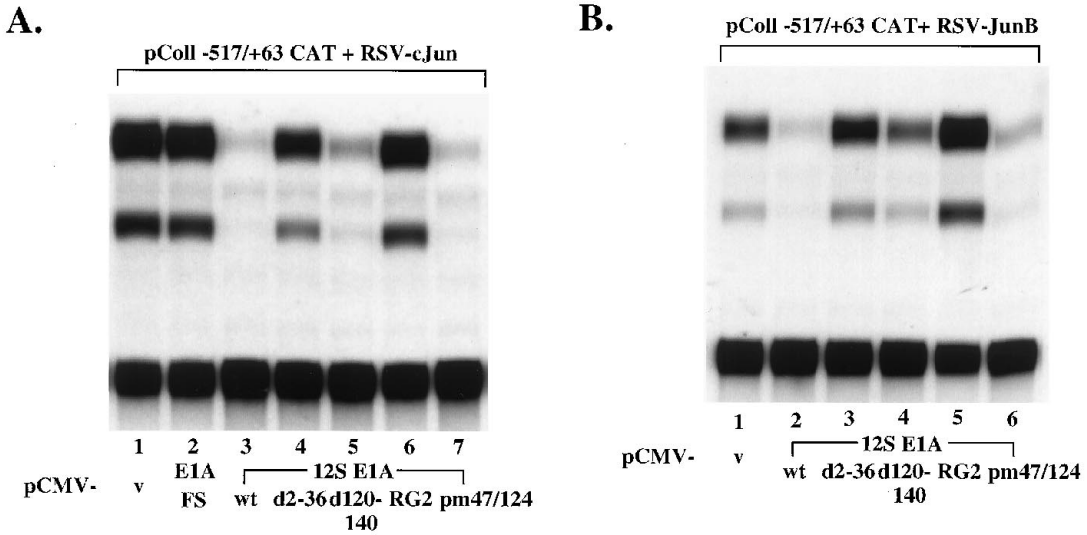


FIG. 2. cJun- and JunB-mediated transcription is repressed by adenovirus E1A. (A) pColl −517/+63 CAT and RSV-cJun (5 μg each) were cotransfected into HeLa cells with 1 μg of cytomegalovirus (CMV) promoter-driven plasmids expressing wild-type E1A and mutant proteins. (B) pColl −517/+63 CAT and RSV-JunB (5 μg each) were cotransfected with 1 μg of E1A expression plasmids into HeLa cells. v, vector alone; wt, wild type; FS, frameshift mutant.

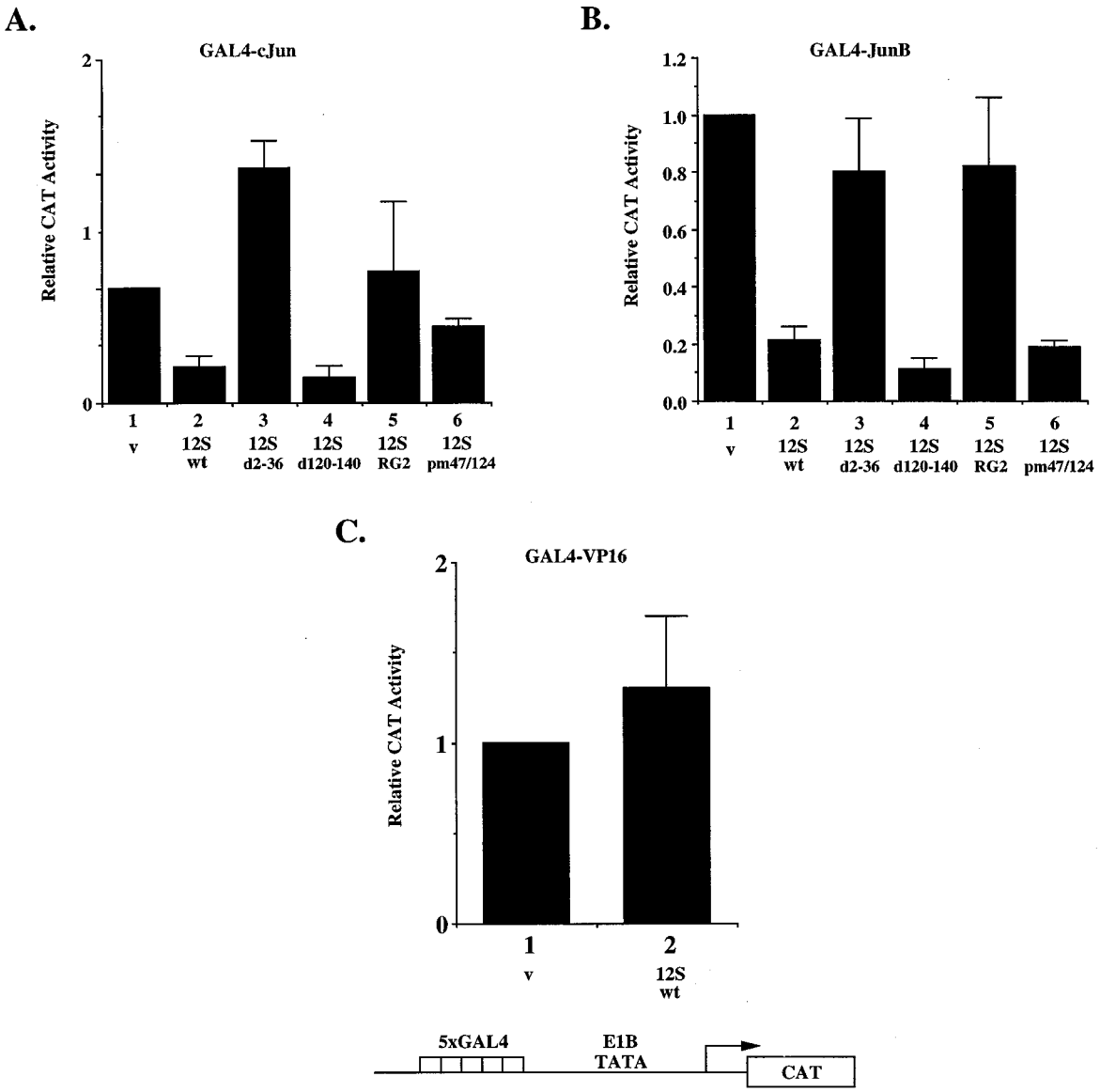


FIG. 3. The repressive effects of E1A on GAL4-cJun- and GAL4-JunB-mediated transcription are specific and correlate with the ability of E1A to bind p300. (A) GAL4-E1BCAT reporter plasmids (10 μg) and GAL4-cJun plasmids (5 μg) were cotransfected into U2OS cells with 1 μg of CMV promoter-driven plasmids expressing wild-type E1A and mutant proteins. The results represent the averages of three independent transfections and CAT assays. A relative CAT activity of 1 represents approximately 21% CAT conversion. (B) GAL4-E1BCAT reporter plasmids (10 μg) and GAL4-JunB plasmids (5 μg) were cotransfected into U2OS cells with 1 μg of CMV promoter-driven plasmids expressing wild-type E1A and mutant proteins. A relative CAT activity of 1 represents approximately 28% CAT conversion. (C) GAL4-E1BCAT reporter plasmids (10 μg) and GAL4-VP16 plasmids (5 μg) were cotransfected into U2OS cells with 1 μg of CMV promoter-driven plasmids expressing wild-type E1A. A relative CAT activity of 1 represents approximately 30% CAT conversion. The architecture of the GAL4-E1BCAT reporter plasmid is shown at the bottom. v, vector; wt, wild type.

examined the effect of p300 on Jun-mediated transcription that is repressed by E1A. As shown in Fig. 4A, overexpression of p300 efficiently overcame the repressive effect of E1A on cJun-mediated transcription. A p300 mutant, p300 *dl*10, which is defective for binding to E1A, was equally capable of relieving the repression of cJun activity by E1A. This result suggested that the ability of p300 to relieve E1A-mediated repression is not simply due to inactivation of E1A proteins by excess p300.

p300 shares significant homology with the transcriptional cofactor CBP (4). It has been shown that both proteins behave similarly in their functional interactions with E1A and the transcription factor CREB (3, 49). To determine whether CBP also plays a role in the cJun-E1A interaction, the ability of CBP to relieve E1A repression of cJun-mediated transcription was examined. As shown in Fig. 4A, at the highest concentration tested, CBP restored cJun-mediated transcription to approximately the basal level, suggesting that CBP may also be involved in the functional interplay between E1A and cJun. Indeed, it has recently been shown that CBP functions as a coactivator of cJun (11). Our protein interaction studies also identified interactions of cJun with both p300 and CBP (see below). While our studies focused on the Jun-p300 functional interaction, our data are consistent with the notion that CBP may also be a cofactor for cJun.

The same experiments were performed to analyze JunB-mediated transcription. As shown in Fig. 4B, the JunB activity

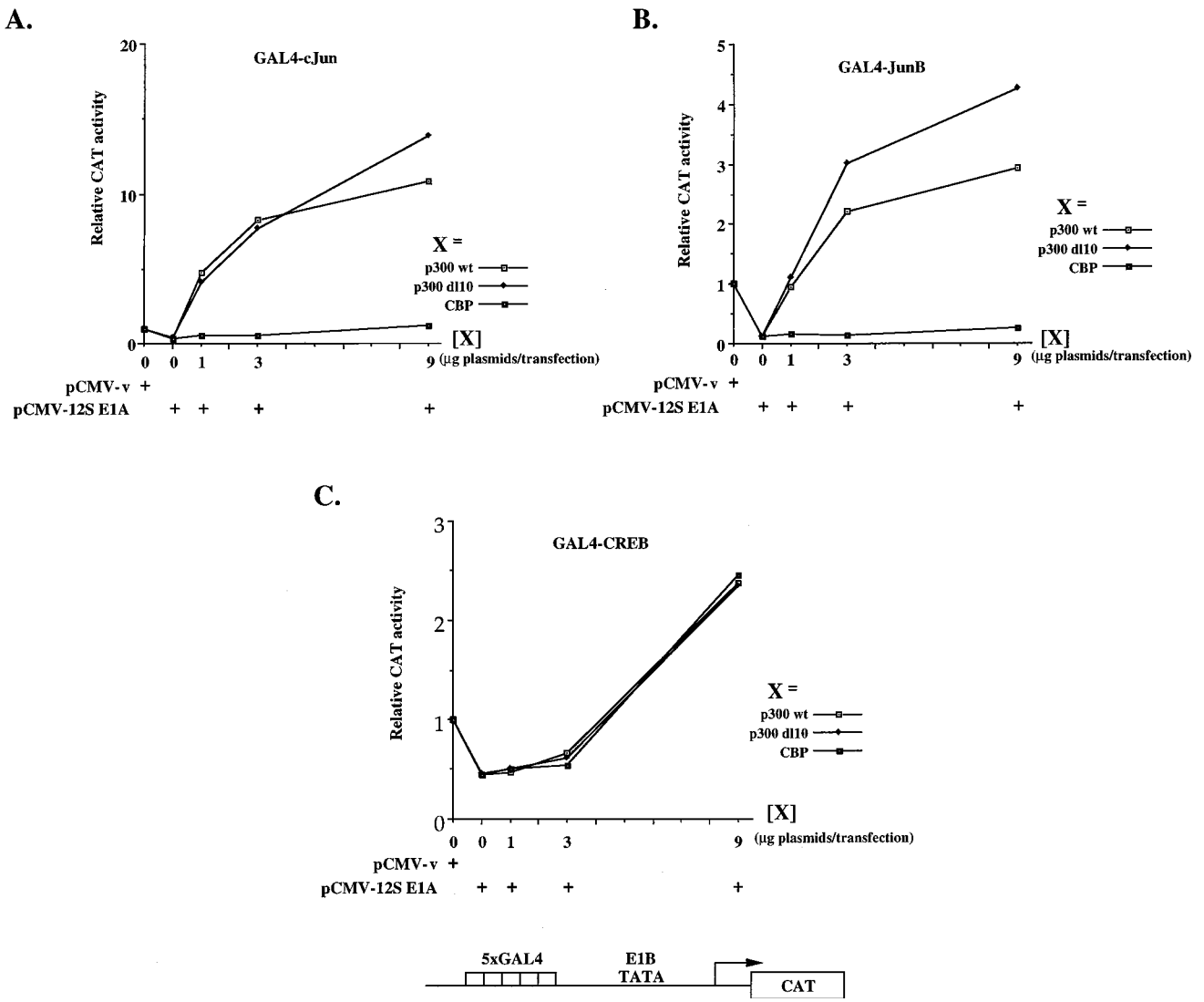


FIG. 4. Overexpression of p300 relieves the repression of GAL4-cJun-, GAL4-JunB-, and GAL4-CREB-mediated transcription by E1A. (A) GAL4-E1BCAT reporter plasmids (10 μg), GAL4-cJun plasmids (5 μg), and CMV promoter-driven 12S E1A plasmids (1 μg) (or the CMV vector [v] alone), as indicated, were cotransfected into U2OS cells with increasing amounts of p300 wild-type (wt), p300 mutant *dl*10, or CBP expression plasmid. The total amount of DNA transfected was adjusted to be equal to that of the parental vector of the expression plasmid. The results represent the averages of three independent transfections and CAT assays. A relative CAT activity of 1 represents approximately 5% CAT conversion. (B) Transfections and CAT assays were carried out essentially the same as in for panel A except that GAL4-JunB was used instead of GAL4-cJun. A relative CAT activity of 1 represents approximately 6% CAT conversion. (C) Transfections and CAT assays were carried out essentially the same as in for panel A except that GAL4-CREB was used instead of GAL4-cJun. A relative CAT activity of 1 represents approximately 3% CAT conversion. The architecture of the GAL4-E1BCAT reporter plasmid is shown at the bottom.

was repressed by E1A and the repression was relieved by overexpression of p300 as well as the p300 mutant *dl*10. In contrast, overexpression of CBP had very little effect on the ability of E1A to repress JunB-mediated transcription. As a control, GAL4-CREB was subjected to the same analyses. As shown in Fig. 4C, CBP, p300, and p300 *dl*10 were equally efficient at rescuing CREB-mediated transcription that was repressed by E1A. This result is consistent with the previous observation that p300 and CBP have similar properties with respect to their ability to function as coactivators of CREB and to interact with E1A (3, 49). Taken together, the results suggest that p300 plays a critical role in mediating the functional interaction between E1A and the Jun proteins. In addition, the findings also suggest that CBP interacts with CREB more readily than with Jun in vivo.

Further experiments were performed to determine whether the results shown in Fig. 4, obtained by using GAL4 fusions, reflected the actions of the native Jun proteins on their natural target promoters. As shown in Fig. 5, activation of the collagenase promoter by cJun and JunB (lanes 1 and 6) was repressed by E1A (lanes 2 and 7) as described previously. Similar to the results of the GAL4-based assays, both wild-type p300 and the mutant p300 *dl*10 were able to relieve E1A-mediated repression (Fig. 5, lanes 3, 5, 8, and 10). In contrast, CBP had very little effect under these assay conditions (Fig. 5, lanes 4 and 9). CREB-mediated transcription was not analyzed in this system since CREB did not activate the collagenase promoter (data not shown). However, the behaviors of CREB in assays using artificial promoters are expected to be comparable to those in assays using native promoters, since it has been shown

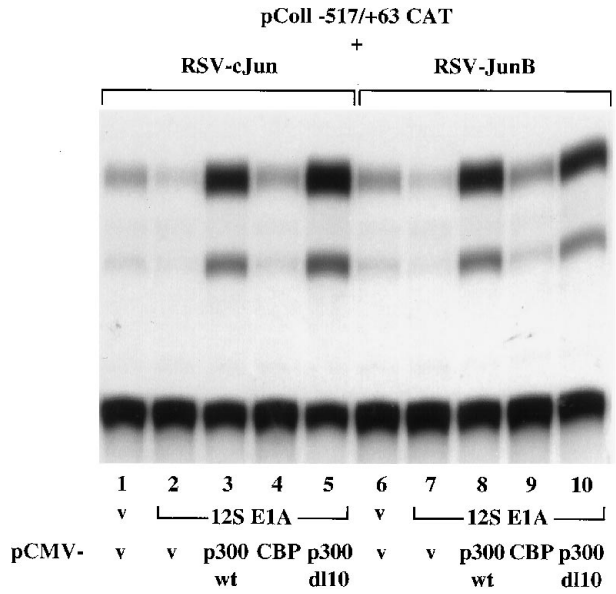


FIG. 5. Overexpression of p300 relieves E1A-mediated repression of native cJun- and JunB-dependent transcription. pColl −517/+63 CAT plasmids (5 μg), RSV promoter-driven cJun or JunB plasmids (5 μg), and CMV promoter-driven 12S E1A plasmids (1 μg) (or the vector [v] alone) were cotransfected into HeLa cells with 5 μg of wild-type (wt) p300, p300 *dl*10, or CBP expression plasmids. The total amount of DNA transfected was adjusted to be equal to that of the parental vector of the expression plasmid. The assays were repeated in three independent experiments, and the result of a representative CAT assay is shown. See text for details.

that both CBP and p300 stimulated CREB-mediated activation of a cyclic AMP response element (CRE) reporter which was repressed by wild-type 12S E1A (49).

To summarize, by using two different reporter systems, we demonstrated that E1A inhibition of cJun- and JunB-mediated transcription can be relieved by overexpression of p300. This ability of p300 was not compromised by an internal deletion that removed the E1A-interacting domain. Intriguingly, under the same assay conditions, although CBP was as efficient as p300 at rescuing CREB-mediated transcription that was repressed by E1A, CBP was much less effective at rescuing cJun and JunB activities. These results suggested that while CBP and p300 share sequence homology and show functional similarity with regard to their interactions with CREB and E1A, there may be subtle differences in their preferences for other transcription factors with which they interact.

p300 functions as a coactivator of cJun and JunB in the absence of E1A. The observation that overexpression of p300 (both wild type and mutant *dl*10) relieved E1A repression of Jun-mediated transcription raised the possibility that p300 is a coactivator of cJun and JunB in the absence of E1A. As shown in Table 1, when cotransfected with GAL4 fusion plasmids into U2OS cells, p300 stimulated the transcriptional activities of GAL4-cJun and GAL4-JunB but had no effect on transcription mediated by the GAL4 DNA-binding domain alone, GAL4-ATF1, or GAL4-VP16. These results demonstrated that p300 could function specifically as a coactivator of cJun and JunB. Under the same assay conditions, CBP had less of an effect, if any, on the activities of cJun and JunB, although CBP can physically interact with the Jun proteins in vitro (see Fig. 8C) and has recently been shown to enhance cJun-mediated activation of the collagenase promoter in F9 cells (11). This apparent paradox will be discussed later (see Discussion). As expected from the results shown in Fig. 4, p300 *dl*10 also was able to stimulate cJun- and JunB-mediated transcription (data not shown). Therefore, the internal deletion that p300 *dl*10 sustained apparently did not affect its ability to function as a coactivator of cJun and JunB.

We next examined the potential cofactor function of p300 by using the collagenase promoter which was shown to be activated by the Jun proteins and is repressed by E1A in a p300-dependent manner (Fig. 1 and 2). As shown in Fig. 6, the wild-type collagenase promoter was activated by p300 but not by the vector DNA alone (lanes 1 and 2). Significantly, the p300-induced activation appeared to be dependent on the TRE site, as the mutant collagenase promoter lacking the TRE was not responsive to p300 (Fig. 6, lanes 3 and 4). The fact that p300 activated this promoter via the TRE site is consistent with the hypothesis that p300 functions as a cofactor for the Jun proteins.

Differential response of Jun-mediated transcription to E1A upon overexpression of wild-type p300 versus that of the mutant *dl*10. The findings that p300 activated cJun- and JunB-mediated transcription (Table 1 and Fig. 6) and that p300 relieved E1A repression of Jun-dependent transcription (Fig. 4 and 5) strongly suggest that p300 is a cofactor for the Jun proteins. The latter experiment was also designed to demonstrate that p300 is the mediator protein between Jun and E1A and that E1A modulates Jun activity through p300. However, because of the fact that p300 activated Jun-dependent transcription, relief of E1A repression of Jun-dependent transcription by p300 could have occurred regardless of whether E1A targeted p300. We therefore performed E1A titration experiments based on the following rationale. If p300 is the part of the Jun transcription complex that is targeted by E1A, when the mutant *dl*10 is expressed in the cells to a significant level, the modified Jun transcription complex containing *dl*10 is expected to be more resistant to the repressive effect of E1A. Increasing amounts of E1A were cotransfected with a fixed amount (9 μg) of p300, p300 *dl*10, or vector DNA, and tran-

TABLE 1. Stimulation of cJun- and JunB-mediated transcription by overexpression of p300[a]

Cotransfected fusion plasmid	% CAT conversion with:			Fold activation with:	
	pCMV-vector	pCMV-p300	pCMV-CBP	p300	CBP
pGAL4-vector	0.5 ± 0.1	0.6 ± 0.3	0.7 ± 0.3	1.2 ± 0.3	1.2 ± 0.3
pGAL4-ATF1	0.8 ± 0.4	0.7 ± 0.2	0.9 ± 0.4	0.9 ± 0.2	1.2 ± 0.5
pGAL4-cJun	3.0 ± 0.7	8.8 ± 2.6	2.6 ± 0.5	2.9 ± 0.3	0.9 ± 0.2
pGAL4-JunB	8.9 ± 3.0	25.1 ± 2.4	12.1 ± 3.5	3.1 ± 1.4	1.5 ± 0.6
pGAL4-VP16	35.3 ± 8.0	40.9 ± 10.4	35.2 ± 13.6	1.2 ± 0.1	1.0 ± 0.3

[a] GAL4-E1BCAT reporter plasmid (10 μg) was transfected into U2OS cells with 5 μg of GAL4 fusion plasmid and 5 μg of plasmid encoding p300 or CBP. Transfections and CAT assays were carried out as described in Materials and Methods. The results are expressed as means ± standard deviations of three independent transfections.

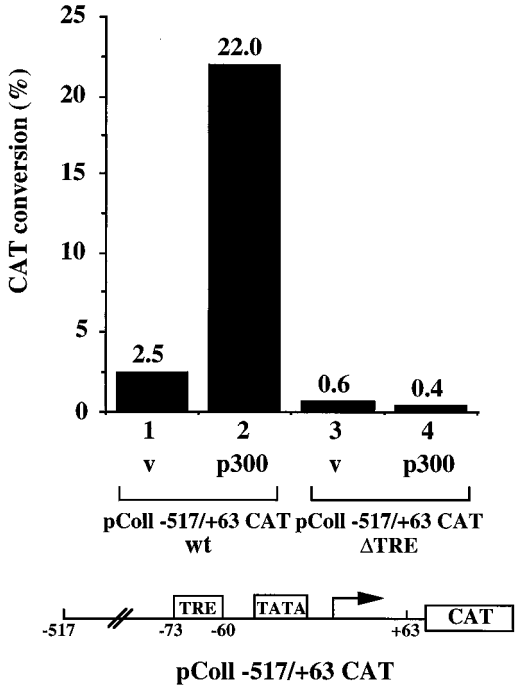


FIG. 6. p300 activates the collagenase promoter. Five micrograms of pColl −517/+63 CAT or pColl −517/+63 CAT ΔTRE was cotransfected into HeLa cells with either the CMVβ vector plasmid (lanes 1 and 3) or CMVβ-p300 (22) (lanes 2 and 4). The percentages of CAT conversion shown above the bars are the averages of two independent transfections and CAT assays. The architecture of the collagenase promoter-driven CAT reporter is shown at the bottom. v, vector; wt, wild type.

scription mediated by GAL4-JunB or GAL4-cJun was measured (Fig. 7). The dose response of GAL4-JunB-mediated transcription to E1A repression differed significantly depending upon the cotransfected plasmid (p300, p300 *dl*10, or the vector). In the absence of transfected p300 (the parental vector CMVβ was added so that the total amount of DNA was identical in all transfections), JunB-mediated transcription was repressed by about 20-fold with only 0.5 μg of the E1A plasmid (Fig. 7A, upper panel, and the vector curve in the lower panel). However, when cotransfected with p300 *dl*10, GAL4-JunB-mediated transcription was virtually unaffected by E1A, even at the highest dose of E1A used in the experiment (2 μg). This is consistent with the prediction that the presumed JunB-p300 *dl*10-containing transcription complex should not respond to E1A. More importantly, this effect of p300 *dl*10 is significantly more pronounced than that of the wild-type p300, as evidenced by the difference between the E1A dose-response curve of GAL4-JunB in the presence of overexpressed p300 and that of GAL4-JunB in the presence of p300 *dl*10 (Fig. 7A). Therefore, the E1A-interacting domain of p300 appeared to be required for repression of JunB activity by E1A. Taken together, these results suggest that p300 is directly involved in the downregulation of JunB activity by E1A.

The same analysis was performed on GAL4-cJun. In the absence of transfected p300, GAL4-cJun activity was repressed by 12S E1A as expected (Fig. 7B, lower panel, vector curve). Similar to the results described above for JunB, when a fixed amount of p300 was cotransfected, GAL4-cJun-mediated transcription became resistant to E1A repression (Fig. 7B, p300 curve). Therefore, in both cases (cJun and JunB), an elevated p300 level within the cells renders Jun-mediated transcription more resistant to E1A repression. This reinforces the notion that the coactivator function of p300 was utilized by E1A to gain control over the transcriptional activities of the Jun proteins. Interestingly, under the conditions when either wild-type p300 or the mutant p300 *dl*10 was overexpressed in cells, E1A not only failed to repress cJun activity but also seemed to activate GAL4-cJun-mediated transcription to a modest extent. The stimulatory effect of E1A was more pronounced when the analysis was performed in cells expressing the p300 mutant *dl*10 (Fig. 7B). At present, it is unclear why JunB and cJun responded to E1A somewhat differently when p300 was overexpressed. As JunB and cJun differ biochemically and functionally in many aspects (7, 58), it is possible that these differences dictate their differential dynamic interactions with p300 and E1A. Finally, the above findings also suggested that the level of p300 within the cell may be an important parameter that determines the outcome of the functional interactions between E1A and the Jun proteins.

Physical interactions of p300 or CBP with c-Jun and JunB. The functional interactions between the Jun proteins and p300 predicted a physical interaction between these proteins. To address this issue, we performed GST affinity matrix-based assays to determine whether p300 physically interacts with cJun and JunB. As shown in Fig. 8A, in vitro-translated, ^{35}S-labeled p300 was captured by GST-12S E1A (lane 7) and GST-YY1 (lane 3), as expected, but not by GST alone (lane 2) or GST-VP16 (lane 6). A substantial amount of p300 was also retained by GST-cJun (lane 4). Intriguingly, although functional assays clearly demonstrated that p300 activated JunB- as well as cJun-mediated transcription, virtually no p300 was retained by the GST-JunB affinity column (lane 5). Consistent with the hypothesis that the interactions between E1A and Jun proteins are mediated by p300, 12S E1A did not interact directly with either cJun or JunB under the same assay conditions (data not shown).

One possibility for the failure to detect JunB-p300 interaction is that posttranslational modifications are necessary for this interaction to occur. We therefore performed the reciprocal GST binding experiment which used JunB produced in reticulocyte lysates by in vitro translation. As shown in Fig. 8B, JunB was captured by both the M (central) and the C-terminal portions of p300 (lanes 2 and 3) but not by either the N-terminal region of p300 or the GST moiety alone (lanes 4 and 1). These results demonstrated that physical interactions between JunB and p300 can occur under certain conditions. This is consistent with the functional data described earlier that suggested an activator-coactivator relationship between JunB and p300. However, more extensive studies are required to further characterize the molecular nature of such interactions.

Next, the interaction of the p300 homolog CBP with the Jun proteins was examined. GST-cJun and GST-JunB were incubated with HeLa whole-cell extracts. After being washed extensively, the bound proteins were analyzed by Western blotting for the presence of CBP, using a CBP-specific polyclonal antibody (C-20). As shown in Fig. 8C, CBP was captured by GST-cJun (lane 3) and, weakly, by GST-JunB (lane 4) but not by GST alone (lane 2). As a positive control, CBP was shown to interact with E1A under these assay conditions (Fig. 8C, lane 5). The interaction between CBP and the Jun proteins is consistent with a role for CBP in Jun-dependent transcription.

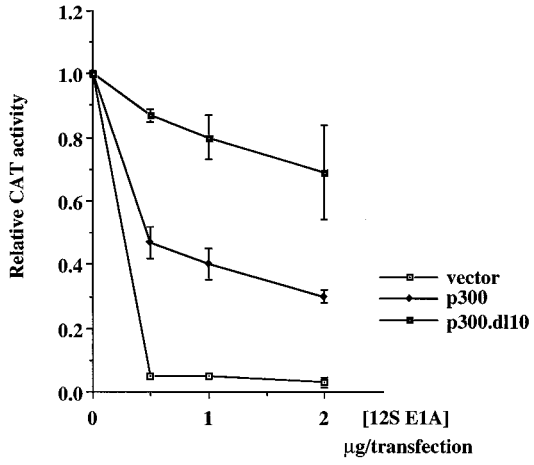
To summarize, we demonstrated that cJun interacts with both p300 and CBP. JunB seemed to also interact with these two proteins, although the binding affinities and the requirements for the binding reactions may be different. The physical interaction of the Jun proteins with p300 or CBP is consistent with the coactivator role of p300 or CBP. Further character-

A. GAL4-JunB

CMVβ-	CAT \ 12S E1A (μg)	0	0.5	1	2
vector	CAT conversion (%)	5.9	0.3	0.3	0.2
	relative activity	1	0.05	0.05	0.03
p300	CAT conversion (%)	27.2	12.4	11.2	9.8
	relative activity	1	0.47	0.40	0.30
p300 dl10	CAT conversion (%)	41.6	36.2	33.3	28.7
	relative activity	1	0.87	0.80	0.69

B. GAL4-cJun

CMVβ-	CAT \ 12S E1A (μg)	0	0.5	1	2
vector	CAT conversion (%)	1.3	0.3	0.3	0.5
	relative activity	1	0.21	0.23	0.38
p300	CAT conversion (%)	11.8	11.1	23.0	26.8
	relative activity	1	0.95	2.05	2.39
p300 dl10	CAT conversion (%)	13.5	25.5	35.1	53.5
	relative activity	1	1.89	2.60	3.97



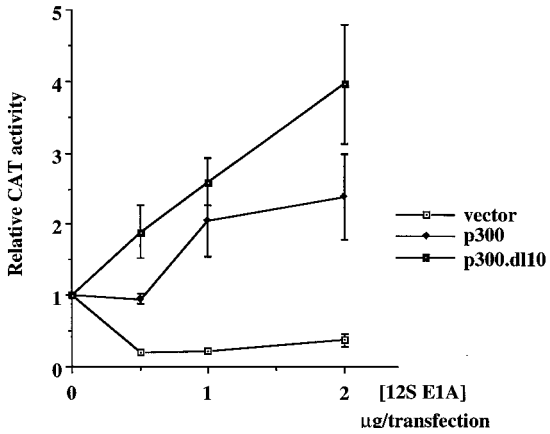


FIG. 7. Differential responses of Jun-mediated transcription to E1A repression upon overexpression of p300 or a p300 mutant defective for interaction with E1A. (A) GAL4-E1BCAT reporter (10 μg), GAL4-JunB (5 μg), and 9 μg of either p300, p300 *dl*10, or the parental vector plasmid (vector) were cotransfected with increasing amounts of CMV promoter-driven 12S E1A plasmids into U2OS cells. The total amount of DNA transfected was adjusted to be equal to that of the parental vector of the expression plasmid. The results shown in the table represent the averages of three independent transfections and CAT assays. Relative CAT activities were calculated by normalizing values of CAT conversion against the ones obtained in the absence of 12S E1A under each condition, i.e., with or without overexpression of wild-type or mutant p300. The lower panel shows a plot of the relative CAT activities based on the same set of results. Thus, a relative CAT activity of 1 represents 5.9% CAT conversion for the vector alone, 27.2% for p300, and 41.6% for *dl*10 transfections. (B) Essentially the same experiments as shown in panel A except that GAL4-cJun was used instead of GAL4-JunB. The results were presented in the same way as for panel A.

ization of the protein-protein interactions focused on cJun and p300, as described below.

Identification of a p300-interacting domain within cJun that coincides with a transcriptional activation domain. To identify the domain within cJun that mediates its physical interaction with p300, various N- and C-terminally deleted cJun proteins were fused to the GST moiety and the fusion proteins were incubated with ^{35}S-labeled p300. As shown in Fig. 9, both aa 1 to 246 (lane 3) and aa 1 to 193 (lane 4) of cJun bound p300. Further C-terminal deletion mutants (aa 1 to 169 and 1 to 117; lanes 5 and 6) significantly reduced the ability of cJun to bind p300, while aa 1 to 79 (lane 7) and the GST moiety alone (lane 2) did not capture a detectable amount of p300. Analyses of two N-terminal deletion mutants showed that GST-cJun aa 96 to 246 bound p300 (lane 9) whereas deletion to aa 138 (GST-cJun aa 138 to 246) significantly reduced its ability to interact with p300 (lane 8). Taken together, these data suggested that the aa 96 to 193 region may contain a domain required for interaction of cJun with p300. Interestingly, this region coincides with activation domain 1 (aa 92 to 196), which has been described previously (8). Although the determination of the functional significance of the interactions between p300 and activation domain 1 of cJun requires further studies, this interaction is nevertheless consistent with the role of p300 as a coactivator of cJun.

Identification of two separate regions of p300 that can mediate the interaction of p300 with cJun. To further explore the cJun-p300 physical interaction, various regions of p300 were analyzed by GST affinity matrix-based assays for their ability to interact with cJun. As shown in Fig. 10A, p300 was divided into three parts, the N-terminal (aa 1 to 596), central (aa 744 to 1571), and C-terminal (aa 1572 to 2370) regions, each of which was fused to GST (43). These GST-p300 fusion proteins were incubated with ^{35}S-labeled, in vitro-translated cJun. As shown in Fig. 10A, cJun was captured by both the N- and the C-terminal portions of the p300 protein but not by the central portion, suggesting that there are two separate regions within p300 that are capable of mediating interaction of p300 with cJun.

The reciprocal-binding experiment was also performed with in vitro-translated p300 and with GST-cJun aa 1 to 246 as the affinity matrix. The N (aa 1 to 1256)- and C (aa 1257 to 2414)-terminal halves of p300 were synthesized and ^{35}S labeled in vitro. As shown in Fig. 10B, both the N- and the C-terminal halves of the p300 protein bound GST-cJun but not the GST moiety alone (lanes 16 to 18 and 4 to 6, respectively), which is

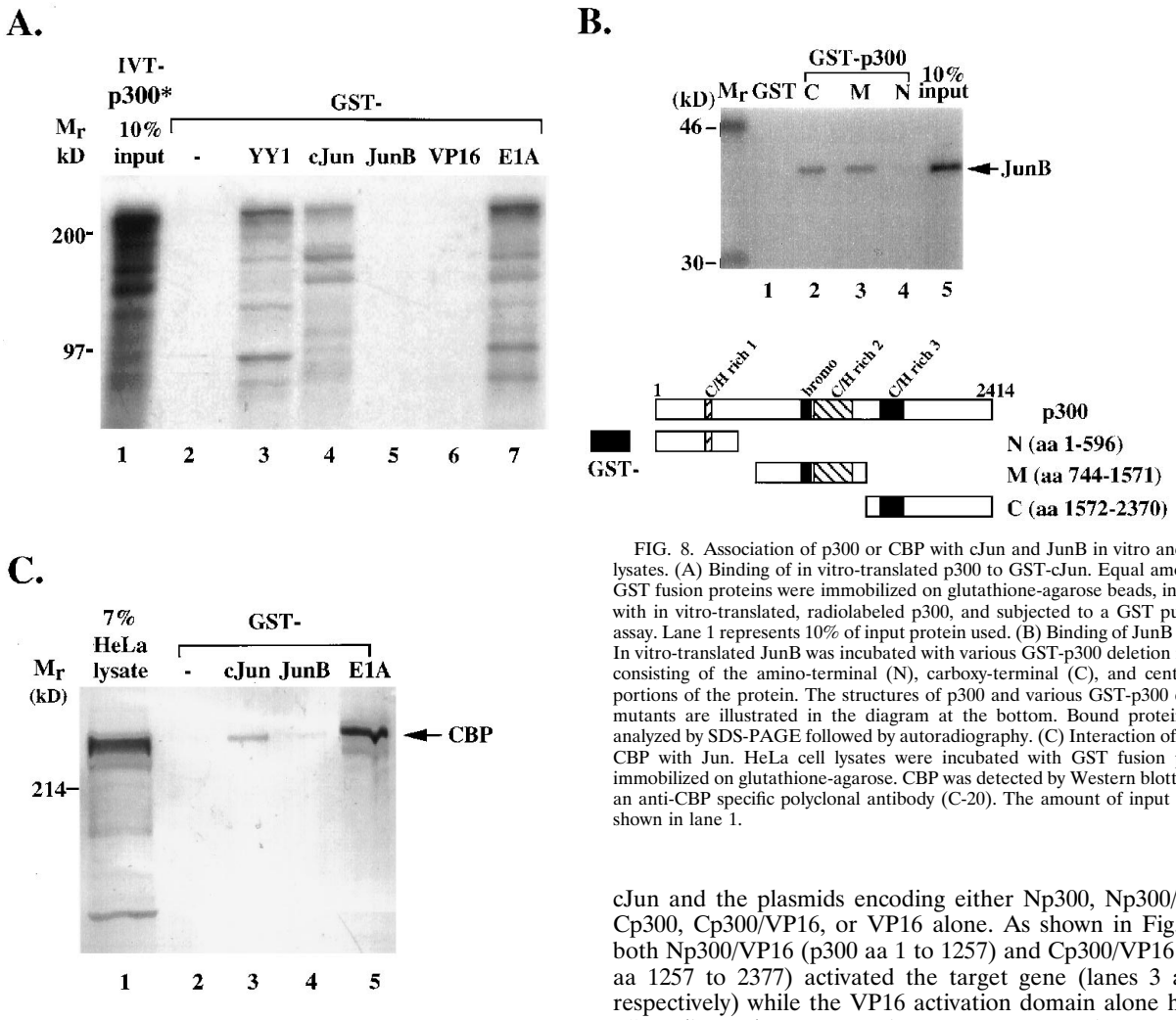


FIG. 8. Association of p300 or CBP with cJun and JunB in vitro and in cell lysates. (A) Binding of in vitro-translated p300 to GST-cJun. Equal amounts of GST fusion proteins were immobilized on glutathione-agarose beads, incubated with in vitro-translated, radiolabeled p300, and subjected to a GST pull-down assay. Lane 1 represents 10% of input protein used. (B) Binding of JunB to p300. In vitro-translated JunB was incubated with various GST-p300 deletion mutants consisting of the amino-terminal (N), carboxy-terminal (C), and central (M) portions of the protein. The structures of p300 and various GST-p300 deletion mutants are illustrated in the diagram at the bottom. Bound proteins were analyzed by SDS-PAGE followed by autoradiography. (C) Interaction of cellular CBP with Jun. HeLa cell lysates were incubated with GST fusion proteins immobilized on glutathione-agarose. CBP was detected by Western blotting with an anti-CBP specific polyclonal antibody (C-20). The amount of input lysate is shown in lane 1.

consistent with the idea that there are at least two independent cJun-interacting domains within p300. The C-terminal half of p300 was further divided into a series of smaller, unidirectional deletion mutants as shown in Fig. 10B. These mutants were in vitro translated, ^{35}S labeled, and incubated with GST-cJun. Removal of aa 1257 to 1752 had no discernible effect on the binding of the p300 mutants to cJun (lanes 4 to 12). However, further deletion from aa 1752 to 1869 (the *Aha*II ATG template) severely reduced the ability of p300 to bind GST-cJun (Fig. 10B, lanes 13 to 15), suggesting that this region of p300 (aa 1752 to 1869) contains an element which is critical for cJun binding.

To determine whether cJun can interact directly with the N- and the C-terminal portions of p300, cJun was purified from bacteria to near homogeneity by heparin-agarose chromatography as described previously (19). The purified bacterial cJun was incubated with GST, GST-Np300, and GST-Cp300 individually. After extensive washing, the bound cJun was detected with α-cJun antibody. As shown in Fig. 10C, both the N- and C-terminal portions of p300 captured purified cJun proteins (lanes 2 and 3), suggesting that both domains are capable of directly interacting with cJun in vitro.

Finally, the cJun-p300 interaction was examined in HeLa cells by a two-hybrid-based assay. The reporter pGAL4-E1BCAT was transfected into HeLa cells together with GAL4-cJun and the plasmids encoding either Np300, Np300/VP16, Cp300, Cp300/VP16, or VP16 alone. As shown in Fig. 10D, both Np300/VP16 (p300 aa 1 to 1257) and Cp300/VP16 (p300 aa 1257 to 2377) activated the target gene (lanes 3 and 5, respectively) while the VP16 activation domain alone had no effect (lane 6). As controls, Np300/VP16 and Cp300/VP16 were shown not to activate GAL4-E1BCAT when the GAL4 DNA-binding domain was used as bait (43) (data not shown). The fact that the N-terminal half of p300 (Np300) without the VP16 activation domain also activated cJun-dependent transcription (Fig. 10D, lane 2) may imply that this region contains both a cJun-interacting protein and a transcriptional activation domain. It is not clear why the C-terminal half of p300 (Cp300) caused a reduction in cJun-mediated transcription (Fig. 10D, lane 4). Regardless, the data presented in Fig. 10D are consistent with the in vitro binding data indicating that both the N- and the C-terminal halves of p300 contain an element that is capable of mediating the interaction between p300 and cJun (Fig. 10C). Taken together, these results demonstrate that the p300 protein contains two separate cJun-interacting domains.

DISCUSSION

p300 mediated the repression of AP-1 activity by E1A. Regulation of AP-1 activity by adenovirus E1A is complicated. It has been suggested that E1A represses AP1-dependent transcription; this is exemplified by the collagenase gene promoter, which is repressed by E1A through an AP-1 site (27, 52). E1A has also been demonstrated to activate transcription through AP-1 sites; e.g., activation of transcription of cJun by E1A is mediated by an AP-1 site (1, 18, 40, 60). It has been proposed that differential dimer formation is responsible for the appar-

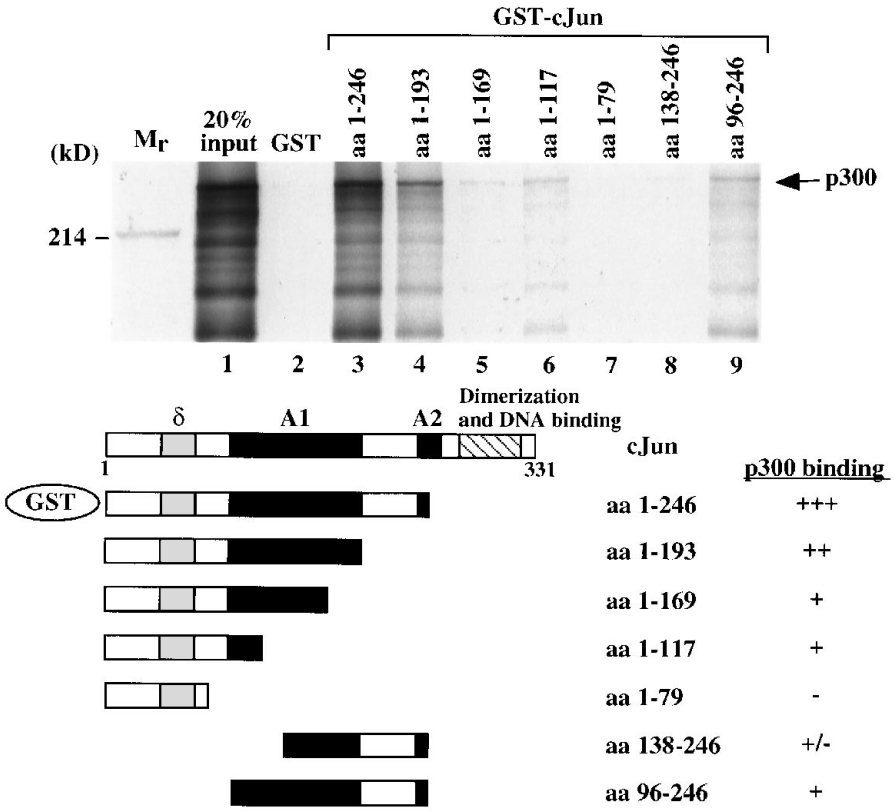


FIG. 9. Identification of a p300-interacting domain within cJun. In vitro-translated p300 was incubated with various GST cJun deletion mutants. The relative binding activities are indicated qualitatively by + and −. p300 is indicated by the arrow on the right. Molecular mass markers are shown on the left. The GST-cJun proteins are shown schematically in a diagram at the bottom; A1 and A2 are activation domains 1 and 2.

ent opposite effects of E1A on AP1-dependent transcription (28). Interestingly, mutational analyses have shown that both effects of E1A require E1A CR1 but not CR2 or CR3 (52, 60). A recent report further demonstrated the importance of the N-terminal region of E1A in the repression of the collagenase promoter (20). It has been established that the N-terminal region and CR1 constitute the p300-interacting domain of E1A while CR1 and CR2 form a binding site for the pRB family of proteins (66, 68).

In this report, evidence that defines a critical role for p300 in the repression of AP-1 activity by E1A is provided. By using deletion and point mutants of E1A, the ability of E1A to downregulate cJun- and JunB-mediated transcription was shown to be correlated with its ability to bind p300. Theoretically, transfection assays using native forms of cJun and JunB may be complicated by the formation of dimers with other AP-1-related proteins. Thus, a parallel assay system in which the Jun proteins were analyzed as GAL4 fusions was designed. These GAL4-Jun proteins lack their own DNA-binding and dimerization domains and are directed to the target promoter via the DNA-binding domain of GAL4. The results of the GAL4-based assays fully recapitulated what was observed for the native forms of cJun (references 20 and 52 and this study) and JunB (this study) or even the composite AP-1 activity (27, 52) with natural promoters. Our results convincingly identified cJun and JunB as two individual members of the AP-1 family whose activities are modulated by E1A in a p300-dependent manner. Furthermore, this study also provided the first evidence that downregulation of cJun- and JunB-mediated transcription is independent of the DNA-binding and dimerization domains of these proteins.

To further explore the functional interactions among the Jun proteins, p300, and E1A, we showed that overexpression of p300 rescued Jun-mediated transcription that was inhibited by E1A (Fig. 4). The ability of p300 to relieve E1A-induced transcriptional repression is not due to inactivation of the E1A proteins since p300 mutant *dl*10, which is defective for E1A binding, was also capable of relieving repression (Fig. 4). Furthermore, overexpression of the mutant *dl*10 reduced the responsiveness of Jun-mediated transcription to E1A repression, indicating that the E1A-binding domain of p300 is directly involved in this process (Fig. 7). Altogether, these results strongly suggest that the endogenous p300 is a natural target for modulation of AP-1 activity by E1A.

JunB is 44% homologous to cJun at the amino acid level, and the two differ biochemically and biologically (7, 58). Despite the differences between JunB and cJun, they share some properties. For instance, both JunB- and cJun-dependent transcription were repressed by E1A in a p300 binding-dependent manner (this study). Under conditions in which cells overexpressed p300, both JunB- and cJun-mediated transcription became more resistant to the repressive effect of E1A (Fig. 7). In the case of JunB, overexpression of p300 *dl*10, which lacks the E1A-interacting domain, rendered JunB-dependent transcription more refractory to E1A repression than did overexpression of the wild-type p300 (Fig. 7A). This result fulfilled the prediction based on the hypothesis that p300 is directly involved in E1A repression of JunB-dependent transcription. An

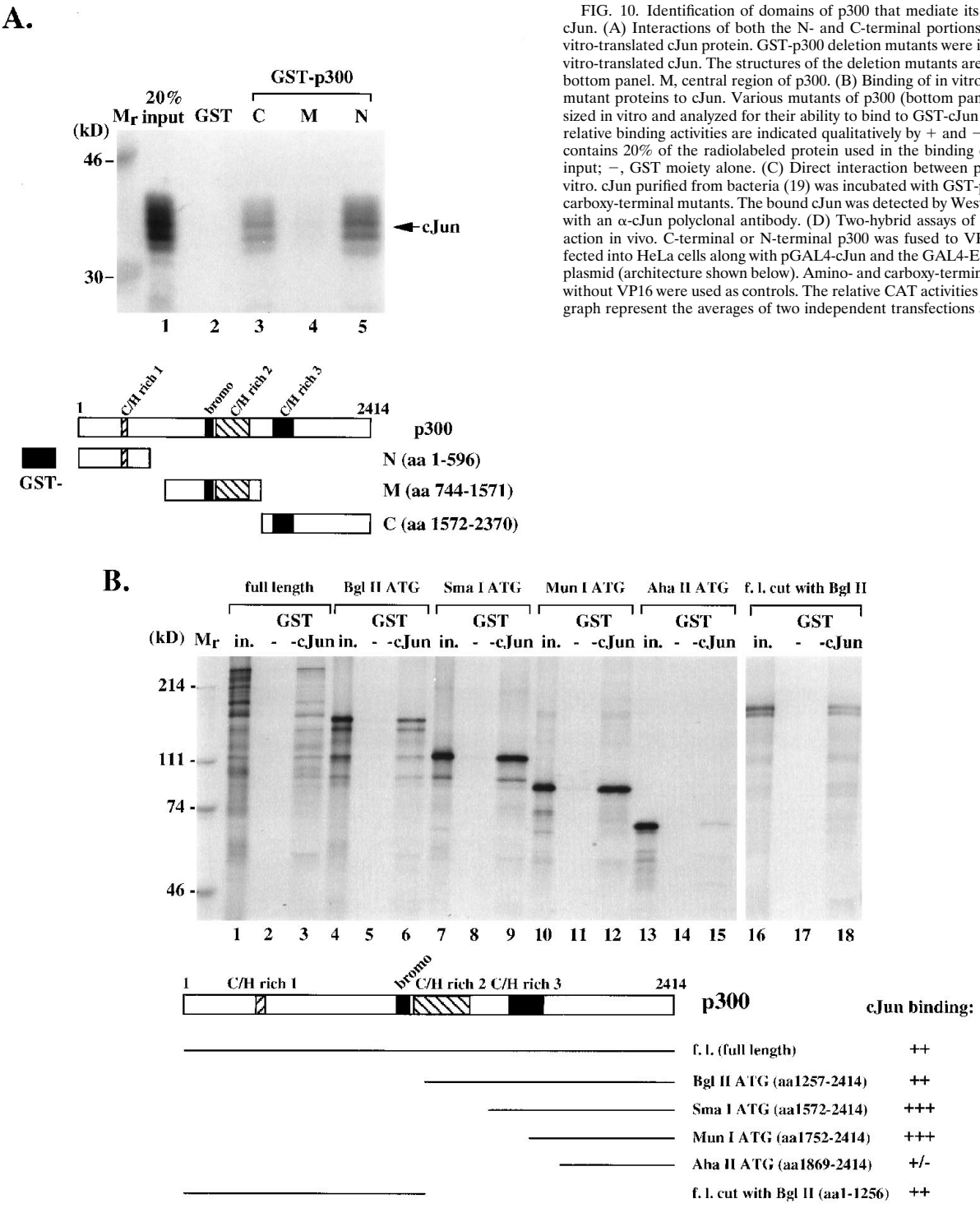
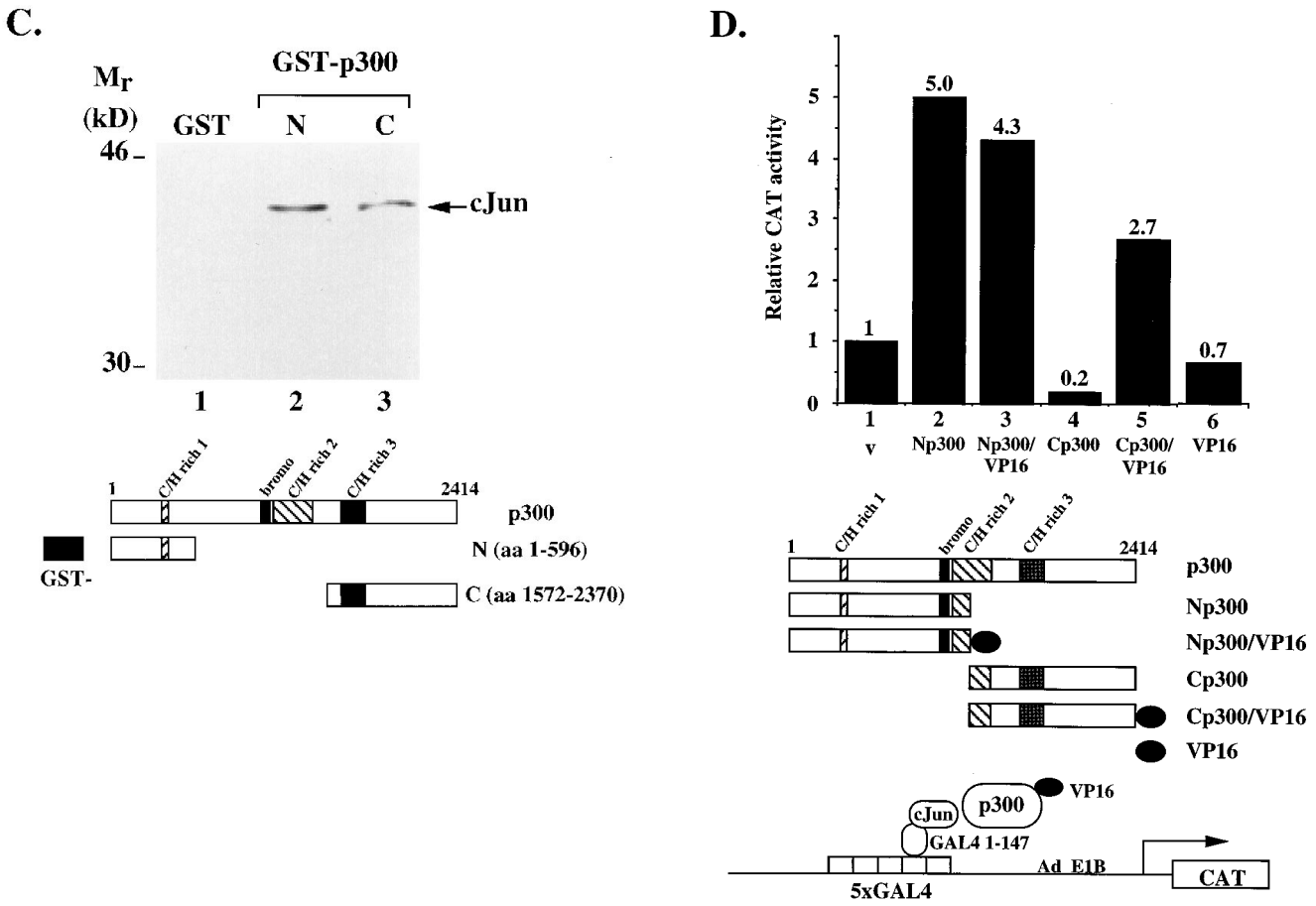


FIG. 10. Identification of domains of p300 that mediate its interaction with cJun. (A) Interactions of both the N- and C-terminal portions of p300 with in vitro-translated cJun protein. GST-p300 deletion mutants were incubated with in vitro-translated cJun. The structures of the deletion mutants are indicated in the bottom panel. M, central region of p300. (B) Binding of in vitro-translated p300 mutant proteins to cJun. Various mutants of p300 (bottom panel) were synthesized in vitro and analyzed for their ability to bind to GST-cJun aa 1 to 246. The relative binding activities are indicated qualitatively by + and −. The input lane contains 20% of the radiolabeled protein used in the binding experiments. in., input; −, GST moiety alone. (C) Direct interaction between p300 and cJun in vitro. cJun purified from bacteria (19) was incubated with GST-p300 amino- and carboxy-terminal mutants. The bound cJun was detected by Western blot analysis with an α-cJun polyclonal antibody. (D) Two-hybrid assays of cJun-p300 interaction in vivo. C-terminal or N-terminal p300 was fused to VP16 and cotransfected into HeLa cells along with pGAL4-cJun and the GAL4-E1BCAT reporter plasmid (architecture shown below). Amino- and carboxy-terminal p300 moieties without VP16 were used as controls. The relative CAT activities shown in the bar graph represent the averages of two independent transfections and CAT assays.

interesting observation that hinted at the differences in behavior of JunB and cJun was made. Specifically, although the response of cJun to E1A in cells that contained elevated levels of p300 shared an important feature with JunB, i.e., both transcription events became refractory to E1A repression, increasing amounts of E1A not only failed to repress cJun-mediated transcription but also induced it to a modest level (Fig. 7B). This result may appear paradoxical since cJun-dependent transcription has been shown to be repressed by E1A under conditions in which p300 was not overexpressed (Fig. 2 to 4A, 5, and 7B). However, it provided a glimpse of the differential behavior of these two proteins, as manifested in their responses to E1A when p300 levels vary. It is possible that the structural differences between JunB and cJun contribute to their differential behavior in the presence of E1A in response to the level of p300 in the cells. Further studies of JunB and



cJun proteins in the context of p300 and E1A should provide insight into the dynamic interactions among these three proteins.

p300 as a coactivator of cJun and JunB. In the absence of E1A, p300 stimulated both cJun- and JunB-mediated transcription (Table 1 and Fig. 6), suggesting that p300 functions as a coactivator of cJun and JunB. This is not surprising since p300 as well as its homolog protein, CBP, has been shown to function as a coactivator of CREB (3, 15, 41, 49). To our knowledge, this study for the first time provided direct evidence to demonstrate the role of p300 as a coactivator of cJun and JunB. There was, however, indirect evidence published previously that is consistent with our conclusion. Using antibody microinjection assays, it has been shown that a TRE (AP-1 site)-containing *lacZ* reporter activity was blocked by CBP antiserum (5). Since the CBP antiserum was raised against a synthetic peptide that is highly conserved between CBP and p300 (CBP amino acids 634 to 648; see references 4 and 49 for sequence comparison), it is possible that the observed effects of the CBP antiserum on TRE-mediated transcription were due to the blocking of both CBP and p300. More recently, CBP was suggested to function as a cofactor for cJun (11), which is consistent with our findings that p300 is a coactivator of the Jun proteins.

Physical interactions between p300 and the Jun proteins. The functional interactions between the Jun proteins and p300 predicted a physical interaction between these proteins. This issue was addressed by both in vitro and in vivo protein-protein interaction assays (Fig. 8 to 10). The finding that both cJun and JunB interacted with p300 provided biochemical evidence to support the hypothesis that p300 functions as a coactivator of the Jun proteins. In this paper, most of the studies focused on the cJun-p300 interaction. We identified a region in cJun that is involved in the interaction with p300, and it corresponds to a transcriptional activation domain identified previously (8). At first approximation, this finding is consistent with the idea that p300 functions as a coactivator of cJun. To firmly establish the significance of the cJun-p300 interaction that may occur at activation domain 1 of cJun, it is necessary to perform an extensive mutagenesis study to determine whether a correlation between the physical and functional interactions of the two proteins exists. Recently, it was reported that aa 1 to 87 of cJun are essential for interaction with CBP (11), while our results indicated that most of this region was dispensable for interaction of cJun with p300 (Fig. 9). It remains to be determined whether the apparent discrepancy between these results is due to differences in experimental design or is a genuine reflection of the intrinsic difference between p300 and CBP.

We also systematically analyzed different regions of p300 that may be involved in the interaction of p300 with cJun. This effort revealed two separate regions (N and C terminal) within p300 that can independently interact with cJun both in vitro (Fig. 10A to C) and in vivo (Fig. 10D). Further deletional analysis of the C-terminal cJun-interacting region showed that 117 amino acids (aa 1752 to 1869) are essential for interaction of this region of p300 with cJun (Fig. 10B, lanes 10 to 15). The other cJun-interacting domain resides in the N-terminal portion of p300 (aa 1 to 596). Recently, Bannister et al. showed

that the CREB-interacting domain of CBP (aa 461 to 662) can interact with cJun in a two-hybrid assay (11). Therefore, it is possible that the interaction we detected between the N-terminal portion of p300 and cJun was also mediated by the CREB-interacting domain.

Since bacterially purified cJun interacts with both GST-Np300 and GST-Cp300 (Fig. 10C), the in vitro cJun-p300 interaction seems direct and can occur without posttranslational modifications, such as phosphorylation. This is similar to the YY1-p300 (43) and c-Myb–CBP (17) interactions reported previously. In both cases, p300 (or CBP) and YY1 (or c-Myb) purified from bacterial have been found to interact with one another in vitro. This type of interaction is different from the inducible CREB-CBP interaction which occurs upon phosphorylation of serine 133 of CREB (5, 15). However, at this stage, we cannot rule out the possibility that the cJun-p300 interaction in vivo requires posttranslational events, such as phosphorylation. It is also possible that phosphorylation of either cJun or p300 modulates the strength of the interaction.

Potential substrate preference of the coactivator CBP. Previously, both CBP and p300 have been shown to function as coactivators of the transcriptional factor CREB (3, 15, 41, 49). Consistent with these reports, our study demonstrated that overexpression of either CBP or p300 relieved the E1A-mediated repression of CREB-dependent transcription, as the dose-response curves for CBP and p300 in this assay appeared identical (Fig. 4C). However, under the same assay conditions, CBP had no significant effect on the transcriptional activity of JunB and a very modest effect on cJun (Fig. 4A and B). Since the same amount of transfected CBP efficiently rescued CREB-dependent transcription but not Jun-dependent transcription that was repressed by E1A, the results suggested that CBP interacts more readily with CREB than with Jun in vivo. Therefore, despite the high degree of sequence homology and functional similarity of these two coactivators, p300 and CBP may have distinct preferences for the transcription factors with which they interact.

Consistent with the idea that CBP may have a preference for CREB versus Jun proteins, CBP was found to have no significant coactivator activity for either cJun or JunB in our cotransfection assays (Table 1). This result may appear contradictory to the recently published finding that CBP functions as a coactivator of cJun (11). It is possible that the discrepancy is attributable to the difference in the cell line used in the studies. The results reported in Table 1 in this study were obtained with U2OS cells, while the study of Bannister et al. was performed with mouse F9 cells. It is possible that the endogenous CBP is not present at a saturating level in F9 cells for Jun-mediated transcription. Therefore, the effect of the transfected CBP was more readily detected. The fact that in this study CBP was found to interact with both cJun and JunB (Fig. 8C) was consistent with a cofactor role for CBP in Jun-dependent transcription.

E1A as a promiscuous transcriptional regulator. Two major roles for E1A make it a critical protein in the adenovirus life cycle. First, E1A is required for transcriptional activation of other adenovirus genes whose products are necessary for the virus to complete its lytic cycle. Second, E1A alters a whole array of host cell functions to prepare a cellular environment favorable for viral DNA replication and virus propagation (for a review, see reference 21). Both functions are closely associated with the ability of E1A to modulate transcription of a wide variety of viral and cellular genes. Since E1A is not a sequence-specific DNA-binding protein (13, 25) and most of the E1A-inducible promoters do not have common sequence elements, it can be predicted that the action of E1A must be indirect and is likely to involve multiple mechanisms requiring protein-protein interactions.

Indeed, two main mechanisms have come to light. One model involves direct physical interactions between E1A and sequence-specific transcription factors, such as ATF-2 (47) and TBP (35, 46). As a result of such interactions, E1A is brought to the promoters for transcriptional activation. The direct interaction occurs between CR3 of E1A and the DNA-binding domain of the respective transcription factors. For instance, it has been shown recently that several unrelated transcription factors, including cJun, Sp1, and USF, interact physically with E1A via their DNA-binding domains (48). It should be noted that the GAL4-cJun fusion used in this study does not contain the DNA-binding domain of cJun and yet is clearly subjected to regulation by 12S E1A, which does not contain CR3. As will be discussed further below, the downregulation of cJun activity by E1A occurs through a distinct mechanism involving the E1A-associated protein p300.

A second model that explains E1A action involves indirect physical interactions between E1A and the sequence-specific transcription factors. This type of interaction is characterized by a requirement for mediator proteins (usually the E1A-associated proteins) and the participation of conserved regions of E1A other than CR3. The most extensively studied example is the RB-E2F complex. E1A targets RB-E2F by binding to RB, which results in the dissociation of the RB-E2F complex (6). The free E2F presumably activates growth-related genes (for a review, see reference 51). More recently, another E1A-associated protein, p300, has been shown to play a crucial role in mediating the transcriptional effects of E1A. We conducted studies that were aimed at understanding the functional interaction between E1A and the transcription factor YY1. In the absence of E1A, YY1 represses transcription. E1A relieves YY1-mediated repression and essentially converts YY1 from a repressor to an activator of transcription (59). By mutational analyses, we demonstrated that the ability of E1A to modulate the activity of YY1 is p300 binding dependent and that p300 is a critical protein that bridges the interaction between E1A and YY1 (43). Other studies by several laboratories demonstrated that p300 and CBP mediate the functional interaction between E1A and CREB (3, 42, 49). One of the AP-1 components, Fos, has also been suggested to be a target of E1A through CBP (10). In this report, we presented experimental evidence that supports the indirect-interaction model and extend the repertoire of transcription factors that are targeted by E1A through p300 and/or CBP to include both cJun and JunB.

The functional interplay between E1A and AP-1 appears to have an important role in transformation and tumor progression. It has been reported that repression of AP-1 activity is tightly linked to the ability of E1A to inhibit expression of several genes thought to be crucial for the invasive and metastatic properties of tumor cells, such as collagenase, stromelysin (53, 61), and an adhesion molecule, CD44 (34). These observations may explain the reduced metastatic potential of tumor cells in the presence of E1A (27, 34). It is more complicated, however, when one considers the role of E1A repression of AP-1 activity in the context of cellular transformation, since both E1A and members of the AP-1 family are known oncoproteins. Therefore, the biological consequences of repression of AP-1 by E1A remain to be established. On the other hand, E1A is also capable of inducing cJun mRNA synthesis through an AP-1 site (60). The apparent opposite effects of E1A on AP-1 activity have been suggested to be a result of the formation of different dimers by cJun and related proteins (28). In this study, we observed that the functional outcome of the interactions among p300, E1A, and cJun can be

affected by the level of p300 relative to that of E1A (Fig. 7B). Delineation of the molecular mechanism underlying this phenomenon should contribute to a better understanding of the complex biological regulation involving E1A and AP-1.

To summarize, we have provided multiple lines of both biochemical and functional evidence demonstrating that p300 is a transcriptional cofactor for both cJun and JunB and that E1A modulates the activities of these transcription factors by targeting p300. Since p300 and CBP appear to be common transcriptional cofactors (3, 5, 22, 43, 49), E1A may thus gain control over multiple transcriptional regulatory pathways through its interactions with p300 or CBP.

ACKNOWLEDGMENTS

We thank members of the laboratory for critical reading of the manuscript and their helpful comments. We are grateful for the gifts of plasmids from Paul Smits and Alex J. van der Eb (Sylvius Laboratory, Leiden, The Netherlands), Michael Mathews (Cold Spring Harbor Laboratory), Jon Licht (Mt. Sinai Medical School), Michael Green (University of Massachusetts Medical Center), R. Goodman (Oregon Health Sciences University), and E. Harlow (Massachusetts General Hospital Cancer Center).

This work was supported by a grant to Y.S. from the NIH (GM53874). Y.S. is the recipient of a Junior Faculty Research Award from the American Cancer Society.

The first two authors contributed equally to this work.

ADDENDUM IN PROOF

After the manuscript was submitted, Smits et al. reported that p300 is involved in E1A repression of cJun-mediated transcription (P. H. M. Smits, L. de Wit, A. J. van der Eb, and A. Zantema, Oncogene **12:**1529–1535, 1996).

REFERENCES

1. **Angel, P., K. Hattori, T. Smeal, and M. Karin.** 1988. The jun proto-oncogene is positively autoregulated by its product, Jun/AP-1. Cell **55:**875–885.
2. **Angel, P., and M. Karin.** 1991. The role of Jun, Fos and the AP-1 complex in cell proliferation and transformation. Biochim. Biophys. Acta **1072:**129–157.
3. **Arany, Z., D. Newsome, E. Oldread, D. M. Livingston, and R. Eckner.** 1995. A family of transcriptional adaptor proteins targeted by the E1A oncoprotein. Nature (London) **374:**81–84.
4. **Arany, Z., W. R. Sellers, D. M. Livingston, and R. Eckner.** 1994. E1A-associated p300 and CREB-associated CBP belong to a conserved family of coactivators. Cell **77:**799–800.
5. **Arias, J., A. S. Alberts, P. Brindle, F. X. Claret, T. Smeal, M. Karin, J. Feramisco, and M. Montminy.** 1994. Activation of cAMP and mitogen responsive genes relies on a common nuclear factor. Nature (London) **370:** 226–229.
6. **Bagchi, S., P. Raychaudhuri, and J. R. Nevins.** 1990. Adenovirus E1A proteins can dissociate heteromeric complexes involving the E2F transcription factor: a novel mechanism for E1A trans-activation. Cell **62:**659–669.
7. **Baichwal, V. R., A. Park, and R. Tjian.** 1992. The cell-type-specific activator region of c-Jun juxtaposes constitutive and negatively regulated domains. Genes Dev. **6:**1493–1502.
8. **Baichwal, V. R., and R. Tjian.** 1990. Control of c-Jun activity by interaction of a cell-specific inhibitor with regulatory domain δ: difference between v- and c-Jun. Cell **63:**815–825.
9. **Bandara, L. R., and N. B. La Thangue.** 1991. Adenovirus E1a prevents the retinoblastoma gene product from complexing with a cellular transcription factor. Nature (London) **351:**494–497.
10. **Bannister, A. J., and T. Kouzarides.** 1995. CBP-induced stimulation of c-Fos activity is abrogated by E1A. EMBO J. **14:**4758–4762.
11. **Bannister, A. J., T. Oehler, D. Wilhelm, P. Angel, and T. Kouzarides.** 1995. Stimulation of c-Jun activity by CBP: c-Jun residues Ser 63/73 are required for CBP induced stimulation in vivo and CBP binding in vitro. Oncogene **11:**2509–2514.
12. **Bohmann, D., T. J. Bos, A. Admon, T. Nishimura, P. K. Vogt, and R. Tjian.** 1987. Human proto-oncogene cjun encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. Science **238:**1386–1392.
13. **Chatterjee, P. K., M. Bruner, S. J. Flint, and M. L. Harter.** 1988. DNA-binding properties of an adenovirus 289R E1A protein. EMBO J. **7:**835–841.
14. **Chellappan, S., S. Hiebert, M. Mudryj, J. M. Horowitz, and J. R. Nevins.** 1991. The E2F transcription factor is a cellular target for the RB protein. Cell **65:**1053–1061.
15. **Chrivia, J. C., R. P. S. Kwok, N. Lamb, M. Hagiwara, M. R. Montimy, and R. H. Goodman.** 1993. Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature (London) **265:**855–859.
16. **Cohen, D. R., P. C. P. Ferreira, R. Gentz, B. R. Franza, and T. Curran.** 1989. The product of a fos related gene, fra-1, binds cooperatively to the AP-1 site with Jun: transcription factor AP-1 is comprised of multiple protein complexes. Genes Dev. **3:**173–184.
17. **Dai, P., H. Akimaru, Y. Tanaka, D.-X. Hou, T. Yasukawa, C. Kanei-Ishii, T. Takahashi, and S. Ishii.** 1996. CBP as a transcriptional coactivator of c-Myb. Genes Dev. **10:**528–540.
18. **de Groot, R., N. Foulkes, M. Mulder, W. Kruijer, and P. Sassone-Corsi.** 1991. Positive regulation of *jun*/AP-1 by E1A. Mol. Cell. Biol. **11:**192–201.
19. **Deng, T., and M. Karin.** 1992. Construction and expression of a monomeric c-Jun protein that binds and activates transcription of AP-1-responsive genes. Proc. Natl. Acad. Sci. USA **89:**8572–8576.
20. **Dorsman, J. C., B. M. Hagmeyer, J. Veenstra, P. Elfferich, N. Nabben, A. Zantema, and A. J. van der Eb.** 1995. The N-terminal region of the adenovirus type 5 E1A proteins can repress expression of cellular genes via two distinct but overlapping domains. J. Virol. **69:**2962–2967.
21. **Dyson, N., and E. Harlow.** 1992. Adenovirus E1A targets key regulators for cell proliferation. Cancer Surv. **12:**161–195.
22. **Eckner, R., M. E. Ewen, D. Newsome, M. Gerdes, J. A. DeCaprio, J. B. Lawrence, and D. M. Livingston.** 1994. Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev. **8:**869–884.
23. **Egan, C., S. T. Bayley, and P. E. Branton.** 1989. Binding of the Rb1 protein to E1A products is required for adenovirus transformation. Oncogene **4:**383–388.
24. **Egan, C., T. N. Jelsma, J. A. Howe, S. T. Bayley, B. Ferguson, and P. E. Branton.** 1988. Mapping of cellular protein-binding sites on the products of early-region 1A of human adenovirus type 5. Mol. Cell. Biol. **8:**3955–3959.
25. **Ferguson, B., B. Krippl, O. Andrisani, N. Jones, H. Westphal, and M. Rosenberg.** 1985. E1A 13S and 12S mRNA products made in *Escherichia coli* both function as nucleus-localized transcription activators but do not directly bind DNA. Mol. Cell. Biol. **5:**2653–2661.
26. **Franza, B. R., Jr., F. J. Rauscher III, S. F. Josephs, and T. Curran.** 1988. The fos complex and fos-related antigens recognize sequence elements that contain AP-1 binding sites. Science **239:**1150–1153.
27. **Frisch, S. M., R. Reich, I. E. Collier, L. T. Genrich, G. Martin, and G. I. Goldberg.** 1990. Adenovirus E1A represses protease gene expression and inhibits metastasis of human tumor cells. Oncogene **5:**75–83.
28. **Hagmeyer, B. M., H. Konig, I. Herr, R. Offringa, A. Zantema, A. J. van der Eb, P. Herrlich, and P. Angel.** 1993. Adenovirus E1A negatively and positively modulates transcription of AP-1 dependent genes by dimer-specific regulation of the DNA-binding and transactivation activities of Jun. EMBO J. **12:**3559–3572.
29. **Hai, T., and T. Curran.** 1991. Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc. Natl. Acad. Sci. USA **88:**3720–3724.
30. **Hai, T., F. Liu, E. A. Allegretto, M. Karin, and M. R. Green.** 1988. A family of immunologically related transcription factors that includes multiple forms of ATF and AP-1. Genes Dev. **2:**1216–1226.
31. **Hamel, P. A., R. M. Gill, R. A. Phillips, and B. L. Gallie.** 1992. Transcriptional repression of the E2-containing promoters EIIaE, c-*myc*, and *RB1* by the product of the *RB1* gene. Mol. Cell. Biol. **12:**3431–3438.
32. **Harlow, E., P. Whyte, B. R. Franza, Jr., and C. Schley.** 1986. Association of adenovirus early-region 1A proteins with cellular polypeptides. Mol. Cell. Biol. **6:**1579–1589.
33. **Hiebert, S. W., S. P. Chellappan, J. M. Horowitz, and J. R. Nevins.** 1992. The interaction of RB with E2F coincides with an inhibition of the transcriptional activity of E2F. Genes Dev. **6:**177–185.
34. **Hofmann, M., W. Rudy, U. Gunthert, S. G. Zimmer, V. Zawadzki, M. Zoller, R. B. Lichtner, P. Herrlich, and H. Ponta.** 1993. A link between ras and metastatic behavior of tumor cells: ras induces CD44 promoter activity and leads to low-level expression of metastatic-specific variants of CD44 in CREF cells. Cancer Res. **53:**1516–1521.
35. **Horikoshi, N., K. Maguire, A. Krelli, E. Maldanado, D. Reinberg, and R. Weinmann.** 1991. Direct interaction between adenovirus E1A protein and the TATA box binding transcription factor IID. Proc. Natl. Acad. Sci. USA **88:**5124–5128.
36. **Houweling, A., P. J. van der Elsen, and A. J. van der Eb.** 1980. Partial transformation of primary rat cells by the leftmost 4.5% fragment of adenovirus 5 DNA. Virology **105:**537–550.
37. **Howe, J. A., J. S. Mymryk, C. Egan, P. E. Branton, and S. T. Bayley.** 1990. Retinoblastoma growth suppressor and a 300-kDa protein appear to regulate cellular DNA synthesis. Proc. Natl. Acad. Sci. USA **87:**5883–5887.
38. **Hsin-Fang, Y.-Y., R. Chiu, and M. Karin.** 1990. Elevation of AP1 activity during F9 cell differentiation is due to increased c-jun transcription. New Biol. **2:**351–361.
39. **Jelsma, T. N., J. A. Howe, J. S. Mymryk, C. M. Evelegh, N. F. Cunnif, and**

S. T. Bayley. 1989. Sequences in E1A proteins of human adenovirus 5 required for cell transformation, repression of a transcriptional enhancer, and induction of proliferating cell nuclear antigen. Virology **171:**120–130.
40. **Kitabayashi, I., R. Chiu, G. Gachelin, and K. Yokoyama.** 1991. E1A dependent up-regulation of cJun/AP-1 activity. Nucleic Acids Res. **12:**479–487.
41. **Kwok, R. P. S., J. R. Lundblad, J. C. Chrivia, J. P. Richards, H. P. Bachinger, R. G. Brennan, S. G. E. Roberts, M. R. Green, and R. H. Goodman.** 1994. Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature (London) **370:**223–226.
42. **Lee, J.-S., X. Zhang, and Y. Shi.** Differential interactions of the CREB/ATF family of transcription factors with p300 and adenovirus E1A. J. Biol. Chem., in press.
43. **Lee, J.-S., K. M. Galvin, R. H. See, R. Eckner, D. Livingston, E. Moran, and Y. Shi.** 1995. Relief of YY1 transcriptional repression by adenovirus E1A is mediated by E1A-associated protein p300. Genes Dev. **9:**1188–1198.
44. **Lee, J.-S., K. M. Galvin, and Y. Shi.** 1993. Evidence for physical interaction between the zinc finger transcription factors YY1 and Sp1. Proc. Natl. Acad. Sci. USA **90:**6145–6149.
45. **Lee, J.-S., R. H. See, K. M. Galvin, J. Wang, and Y. Shi.** 1995. Functional interactions between YY1 and adenovirus E1A. Nucleic Acids Res. **23:**925–931.
46. **Lee, W. S., C. C. Kao, G. O. Bryant, X. Liu, and A. J. Berk.** 1991. Adenovirus E1A activation domain binds the basic repeat in the TATA box transcription factor. Cell **67:**365–376.
47. **Liu, F., and M. R. Green.** 1990. A specific member of the ATF transcription factor family can mediate transcription activation by the adenovirus E1A protein. Cell **61:**1217–1224.
48. **Liu, F., and M. R. Green.** 1994. Promoter targeting by adenovirus E1a through interaction with different cellular DNA-binding domains. Nature (London) **368:**520–525.
49. **Lundblad, J. R., R. P. S. Kwok, M. E. Laurance, M. L. Harter, and R. H. Goodman.** 1995. Adenoviral E1A-associated protein p300 as a functional homologue of the transcriptional co-activator CBP. Nature (London) **374:**85–88.
50. **Nakabeppu, Y., K. Ryder, and D. Nathans.** 1988. DNA binding activities of three murine jun proteins: stimulation by fos. Cell **55:**907–915.
51. **Nevins, J. R.** 1992. E2F: a link between the Rb tumor suppressor protein and viral oncoproteins. Science **258:**424–429.
52. **Offringa, R., S. Gebel, H. van Dam, M. Timmers, A. Smits, R. Zwart, B. Stein, J. L. Bos, A. van der Eb, and P. Herrlich.** 1990. A novel function of the transforming domain of E1A: repression of AP-1 activity. Cell **62:**527–538.
53. **Offringa, R., A. M. M. Smits, A. Houweling, J. L. Bos, and A. J. van der Eb.** 1988. Similar effects of adenovirus E1A and glucocorticoid hormones on the expression of the metalloprotease stromelysin. Nucleic Acids Res. **16:**10973–10984.
54. **Pugh, B. F., and R. Tjian.** 1990. Mechanism of transcriptional activation by Sp1: evidence for coactivators. Cell **61:**1187–1197.
55. **Rauscher, F. J., III, L. C. Sambucetti, T. Curran, R. J. Distel, and B. Spiegelman.** 1988. A common DNA binding site for fos protein complexes and transcription factor AP-1. Cell **52:**471–480.
56. **Rauscher, F. J., III, P. J. Voulalas, B. R. Franza, and T. Curran.** 1988. Fos and jun bind cooperatively to the AP-1 site: reconstitution in vitro. Genes Dev. **2:**1687–1699.
57. **Ruley, H. E.** 1983. Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. Nature (London) **304:**602–606.
58. **Schutte, J., J. Viakket, M. Nau, S. Segal, J. Fedorko, and J. Minna.** 1989. jun-B inhibits and c-fos stimulates the transforming and trans-activating activities of c-jun. Cell **59:**987–997.
59. **Shi, Y., E. Seto, L. S. Chang, and T. Shenk.** 1991. Transcriptional repression by YY1, a human GLI-Kruppel related protein, and relief of repression by adenovirus E1A protein. Cell **67:**377–388.
60. **van Dam, H., R. Offringa, I. Meijer, B. Stein, A. M. Smits, P. Herrlich, J. L. Bos, and A. J. van der Eb.** 1990. Differential effects of the adenovirus E1A oncogene on members of the AP-1 transcription factor family. Mol. Cell. Biol. **10:**5857–5864.
61. **van Dam, H., R. Offringa, A. M. Smits, J. L. Bos, N. C. Jones, and A. van der Eb.** 1989. The expression of the growth factor-inducible genes JE, c-myc and stromelysin by adenovirus E1A is mediated by conserved region 1. Oncogene **4:**1207–1212.
62. **van der Eb, A. J., H. van Ormondt, and P. I. Schrier.** 1979. Structure and function of the transforming genes of human adenoviruses and SV40. Cold Spring Harbor Symp. Quant. Biol. **44:**383–399.
63. **van der Elsen, P., S. de Parter, A. Houweling, J. van der Veer, and A. J. van der Eb.** 1982. The relationship between regions E1a and E1b of human adenoviruses in cell transformation. Gene **18:**175–185.
64. **van der Elsen, P. J., A. Houweling, and A. J. van der Eb.** 1983. Morphological transformation of human adenoviruses is determined to a large extent by gene products of region E1a. Virology **131:**242–246.
65. **Vogt, P. K., and T. J. Bos.** 1990. Jun: oncogene and transcription factor. Adv. Cancer Res. **55:**1–35.
66. **Wang, H.-G. H., Y. Rikitake, M. C. Carter, P. Yaciuk, S. E. Abraham, B. Zerler, and E. Moran.** 1993. Identification of specific adenovirus E1A N-terminal residues critical to the binding of cellular proteins and to the control of cell growth. J. Virol. **67:**476–488.
67. **Weintraub, S. J., C. A. Prater, and D. C. Dean.** 1992. Retinoblastoma protein switches the E2F site from positive to negative element. Nature (London) **358:**259–261.
68. **Whyte, P., N. M. Williamson, and E. Harlow.** 1989. Cellular targets for transformation by the adenovirus E1A proteins. Cell **56:**67–75.
69. **Yee, S. P., and P. E. Branton.** 1985. Detection of cellular proteins associated with human adenovirus type 5 early region 1A polypeptides. Virology **147:**142–153.
70. **Zamanian, M., and N. B. LaThangue.** 1992. Adenovirus E1a prevents the retinoblastoma gene product from repressing the activity of a cellular transcription factor. EMBO J. **11:**2603–2610.
71. **Zerial, M., L. Toschi, R. P. Ryseck, M. Schuermann, R. Mueller, and R. Bravo.** 1989. The product of a novel growth factor activated gene, fosB, interacts with JUN proteins enhancing their DNA binding activity. EMBO J. **8:**805–813.