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Distinct Domains of Adenovirus E1A Interact with Specific Cellular Factors To Differentially Modulate Human Immunodeficiency Virus Transcription

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Transcription of human immunodeficiency virus (HIV) type 1 and other viruses is regulated by the transcription factor NF-κB, which interacts with the multifunctional cellular protein p300. p300, originally identified by its ability to bind adenovirus early region 1A (E1A), has also been shown to regulate HIV transcription through its interaction with NF-κB. The 13S form of E1A activates HIV gene expression, while the 12S form represses its transcription. In this report, we have investigated whether these divergent effects of E1A are dependent upon common or distinct cellular cofactors, including p300, pRb, and the TATA box-binding protein (TBP). Unlike activation in the absence of E1A, cooperative stimulation of HIV gene expression by 13S E1A and RelA was independent of the ability of E1A to bind p300 but was critically dependent on the E1A CR3 region which associates with TBP. In contrast, inhibition of basal HIV gene expression by the 12S form of E1A was dependent on p300 but not pRb or TBP. Interestingly, mutation of the CR2 region of 12S E1A responsible for pRb binding abolished the repression of HIV transcription stimulated by tumor necrosis factor alpha, suggesting that repression of cytokine-activated transcription involves cofactors different from those used in unstimulated cells. Repression and activation of HIV transcription by different forms of E1A are mediated by distinct sets of cellular cofactors. These findings suggest that E1A has evolved to interact by alternative mechanisms with a transcriptional coactivator and its associated cofactors to differentially modulate cellular and viral gene expression.

The adenovirus early region 1A (E1A) gene products are the major regulatory proteins synthesized early during adenovirus infection. These proteins play a central role in the deregulation of cell proliferation and regulate the transcription of a number of cellular genes, in addition to stimulating expression from the early adenovirus promoters (reviewed in references 5, 24, and 44). Two mRNAs, 13S and 12S, encoding E1A proteins of 289 and 243 amino acids (289R and 243R), respectively, are generated through the alternative splicing of a single pre-mRNA (10). Common to both 289R and 243R E1A are two regions required for transformation and cell growth regulation that are conserved between different adenovirus serotypes: CR1 and CR2. The binding of several cellular regulatory products to well-characterized sites in CR1, CR2, and the comparatively nonconserved amino terminus is closely linked to the cell growth functions of E1A (34, 35, 38, 55). CR2 and the amino terminus of CR1 form a noncontiguous site for the direct binding of the family of proteins related to the product of the retinoblastoma (pRb) tumor susceptibility gene (17, 54). CR1 and the extreme amino terminus of E1A form a binding site for the 300-kDa cellular product, p300 (52, 56) (see Fig. 1A). A third conserved region of 46 amino acids, CR3, is present only in 289R, and while superfluous for transformation activity, CR3 has been shown to elicit strong transcriptional activation of both viral and cellular genes (5, 24, 44).

Mutagenesis and protein fusion experiments have revealed that the CR3 region is itself functionally separable into two activities: an NH_2-terminal, zinc-binding, transcriptional activation region and a carboxyl-terminal portion which has been hypothesized to stimulate a number of diverse promoters by association with several classes of DNA binding domains (31, 32, 53). Interaction of the activation domain of CR3 with a critical component of the basal transcriptional machinery, the TATA box-binding protein (TBP), has been demonstrated and is believed to be important for the transcriptional activation function of 289R (16, 28). In contrast, 243R E1A has the ability to repress the transcription of several viral promoters and a number of cellular genes involved in control of cell growth and differentiation (9, 14, 19, 20, 48, 50, 51). While transcriptional repression by E1A remains enigmatic, the NH_2-terminal regions of E1A that have been shown by mutagenesis studies to be involved in repression are also required for E1A-mediated cell differentiation and transformation (48, 52), suggesting a close correlation between transcriptional repression and transformation.

Previous reports demonstrate that 289R E1A activates human immunodeficiency virus type 1 (HIV-1) enhancer-dependent transcription, while 243R E1A represses its basal activity (36, 40, 46, 51, 52). Another regulator of HIV gene expression is NF-κB, a member of the family of Rel-related cellular transcription factors important to the organism's response to stress and infection (reviewed in references 18 and 45). We have recently found that p300, a coactivator recruited to a number of unrelated promoters by protein-protein interactions, physi-

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cally associates with RelA and serves as a coactivator for κB-dependent activation of the HIV enhancer (39). Here, we have investigated the requirement of p300 and other cellular cofactors for activation or repression of HIV by E1A. We demonstrate that there is cooperative activation of HIV gene expression by RelA and 289R E1A and that this effect is independent of E1A binding to p300 but requires the region of CR3 which binds to TBP. In contrast, repression of HIV gene expression by 243R was dependent upon the ability of E1A to bind p300. Hence, we conclude that distinct forms of E1A mediate activation and repression of HIV transcription through their ability to interact with different cellular transcription factors and coactivators.

MATERIALS AND METHODS

Plasmids. The reporter constructs HIV-CAT and ΔκB HIV-CAT have been previously described (29). The Rous sarcoma virus (RSV) expression vectors for both IκB-α and RelA have also been reported elsewhere (12, 43). The wild-type adenovirus serotype 5 289R E1A expression plasmid and mutants *pm*563 and *dl*922/947 were kind gifts from E. Harlow, and their construction has been previously reported (55). Mutant *pm*563 has a substitution of glycine for arginine at amino acid 2 in the amino terminus. *dl*922/947 has a deletion of amino acids 122 to 129 within CR2. Similarly, the wild-type 243R E1A expression plasmid and the mutant Cxdl have been described (35). Mutant Cxdl has a deletion of amino acids 121 to 150 that disrupts conserved region 2 and deletes the 12S splice donor site. The cytomegalovirus (CMV) p300 vector has been previously described (39).

Mutant 12S *pm*563 was prepared by digesting wild-type 243R cDNA with restriction enzymes *Bsm*I and *Kpn*2I (New England Biolabs and GIBCO BRL, respectively). The resulting *Bsm*I-*Kpn*2I fragment was gel purified and ligated into *Bsm*I-*Kpn*2I-linearized *pm*563. For analysis of the effects of single amino acid substitutions within CR3 upon E1A-mediated transactivation of the HIV long terminal repeat (LTR), E1A constructs 289R, 176MK, 188ST, 147VL, and 157CS (kindly provided by R. Ricciardi [53]) were subcloned into the 243R expression vector described above to create 13S expression vectors bearing single site mutations within CR3. Again, E1A plasmids 289R, 176MK, 188ST, 147VL, and 157CS were digested with *Kpn*2I and *Bsm*I, and the CR3-containing restriction fragment was gel purified and ligated into *Kpn*2I-*Bsm*I-linearized 243R expression plasmid.

Cell culture. Jurkat T cells were grown under 5% CO_2 in RPMI medium (GIBCO BRL) supplemented with 5% bovine fetal calf serum. UM-449 human melanoma cells (30) were grown under 5% CO_2 in RPMI medium supplemented with 10% bovine fetal calf serum.

Transfections and CAT assays. Jurkat T cells were transfected with DEAE-dextran essentially as described previously (29). The amounts of transfected chloramphenicol acetyltransferase (CAT) reporter construct and E1A plasmid used are noted in the appropriate figure legends. In each case a control plasmid (RSV expression vector for alcohol dehydrogenase) was included such that each condition contained the same concentration of DNA. Where indicated, 16 h posttransfection, cells were treated with 200 U of tumor necrosis factor alpha (TNF-α) ml^{-1}. At 24 h posttransfection, cells were harvested and CAT assays were performed as detailed previously (42).

UM-449 cells were transfected with γAP-DLRIE/DOPE 50/50 (Vical). Specifically, cells were plated to 60% confluency 1 day prior to transfection, washed twice with Opti-MEM (GIBCO BRL), and transfected with a lipid-DNA solution made by combining 1.5 ml of Opti-MEM containing 15 μg of DNA and 1.5 ml of Opti-MEM containing 30 μl of a 2-mg/ml lipid solution. Cells were incubated for 4 h at 37°C, and the lipid solution was then removed and replaced with fresh medium.

Western immunoblot analysis. Jurkat T cells were transfected with 25 μg of E1A plasmid as previously described (29). Twenty-four-hour-posttransfection nuclear extracts of transfected cells were prepared essentially as described previously (37). Nuclear extracts were boiled in Laemmli sample buffer and subjected to reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The transfer and immunoreaction conditions have been described previously (42). The anti-E1A antibody used (sc-430; Santa Cruz Biotechnology, Inc.) was a rabbit polyclonal immunoglobulin G recognizing adenovirus 2 and 5 E1A antigens (antibody raised against full-length 13S splice variant). Horseradish peroxidase-conjugated anti-rabbit antibody (Cappel) was used as a secondary antibody at a dilution of 1:5,000. Incubation with anti-E1A antibody was for 3 h at room temperature and for 1 h at room temperature with secondary antibody. Immunocomplexes were then detected by chemiluminescence with ECL Western blotting reagents (Amersham) according to the manufacturer's instructions.

Biosynthetic labeling of proteins and immunoprecipitation. UM-449 cells were transfected with 15 μg of E1A expression vector. Forty-hour-posttransfection cells were washed twice and then grown for 1 h in serum-free RPMI medium deficient in both Met and Cys. A mix of [^{35}S]methionine and [^{35}S]cysteine (Promix; Amersham) was added at a concentration of 100 μCi ml^{-1} to label for 4 h at 37°C. After removal of medium cells were washed twice in cold phosphate-buffered saline, and nuclear extracts were prepared essentially as described previously (37). For immunoprecipitation, nuclear extracts were diluted into 500 μl of IP buffer (20 mM HEPES [pH 7.9], 75 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.1% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride, 1-μg ml^{-1} apoprotinin, 1-μg ml^{-1} pepstatin A, 2-μg ml^{-1} leupeptin, and 1 mM sodium vanadate) and incubated for 2 h at 4°C in the presence of 1 μg of either anti-E1A (sc-430; Santa Cruz Biotechnology, Inc.) or a rabbit immunoglobulin G antibody used as a control. Ten microliters (packed volume) of protein G-agarose beads was added prior to incubation for a further 1 h. Immunocomplexes were washed three times in 500 μl of cold IP buffer. Bound proteins were then eluted by boiling beads in 20 μl of Laemmli sample buffer for 3 min. The supernatant was then subjected to SDS-PAGE (10% gel). Gels were fixed in 5% isopropanol–5% acetic acid, enhanced in Autofluor (Molecular Dynamics) for 1 h, dried, and exposed to X-Omat film (Kodak) at −70°C.

RESULTS

Activation of HIV gene expression by 289R E1A is independent of p300. To investigate whether p300 binding is required for activation of the HIV LTR by the 289-amino-acid form of E1A (289R), we studied the ability of 289R mutants (Fig. 1A) to activate HIV transcription. Jurkat T cells were cotransfected with an HIV reporter plasmid and a 289R E1A expression plasmid. Consistent with previous reports (7, 8, 27, 36, 40), we observed that 289R E1A is a weak activator of the HIV enhancer (Fig. 1B). In contrast to other κB-dependent viral activators, such as human T-cell leukemia virus Tax protein or Epstein-Barr virus LMP1 protein, transactivation by E1A did not require the presence of κB sites (Fig. 1C) (36). To confirm that E1A transactivation was independent of NF-κB, we performed cotransfection with an expression vector encoding IκB-α. Cotransfection with this vector did not inhibit transactivation of the HIV enhancer stimulated by 289R E1A (Fig. 1B and C).

Cotransfection of plasmids encoding 289R and RelA induced an increase in HIV transcriptional activation which was greater than additive (Fig. 2). To address whether binding of p300 to E1A contributed to HIV transcriptional activation as it does in the absence of E1A, we tested the ability of previously described mutants of 289R to activate HIV gene expression. Point mutant 563 (*pm*563) has a substitution of glycine in place of arginine at amino acid 2 which was shown previously to specifically disrupt the association of E1A with p300 and to abolish the transforming activity of E1A (56). Deletion mutant 922/947 (*dl*922/947), which has a deletion of amino acids 122 to 129 within CR2, retains the ability of wild-type E1A to bind p300 yet is unable to efficiently bind pRb (56). Both the p300 and the pRb-binding-defective 289R E1A mutants displayed near wild-type capacities to activate the HIV enhancer in transient-transfection studies. Furthermore, cooperative activation of HIV gene expression by 289R and RelA was independent of the ability of 289R to associate with either p300 or p105-pRb (Fig. 2).

The TBP-binding CR3 region of 289R E1A is required for activation of HIV gene expression. E1A-mediated activation of adenovirus early genes has been shown previously to require an association of TBP with the CR3 region (16, 28). To determine whether this region contributes to activation by NF-κB, the ability of CR3 region mutants to stimulate HIV gene expression was analyzed in the presence or absence of cotransfected RelA. Mutant 147VL, which contains a conservative substitution within the Cys_4 zinc finger region at the N terminus of CR3, has previously been shown to have reduced TBP binding in vitro (16). Transfection revealed that this substitution was also sufficient to abolish E1A-mediated activation of the HIV enhancer (Fig. 3A). Similarly, this mutant was unable to enhance HIV transcriptional activation by cotransfected RelA (Fig. 3A). Conservative substitution within the finger region of CR3 of each of the four cysteines that are required

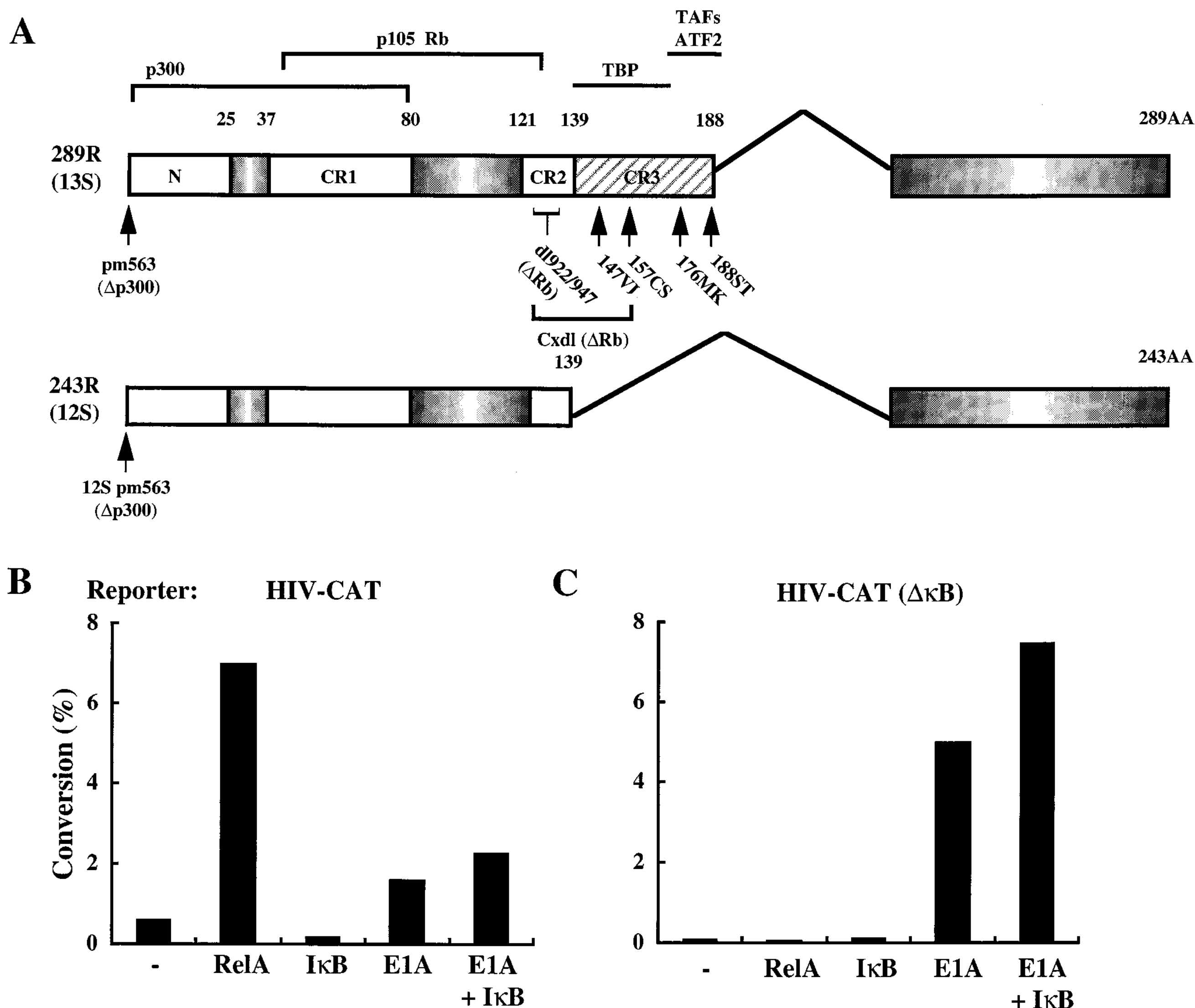


FIG. 1. Activation of HIV gene expression by 289R E1A is independent of NF-κB. (A) Schematic representation of 13S (upper) and 12S (lower) E1A expression vectors and mutants. (B and C) Jurkat T-leukemia cells were transfected with 5 μg of the HIV-CAT (panel B) or ΔκB HIV-CAT (panel C) reporter plasmid, together with the indicated combinations of RSV-RelA (0.2 μg), RSV-IκB-α (0.5 μg), and 289R E1A (0.5 μg) expression plasmids. Transfections were performed by the DEAE-dextran method as described in Materials and Methods. A control plasmid was included such that each condition contained a total of 1 μg of expression plasmid. For panel C, CAT assay sensitivity was enhanced through the extension of the incubation time from 2 to 6 h, with the addition of acetyl coenzyme A every 2 h, so that a significant stimulation of ΔκB HIV-CAT could be determined. The results shown are representative of two independent transfections.

for coordinating zinc has been shown both to impair TBP binding and to abolish transactivation of the adenovirus E3 promoter (16, 53). We tested the ability of point mutant 157CS, which has a substitution of the Zn(II) ligand C157, to stimulate HIV-CAT activity and found that it, too, was unable to transactivate the HIV enhancer or to interact with RelA in a cooperative manner (data not shown). Interestingly, mutant 176MK, which has a nonconservative substitution within the finger region and retains a near wild-type ability to bind TBP, was also unable to activate HIV gene expression. This mutant also has a greatly reduced capacity to transactivate the adenovirus E3 promoter. This finding suggests that the association of TBP with the activation domain of E1A is not required for transcriptional activation of the HIV enhancer or, alternatively, that additional factors are needed to achieve this effect.

The ability of enhancer binding proteins, such as the ATF family of transcription factors, to bind E1A has been mapped to the carboxyl-terminal region of CR3 implicated in promoter targeting (32). The association of this region with TBP-associated factors (TAFs) has also been shown (15, 33). Conservative substitution of amino acid S188 within the carboxyl-terminal region of CR3 did not, however, significantly affect the ability of E1A to transactivate the HIV enhancer (Fig. 3A). This mutant (188ST) retained a wild-type ability to bind TBP yet does not activate transcription of an adenovirus promoter in vivo (16, 53). Its ability to activate HIV gene expression suggests that distinct TAFs may be required for activation of different E1A-inducible promoters (note that the decreased protein level of mutant 188ST relative to the wild type [Fig. 3B] may account for the slightly reduced ability of 188ST to activate HIV gene expression in both the presence and absence of cotransfected RelA). Western blot analysis confirmed that the other mutants were expressed at levels similar to that of wild-type E1A in transfected Jurkat cells (Fig. 3B).

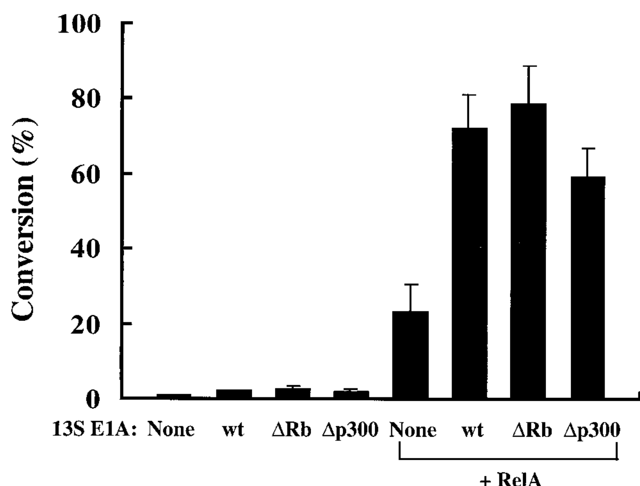


FIG. 2. Activation of HIV gene expression by 289R in both the presence and absence of RelA is independent of its p300 and pRb binding regions. Jurkat T cells were cotransfected with 5 μg of reporter plasmid (HIV-CAT) and 0.5 μg of the indicated expression plasmid encoding RelA (none), 289R (wt [wild type]), *dl*922/947 (ΔRb), or *pm*563 (Δp300). Control plasmid was included such that each condition contained 1 μg of expression plasmid. The results shown are representative of three independent transfections, and error bars represent standard error values.

E1A-mediated suppression of TNF-α-induced transactivation. E1A 243R is able to repress gene expression from a number of unrelated promoters (9, 14, 19, 20, 48, 50, 51). The involvement of several of the gene products repressed by 243R in cellular differentiation and transformation suggests that E1A-mediated transcriptional repression may be an important determinant of the pleiotropic effects of E1A on cell growth. 243R has been shown to repress basal transcription from the HIV LTR in vivo (51, 52) and to suppress Tat-activated transcription in vitro (47). Here, transfection with the HIV-CAT reporter confirmed the previous findings that, in unstimulated cells, 243R represses HIV transcription (Fig. 4A, left). Cotransfection of an expression plasmid for 243R was also sufficient to repress activation by cotransfected RelA (Fig. 4A, middle), suggesting that E1A could exert this effect, directly or indirectly, through NF-κB. This finding is in contrast to the ability of 289R, which contains the 46-amino-acid CR3 region that binds to TBP, to synergize with cotransfected RelA. To determine whether E1A could repress cytokine-stimulated transcription mediated by NF-κB, we examined the effect of transfected E1A upon HIV-CAT activity in the presence of TNF-α. Activation of the HIV enhancer by treatment with TNF-α was also suppressed by 243R E1A (Fig. 4A, right). In fact, TNF-α-induced HIV enhancer activity was repressed nearly to its basal level of activity found in unstimulated cells, suggesting that E1A can overcome activation of NF-κB by TNF-α. To examine the role of p300 in the repression of the HIV enhancer by 243R E1A, p300 was overexpressed in cotransfections with the HIV-CAT reporter, RelA, and 243R E1A (Fig. 4B). Repression by 243R E1A was relieved by p300 in a dose-dependent fashion, suggesting that repression by 243R E1A occurs through its ability to sequester p300.

The p300 binding domain of 12S E1A is required for repression of both basal and TNF-α-stimulated HIV transcription. The NH_2-terminal region of E1A is responsible for binding to p300 and is required for repression of the transcriptional activation of a number of promoters (23, 41, 46–48, 52). To define the requirements for p300 or pRb binding in repression of both basal and cytokine-activated HIV transcription, cotransfection of HIV-CAT with expression vectors encoding either wild-type or mutant E1A was performed. The binding of these mutants to p300 was confirmed by immunoprecipitating radiolabeled proteins from a highly transfectable line, UM-449. Cells were transfected with the relevant wild-type or mutant E1A expression vectors, and cell lysates were incubated with an E1A-specific polyclonal antibody. Western blot analysis confirmed that the expression of both mutants and wild-type E1A was comparable and that the labeled 300-kDa protein migrated with a protein that reacted with anti-p300 antibodies (data not shown). The pRb-binding-defective CR2 mutant, Cxdl, associated with p300 at a level comparable to that of wild-type E1A. In contrast, the 12S version of *pm*563 was unable to bind p300 (Fig. 5D). The band that comigrates with p300 in these experiments is likely an E1A binding protein that is different from more recently described higher-molecular-weight counterparts of p300. Transient transfection of these mutants into Jurkat cells showed that wild-type 243R and the pRb-binding-defective mutant, Cxdl, inhibited basal HIV gene expression. In contrast, the p300-binding-defective mutant, 12S *pm*563, failed to suppress HIV-CAT activity (Fig. 5A).

Similar to its effect on basal expression, the mutant deficient in binding to p300, 12S *pm*563, failed to inhibit TNF-α-induced transcriptional activation of the HIV enhancer (Fig. 5B). Interestingly, however, the pRb-binding-defective mutant, Cxdl, which retains its ability to repress basal transcription of the HIV enhancer, was unable to repress TNF-α-induced tran-

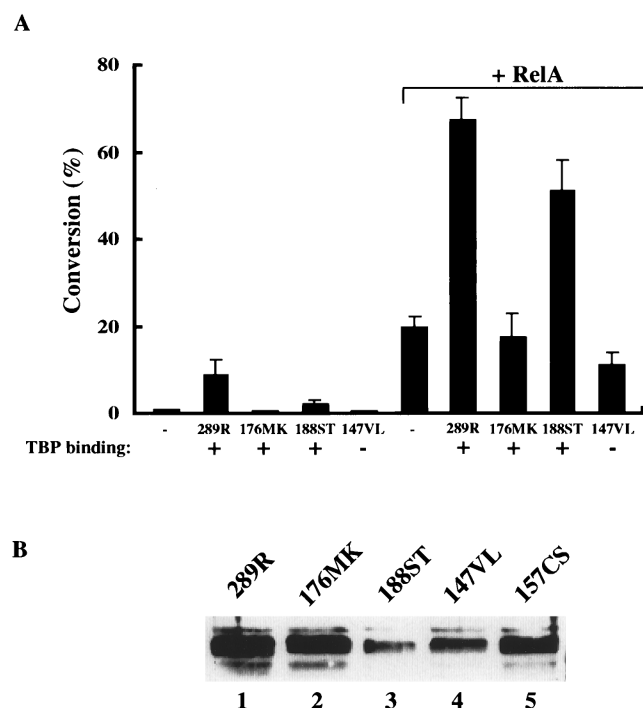


FIG. 3. Association of CR3 with cellular factors is essential for HIV transactivation by E1A. (A) Jurkat T cells were cotransfected with 5 μg of HIV-CAT together with the indicated combination of RSV-RelA (0.5 μg) and E1A (0.5 μg) expression plasmids. Control plasmid (indicated by minus sign) was included such that each condition contained a total of 1 μg of expression plasmid. The results shown are representative of at least three independent transfections, and error bars represent standard error values. (B) Western blot analysis of wild-type and CR3 mutant E1A proteins. Jurkat T cells were transfected with 25 μg of expression plasmid for E1A or its mutants. Western immunoblot analysis was performed as described in Materials and Methods with an anti-E1A rabbit polyclonal antibody.

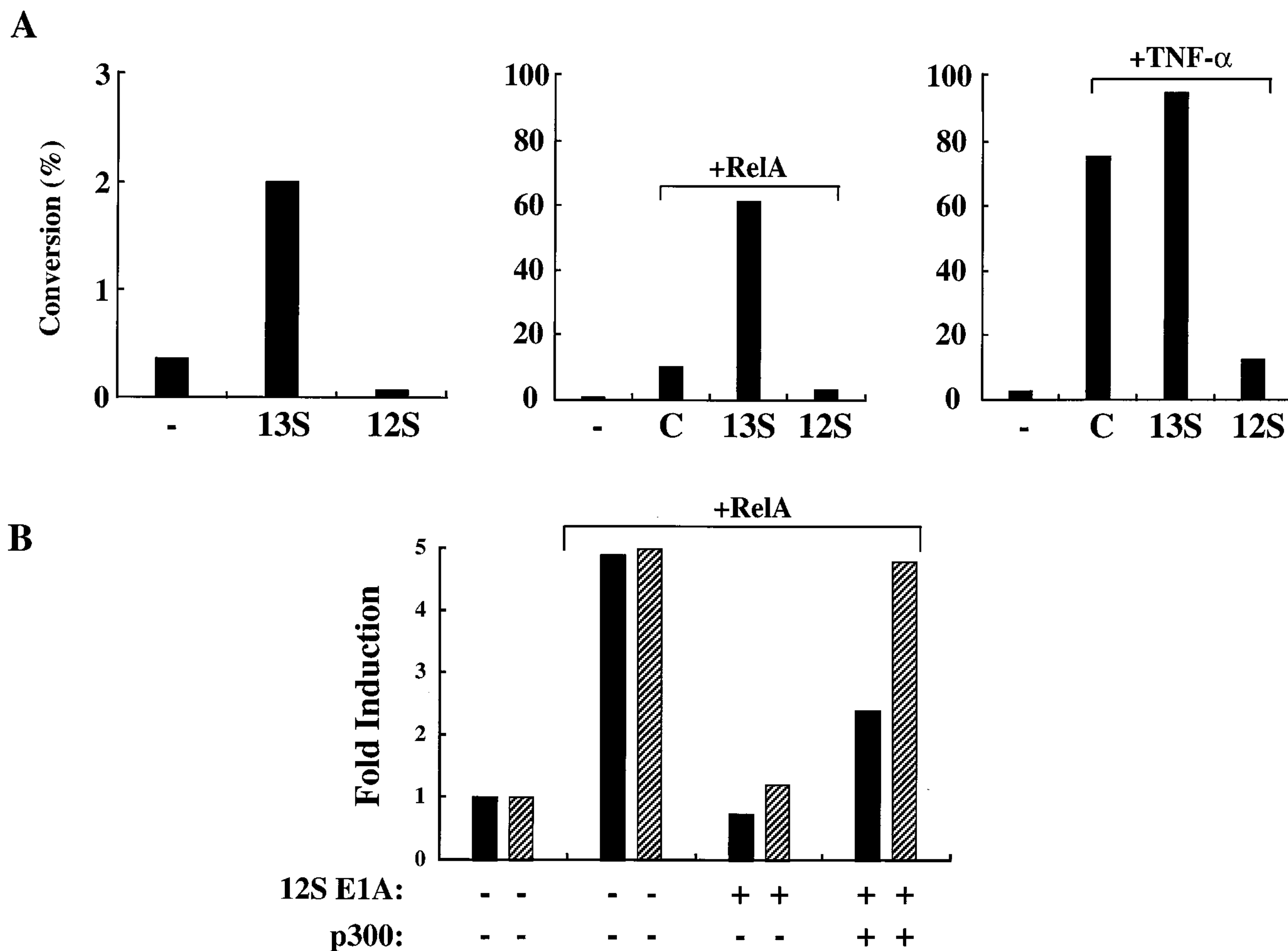


FIG. 4. 243R E1A represses both basal and TNF-α-stimulated HIV gene expression, and p300 overcomes the repression. (A) Jurkat T cells were cotransfected with 5 μg of HIV-CAT reporter together with the indicated combinations of expression plasmids for RelA (0.5 μg; middle panel), 289R E1A (0.5 μg; 13S), or 243R E1A (2.5 μg; 12S). Where indicated, cells were treated for 8 h with 200 U of TNF-α ml^{-1}. Control plasmid (indicated by minus sign) was included such that each transfection contained 3 μg of expression plasmid. The results shown are representative of at least two independent transfections. Note scale change between figures. (B) Jurkat T cells were cotransfected with 2 μg of HIV-CAT reporter plasmid alone or together with the combinations of RSV-RelA (0.2 μg), 243R E1A (2 μg), and CMV p300 (2 or 5 μg, filled or hatched bars, respectively) indicated by plus and minus signs underneath the bars. Appropriate control plasmids were used such that samples contained either 4.2 μg (filled bars) or 7.2 μg (hatched bars) of the indicated expression plasmid.

scription (Fig. 5B), suggesting that TNF-α alters the association of cellular cofactors for NF-κB, likely incorporating active pRb binding into the complex after cytokine stimulation. These results demonstrate the requirement of p300 binding for 243R-mediated repression of both basal and TNF-α-stimulated HIV LTR-directed gene expression.

It has previously been speculated that one mechanism of repression by E1A may involve inhibition of p300 or its sequestration in a form in which it is unable to function as a coactivator for transcriptional activators. Recently, striking progress has been made towards a better understanding of the physiological role of the p300/CBP family (reviewed in reference 22), which serve as coactivators for a number of unrelated transcription factors. These studies support the hypothesis that a common feature of E1A repression is a dependence on p300 as a coactivator. p300 can serve as a coactivator for NF-κB-mediated activation of the HIV LTR (39), and some previous studies indicate that repression of the HIV LTR by 243R is mediated by enhancer elements (51, 52). However, more recent reports support an alternative mechanism for E1A-mediated repression that is enhancer independent and mediated by an interaction of E1A with the general transcription machinery (47, 49). To address this question, repression of basal transcription by 243R from either the wild-type or mutant κB HIV-CAT reporter was analyzed. Mutation of the κB sites abolished the ability of 243R to repress transcriptional activity (Fig. 5C). In addition, the HIV enhancer containing mutant κB sites showed expression that was 10-fold lower than that of the wild-type enhancer (data not shown), suggesting that a low level of nuclear endogenous NF-κB which cannot activate the κB mutant enhancer is present in these Jurkat cells. These data suggest that the κB elements mediate enhancer-dependent repression of HIV transcription by 243R and support the hypothesis that E1A-mediated repression results from inhibition of p300 so that it is no longer able to function as a coactivator for NF-κB-dependent transcriptional activation.

DISCUSSION

In this report, we have investigated E1A regulation of HIV transcription and its dependence upon cellular gene products. These studies show that activation of HIV transcription by

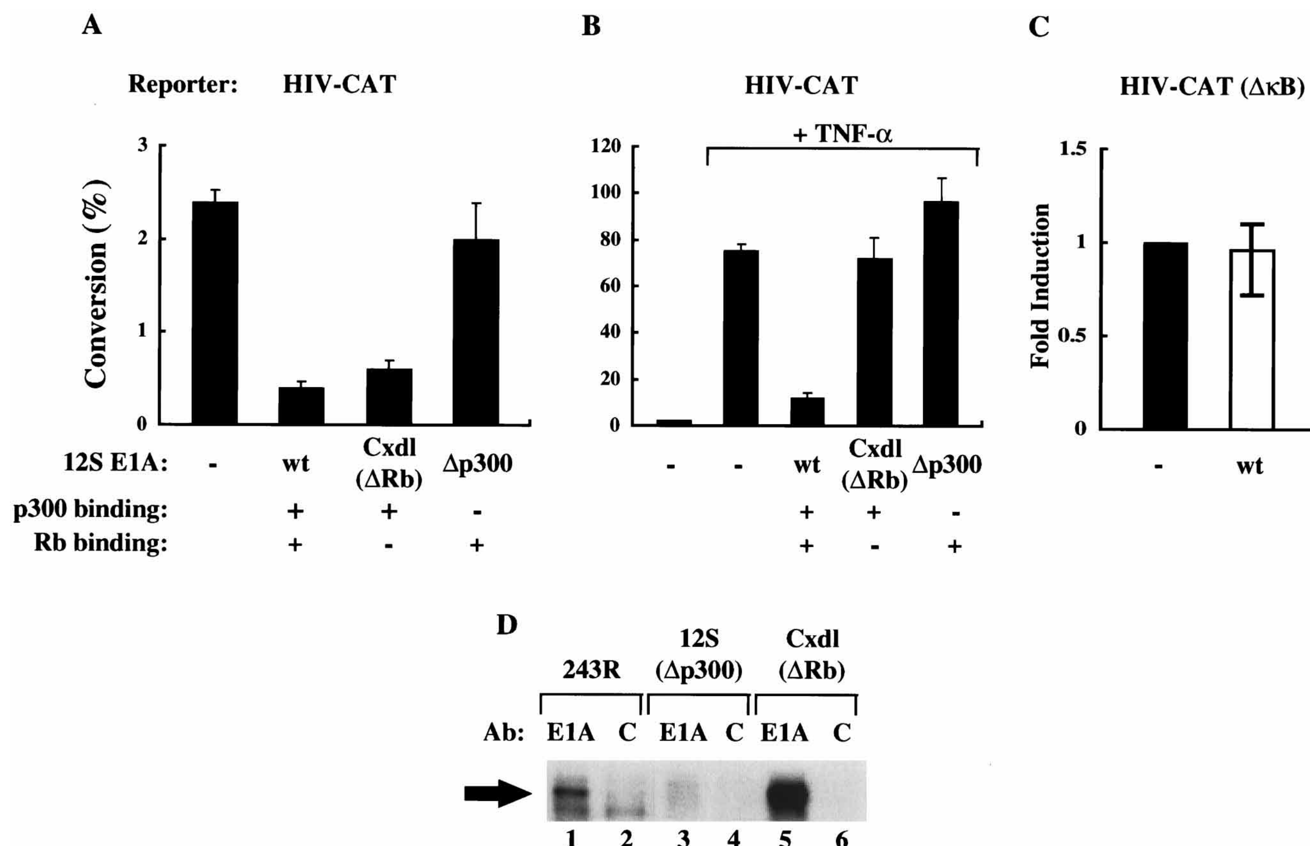


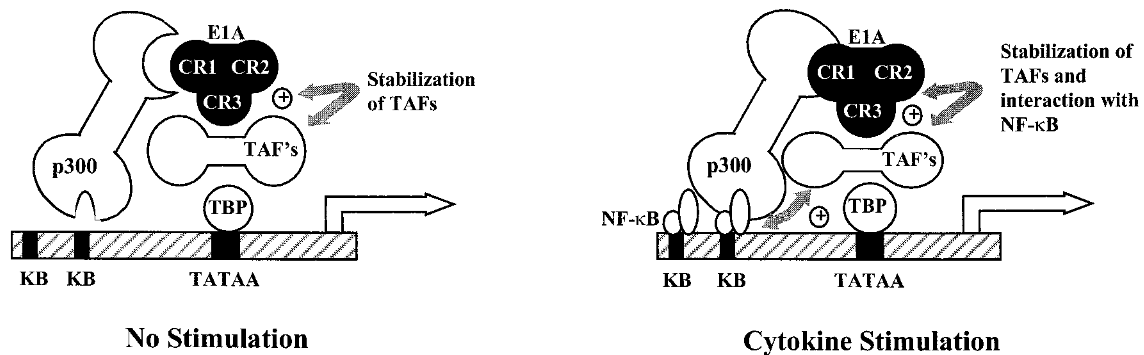
FIG. 5. Different cellular factors mediate repression of basal and TNF-α-stimulated gene expression. Jurkat T cells were transfected with 2 μg of HIV-CAT (A and B) or 2 μg of HIV-CAT (ΔκB) (C). Cells were cotransfected with 5 μg of expression plasmids for 243R E1A (wt) or the mutant E1A protein Cxdl (ΔRb) or 12S pm563 (Δp300). For the results shown in panel B, cells were treated for 8 h with 200 U of TNF-α ml^{-1}. Control plasmid was included such that each transfection contained a total of 5 μg of expression plasmid. Results shown are representative of at least three independent transfections, and for panels A and B, error bars represent the standard errors. Panel C is representative of two independent transfections, and the error bar represents exact values from each experiment. Note scale change between panels. (D) UM-449 cells were transfected with expression plasmid for E1A or the indicated E1A mutants and biosynthetically labeled. Immunoprecipitations were performed either with an anti-E1A antibody or with a control antibody, as described in Materials and Methods. The arrow marks the approximate position of a 300-kDa protein.

289R E1A is dependent upon association with components of the general transcription complex through its CR3 region. Activation is independent of the κB sites in the HIV enhancer and association with the cellular proteins p300 and pRb (Fig. 6A). By contrast, 243R-mediated repression of basal HIV transcription is dependent upon its association with p300 and requires the κB sites (Fig. 6B). In light of recent observations that p300 acts as a coactivator for NF-κB-mediated activation of the HIV enhancer (39), these data support a mechanism of repression of HIV gene expression by which E1A inhibits p300 so that it is no longer able to serve as a coactivator for NF-κB.

Consistent with previous findings, we have found that HIV transcriptional activation by 289R E1A is independent of NF-κB but will function cooperatively with NF-κB when this transcription factor is induced (7, 27, 36). Cooperative transcriptional activation, which is thought to be a consequence of protein-protein interactions between enhancer binding proteins and the basal transcription complex, has been reported for a number of cellular transcription factors. The synergy between 289R and RelA is likely to be the result of such an interaction, possibly facilitated by binding of E1A to TBP, as well as the interaction of RelA with p300 (Fig. 6A, right).

Previous studies have shown that the CR3 region of E1A targets multiple components of the TFIID complex (15, 16, 28, 33) in vitro. The zinc finger region at the NH_2 terminus of CR3 binds TBP, and the functional significance of this interaction has been defined by mutagenesis studies which demonstrate a correlation between reduced TBP binding and a diminished capacity to transactivate the adenovirus E3 promoter (16). In this study, we have utilized a series of E1A mutants to test the significance of the TBP-E1A interaction for transactivation of the HIV enhancer. Mutants previously shown to be unable to bind TBP in vitro (16) were also defective in their capacity to stimulate HIV-CAT activity. Interestingly, one finger region mutant which retained a wild-type capacity to bind TBP (16) displayed an abrogated transactivation capacity. It has been proposed that one or more other factors in addition to TBP are required for E1A-mediated transactivation. The inability of this mutant to activate transcription from either the HIV enhancer or the E3 promoter suggests that this as yet unidentified factor may be required for transactivation of several or all E1A-inducible promoters. Other TAFs seem likely candidates to bind this putative finger region; however, those TAFs currently known to interact with E1A, $dTAF_{II}110$ and $hTAF_{II}250$, do so within the carboxyl region of CR3 (15). A mutation within the carboxyl-terminal, promoter-targeting region of CR3, reported previously to abrogate activation of the E3 promoter (16, 53), did not affect HIV enhancer function. Thus, it seems likely that the transcriptional activation of these different promoters by E1A is dependent either upon interaction

A. Activation - 289R E1A



B. Repression - 243R E1A

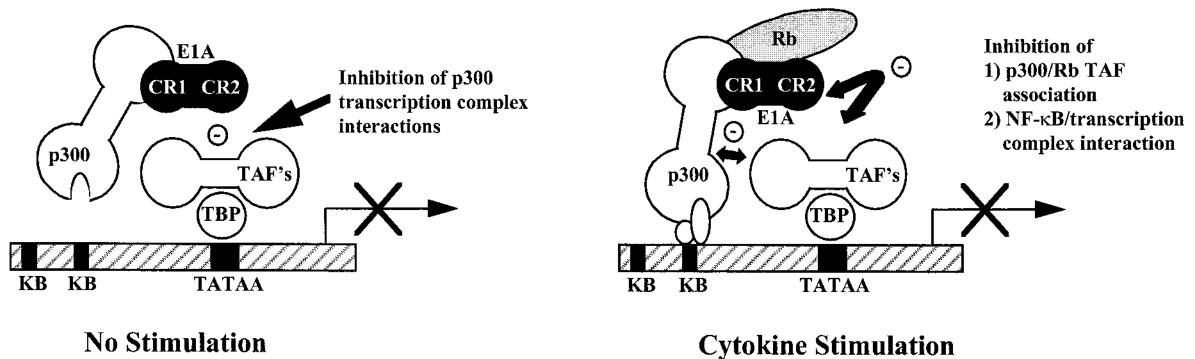


FIG. 6. Modulation of HIV transcription by different forms of E1A through specific interactions with cellular factors. (A) Activation of gene expression by 289R E1A is mediated by interaction of the CR3 domain with TBP and TAFs and does not require p300 or NF-κB (left). After cytokine stimulation with TNF-α, a cooperative activation is observed, presumably by recruitment of NF-κB/p300 into this complex (right). (B) Repression of basal transcription by 243R E1A requires p300 binding which may prevent its interaction with the TBP and the TFIID complex (left). After cytokine stimulation, E1A inhibits p300/pRb-dependent interactions that facilitate NF-κB-dependent interactions with the basal transcription complex (right).

with different TAFs or upon association with different enhancer binding factors required to recruit E1A to the E3 promoter.

Previous studies have also reported the ability of the 243R adenovirus E1A gene product to repress HIV LTR-directed transcription (51, 52). The findings of this work, in which we utilize an E1A point mutant that does not bind p300, concur with prior studies which demonstrated that repression is largely dependent upon the binding of p300 to CR1 and the nonconserved amino terminus of 243R. The importance of p300 binding in E1A-directed enhancer repression has now been shown in several different promoters in addition to HIV-1, including the insulin enhancer (48). Here, the ability of E1A mutants defective in the ability to bind p300 or pRb to repress cytokine-activated HIV transcription was also studied. We find that the CR2 region of E1A, which binds to pRb, and the p300 binding region are required for E1A-mediated repression of TNF-α-stimulated HIV gene expression (summarized in Fig. 6B, right). Because this CR2 region is not required for p300 binding, we conclude that repression of cytokine-induced HIV transcription is dependent upon additional cellular factors other than p300, specifically pRb or the related proteins p107 and p130. In addition, it is possible that the binding of additional undefined proteins is affected by the Cxd1 mutation and that these proteins also play a role in the repression of TNF-α-stimulated NF-κB.

Several cellular targets of repression by 243R have important roles in the regulation of cell growth. Because there is a strong correlation between the ability of E1A to bind p300 and to immortalize cells, E1A-mediated repression of cellular genes seems likely to play an important role in cell transformation and proliferation. The mechanism by which repression is achieved remains poorly understood. Recent in vivo and in vitro studies suggested an enhancer-independent mechanism of repression in which the amino terminus of 243R directly targets the general transcription machinery (47, 49). While attractive, such a model was in contradiction to previous observations that demonstrate the dependence of repression by 243R upon upstream enhancer elements (51, 52). Our findings suggest that the κB sites of the HIV enhancer are required for repression of HIV gene expression in vivo and implicate p300 in this process. Because p300 also interacts with factors in the basal transcriptional complex, such as TBP (1), the additional complexity of its protein-protein interactions may explain the apparently conflicting interpretations of previous studies.

We have recently described the previously unrecognized as-

sociation between the transactivation domain of RelA and p300. Cotransfection of an expression vector for p300 enhanced HIV transcription stimulated by RelA, and a model for κB-dependent transcriptional activation in which NF-κB is regulated by cyclin-dependent kinases through the intermediacy of the p300 and CBP coactivators has been described (39). The data presented in this report are consistent with these findings and suggest that transcriptional repression by 243R results from the inhibition of p300 function by E1A. We hypothesize that the association of p300 with E1A renders p300 unable to enhance NF-κB-mediated transcription. The interaction between p300 and E1A may also interfere with the ability of p300 to interact with basal transcription factors, since p300 binds to RelA and E1A through distinct domains (references 6 and 39 and unpublished observations). The observation that the pRb-binding CR2 region is required for repression of TNF-α-induced HIV transcription raises the possibility, however, that the mechanism of transcriptional repression by E1A is more complex than simply the sequestration of a single cellular product and that these associated cellular proteins differ in unstimulated versus cytokine-activated cells (Fig. 6B, right).

p300 has a high degree of homology to CBP (2), a transcriptional activator which binds the cyclic AMP response element (CRE) binding protein (CREB) and has recently been shown to regulate the activity of several other enhancer elements in addition to CRE (3). p300 and CBP have been shown to bind the basal transcription factors TFIIB and TBP (1, 26) and to serve as coactivators of transcription, bridging basal transcription factors with a number of upstream DNA-binding activator proteins in addition to NF-κB, including c-Myb, CREB, c-Fos, Sap-1a, and the nuclear receptor family (4, 11, 21, 25, 26). In analogous studies, the ability of E1A to repress Ap1 activity has been shown to result from the sequestration of the coactivator CBP from c-Fos (4). Additionally, a mutant form of p300 which is resistant to E1A can overcome its repression of the simian virus 40 promoter (13), and CREB has been shown to inhibit nuclear receptor activation (25) presumably through its interaction with CBP, which serves as a coactivator for its nuclear receptors. Despite the fact that the viral and cellular genes repressed by E1A lack a common enhancer element, the emerging promiscuity of the p300/CBP family of transcriptional coactivators makes it now seem increasingly plausible that transcriptional repression by E1A is accounted for by its ability to inhibit interactions with cellular proteins related to the p300/CBP family.

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