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Transcription factor TFIID is a direct functional target of the adenovirus E1A transcription–repression domain

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ABSTRACT **The 243-amino acid adenovirus E1A oncoprotein both positively and negatively modulates the expression of cellular genes involved in the regulation of cell growth. The E1A transcription repression function appears to be linked with its ability to induce cellular DNA synthesis, cell proliferation, and cell transformation, as well as to inhibit cell differentiation. The mechanism by which E1A represses the transcription of various promoters has proven enigmatic. Here we provide several lines of evidence that the "TATA-box" binding protein (TBP) component of transcription factor TFIID is a cellular target of the E1A repression function encoded within the E1A N-terminal 80 amino acids. (*i*) The E1A N-terminal 80 amino acids [E1A-(1–80)protein] efficiently represses basal transcription from TATA-containing core promoters *in vitro*. (*ii*) TBP reverses completely E1A repression *in vitro*. (*iii*) TBP restores transcriptional activity to E1A-(1–80) protein affinity-depleted nuclear extracts. (*iv*) The N-terminal repression domain of E1A interacts directly and specifically with TBP *in vitro*. These results may help explain how E1A represses a set of genes that lack common upstream promoter elements.**

Two major regulatory proteins of 243 and 289 amino acid residues (243R and 289R) are first expressed from early gene 1A (E1A) upon infection by group C adenoviruses. These proteins are involved in several cellular functions, including transcriptional activation, transcriptional repression, the induction of cellular DNA synthesis, cell immortalization, cell transformation, and the inhibition of metastasis and of cell differentiation (for reviews, see refs. 1 and 2). Amino acid sequence comparisons of E1A proteins from different serotypes reveal three conserved regions, CR1 (amino acids 41–80), CR2 (amino acids 121–139), and CR3 (amino acids 140–188) (1, 2). E1A 243R and E1A 289R are identical except for CR3, a transactivation domain unique to 289R. CR3 is essential for transcriptional activation of adenovirus early genes during productive viral infection but is not required for cell transformation.

Three regions encoded within E1A 243R are required for the growth regulatory functions of E1A. These include conserved regions CR1 and CR2 and the relatively nonconserved N-terminal region (amino acids ≈1–40). These E1A domains function by directly targeting cellular regulatory proteins and modulating their activities. For example, by targeting different sets of cellular proteins, E1A can induce S-phase DNA synthesis through two distinct pathways. The first is the Rb/E2F pathway, which requires contact sites within CR2 and the N-terminal portion of CR1. The second is the N-terminal pathway, which involves contact sites within the nonconserved N terminus and a portion of CR1. Both pathways are required for the cell immortalization and cell transformation functions of E1A. The Rb/E2F pathway has been well studied. In contrast, the cellular regulatory proteins interacting with the nonconserved N terminus and their functions are only beginning to be elucidated. An intriguing function of 243R is its ability to transcriptionally repress cellular genes involved in growth regulation and differentiation as well as several viral promoters (1, 2). The mechanism of E1A repression is poorly understood. A puzzling feature is the ability of E1A to transcriptionally repress the expression of a diverse group of cellular and viral genes that contain no apparent upstream promoter elements in common. We have previously shown that the adenovirus E1A N-terminal 1–80 amino acids [E1A-(1–80)] are sufficient for transcriptional repression of several promoters *in vivo* and *in vitro* (3, 4). In this report, we investigate the cellular target(s) of E1A repression by use of an *in vitro* transcription repression system. We show that the E1A-(1–80) protein represses transcription from basal promoters, thus implicating a general transcription factor. We then demonstrate by transcription–depletion analysis and rescue experiments that the general transcription component directly targeted by the E1A repression domain is the "TATA-box" binding protein (TBP) component of transcription factor TFIID.

MATERIALS AND METHODS

***In Vitro* Transcription Repression.** *In vitro* transcription reactions were performed as described (3). RNA transcripts were analyzed by primer extension. Our previous studies have shown that transcription repression by E1A is dependent upon the relative amounts of nuclear extract and E1A protein in the transcription reaction (3, 4). For a fixed amount of nuclear extract, the degree of repression is proportional to the amount of added E1A, and E1A-repressed transcription is overcome by the addition of more nuclear extract (3). To maximize the measurement of E1A repression, a minimal amount of nuclear extract (5 μl) was used for *in vitro* transcription in these studies. Under these conditions, addition of more nuclear extract or recombinant human TBP results in a dose-dependent increase in the transcription signal until a saturation level is achieved. The reversal of E1A repression by added TBP (under the conditions of saturating template and limiting nuclear extract) presumably reflects restoration of the basal activity of the promoter. Recombinant TBP was purchased from Promega. Recombinant E1A proteins were expressed in and purified from bacteria as described (4). When the (-533) human immunodeficiency virus (HIV) long terminal repeat (LTR) construct pBennCAT (5), (-117)HIV LTR, (-83)HIV LTR (6), ATF(-31)HIV LTR (6), and USF(-31)HIV LTR (6) were used as templates, exposure of autoradiograms was for approximately 12 h. When (-31)HIV LTR, (-31)HIV LTR ΔTAR (6), pTATAA chloramphenicol acetyltransferase (CAT) (7), E1B TATA (8), and MLPCAT (9) were used as templates, exposure times were increased to approximately 48 h.

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Abbreviations: TBP, "TATA-box" binding protein; HIV, human immunodeficiency virus; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase.

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E1A-(1–80) Protein Affinity-Depletion Analysis of Nuclear Transcription Extracts. Affinity columns were prepared by immobilization of recombinant proteins to Affi-Gel 10 in 20 mM Hepes (pH 7.6), according to the manufacturer's instructions (Bio-Rad). One milliliter of HeLa cell nuclear extract (3) was recirculated at 4°C through 250 μl of a packed Affi-Gel 10 affinity column containing 1.5 mg of wild-type E1A-(1–80) or E1A-(1–80) with residues 4–25 deleted [E1A-(1–80 Δ4–25)], for 2 h at a flow rate of 0.1 ml/min. Protein concentrations were determined with a Bio-Rad protein assay kit, and equal amounts of protein from the nuclear extract or column flow-through were used for the *in vitro* transcription assay. Ten-microliter amounts of the initial nuclear extract and flow-through fractions were examined by immunoblot analysis using mouse monoclonal antibody to TBP (Santa Cruz Biotechnology) and enhanced chemical luminescence (ECL) (Amersham). TFIIB was a generous gift from T. Roberts and M. R. Green (Program in Molecular Medicine, University of Massachusetts Medical Center, Worcester).

***In Vitro* Binding Assays.** Recombinant proteins were immobilized on Affi-Gel 10 as described above. pGEM3-hTBP (10) was translated *in vitro* by using a Promega TNT reticulocyte kit, Sp6 polymerase, and ^{35}S-labeled methionine. Affi-Gel 10 preparations containing 1, 2, and 4 μg of E1A-(1–80), 4 μg of E1A-(1–80 Δ4–25), and 12 μg of E1A 243R were preincubated for 60 min at 4°C in 300 μl of Nonidet P-40 binding buffer [40 mM Hepes, pH 7.5/150 mM KCl/5 mM $MgCl_2$/0.5 mM EDTA/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride/0.2% Nonidet P-40/sonicated and clarified *Escherichia coli* extract (0.5 mg/ml)] (11). *In vitro*-translated TBP (3 μl) was added and the incubation was continued for an additional 60 min. Beads were washed four times with 1 ml of Nonidet P-40 buffer lacking *E. coli* extract, and bound protein was eluted with SDS/sample buffer, resolved by SDS/PAGE, and quantitated by PhosphorImager analysis.

RESULTS

The E1A-(1–80) Protein Represses Basal Transcription from Core Promoters *in Vitro*. We have shown that both HIV-1 Tat independent and Tat-activated transcription of the HIV-1 LTR promoter are strongly and specifically repressed by E1A-(1–80) protein *in vitro* (3). We attempted to delineate the promoter elements responsible for E1A repression of the HIV LTR. As shown in Fig. 1, each sequential deletion of upstream promoter element yielded a template that was still repressed by E1A. The degree of repression ranged from 74% to 96% in these experiments, as determined by densitometric analysis (see Fig. 1). Remarkably, the core promoter of HIV LTR is repressed by the E1A-(1–80) protein. Strong E1A repression was also observed *in vitro* when other transcription binding sites were fused to the HIV LTR core promoter, including ATF (-31)HIV LTR and USF (-31)HIV LTR (data not shown). Additionally, when (-31)HIV LTR ΔTAR (which lacks the downstream TAR element) was used as template, strong repression by the E1A-(1–80) protein was still observed (data not shown). pTATAA CAT, which contains the E1A-repressible fibronectin gene (12) from positions −36 to +8 in which the TATA sequence is the only known element (7), was similarly repressed (data not shown). Finally, the synthetic promoter E1B TATA CAT (8), consisting of the E1B TATA box (AGGGTATATAATG) inserted immediately upstream of the CAT gene, is almost totally repressed *in vitro* by the E1A-(1–80) protein (Fig. 2). The concurrent lack of repression of the MLP template in the same reaction mixture demonstrates the specificity of E1A repression. The above results showing that E1A can repress a variety of core promoters suggest that no specific upstream or downstream element(s) is required for E1A repression.

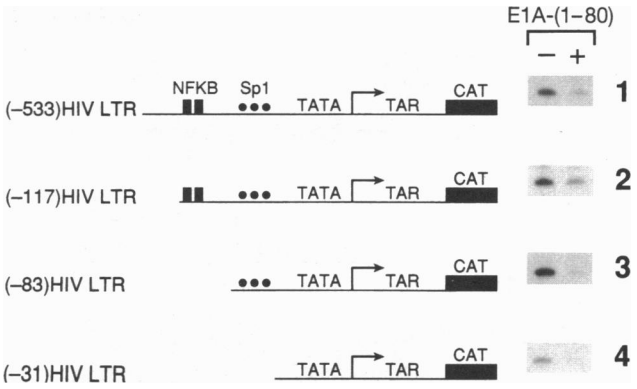

FIG. 1. No specific upstream promoter elements are required for E1A transcriptional repression of the HIV-1 LTR promoter *in vitro*. Various deletion mutants of the HIV-1 LTR promoter were used as templates in the E1A transcription repression reaction *in vitro* to identify sequences in HIV-1 LTR that can mediate E1A repression. To the left is shown the HIV-1 LTR promoter mutants used. To the right is shown the *in vitro* transcription results in the absence (−) or presence (+) of 400 ng of E1A-(1–80) protein. The reactions contained 500 ng of DNA template. RNA transcripts were analyzed by primer extension. The optical density of bands on autoradiographs was quantitated by using a Molecular Dynamics personal densitometer and IMAGEQUANT software. E1A-(1–80) repressed expression from the (-533)HIV LTR by 85%, from the (-117)HIV LTR by 74%, from the (-83)HIV LTR by 93%, and from the (-31)HIV LTR by 85%. These experiments were repeated three times with similar results.

The TATA-Box Binding Factor TBP Can Overcome Transcription Repression *in Vitro* by the E1A 243R and E1A-(1–80) Proteins in a Dose-Dependent Manner. The repression of TATA-driven basal transcription implies that the general transcription machinery may be the direct target of E1A repression. We have found that the TFIID fraction from a phosphocellulose column was able to reverse E1A repression *in vitro* (data not shown). To confirm TFIID involvement and to determine whether TBP is sufficient to overcome E1A repression, recombinant TBP was assayed in transcription reactions repressed by E1A. TBP was able to overcome completely repression by both E1A 243R (Fig. 3*A*) and by E1A-(1–80) (Fig. 3*B*). Significantly, restoration of transcription by TBP is overridden by increasing the concentration of E1A 243R or E1A-(1–80) (Fig. 3 *A* and *B*, see legends for quantitation). TBP overcame repression by E1A-(1–80) in a dose-dependent manner (Fig. 3*C*). These findings provide strong presumptive evidence that the TBP subunit of TFIID is the cellular target of E1A repression.

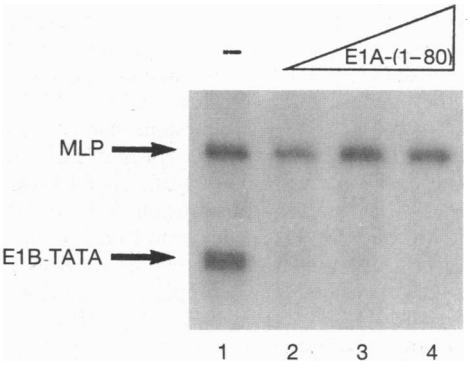

FIG. 2. Synthetic E1B TATA promoter is repressed *in vitro* by E1A-(1–80), whereas the major late promoter (MLP) in the same reaction mixture is not repressed. Lanes 2, 3, and 4 contained 200 ng, 400 ng, and 600 ng of E1A-(1–80) protein, respectively. Reaction mixtures contained 100 ng of MLPCAT and 1 μg of E1B TATA CAT. RNA transcripts were analyzed by primer extension. These experiments were repeated three times with identical results.

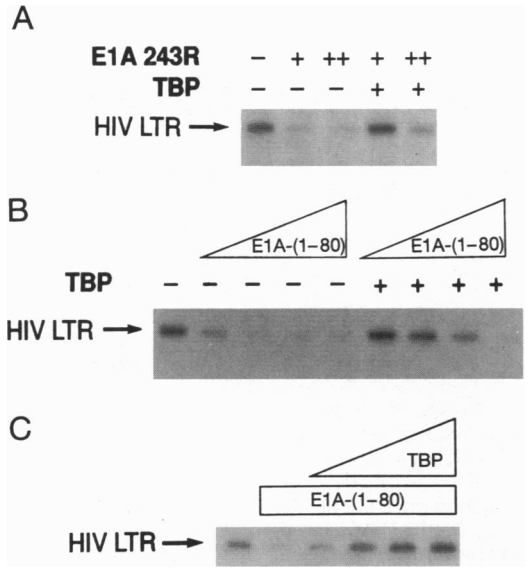

FIG. 3. TBP can overcome transcriptional repression *in vitro* by E1A 243R or E1A-(1–80) in a dose-dependent manner. (*A*) TBP can reverse E1A 243R repressed transcription. Addition of 900 ng (+) and 1200 ng (++) of recombinant E1A 243R protein repressed HIV LTR transcription by 81% and 88%, respectively, as shown by densitometric analysis. TBP (5 ng) overcomes repression by 900 ng but not by 1200 ng of E1A 243R (repressed by 76%). (*B*) The addition of E1A-(1–80) (200, 400, 600, and 800 ng in lanes 2–5, respectively) to the reaction mixture results in strong repression of HIV-1 LTR transcription (75%, 90%, 91%, and 93%, respectively). TBP (5 ng) can completely overcome repression by 200 ng and 400 ng of E1A-(1–80) (lanes 6 and 7) but not by 600 ng (lane 8) or 800 ng (lane 9) of E1A-(1–80). (*C*) The repression of transcription by 600 ng of E1A-(1–80) can be completely overcome by increasing the amount of TBP (10, 20, 40, and 80 ng of TBP in lanes 3–6, respectively). *In vitro* transcription reactions were performed as described in Fig. 1 by using pBennCAT as template. These experiments were repeated three times with similar results.

TBP Can Restore the Transcriptional Activity of E1A-(1–80) Affinity-Depleted Nuclear Extracts. To further elucidate the functional interaction between E1A-(1–80) and TFIID, affinity column depletion analysis of transcription extracts was performed by using the wild-type E1A-(1–80) protein as immobilized ligand. As control, the deletion mutant protein E1A-(1–80 Δ4–25), which lacks amino acids 4–25 and was shown (3, 4) to be defective in repression *in vitro*, was used as ligand. The flow-through from the E1A-(1–80) column was unable to support transcription, whereas that from the E1A-(1–80 Δ4–25) column was as active as the original extract (Fig. 4*A*). Importantly, the addition of TBP (Fig. 5, compare lanes 4 and 5) efficiently restored transcriptional activity to the transcription-deficient E1A-(1–80)-depleted extract. In contrast, another general transcription factor, TFIIB, did not restore activity (Fig. 5, lane 7). Of significance, transcription activity restored by TBP was in turn repressible by E1A-(1–80) (Fig. 5, lane 6). This finding indicates that E1A can interact with added TBP to repress transcription. Similar results were obtained with the E1B TATA promoter and the collagenase promoter (data not shown). Immunoblot analysis with anti-TBP antibody revealed the complete depletion of TBP (TFIID) by the E1A-(1–80) column (Fig. 4*B*), concomitant with the loss of transcriptional activity (Fig. 4*A*). Significantly, TBP was not depleted by affinity chromatography with E1A-(1–80 Δ4–25) (Fig. 4*B*), which is defective in E1A repression (3, 4). TBP was strongly bound to the E1A-(1–80) column but not to the E1A-(1–80 Δ4–25) column (data not shown). In conclusion, E1A-(1–80) specifically removes TBP (TFIID) from the nuclear transcription extract, as predicted by the ability of TBP to rescue transcriptional activity.

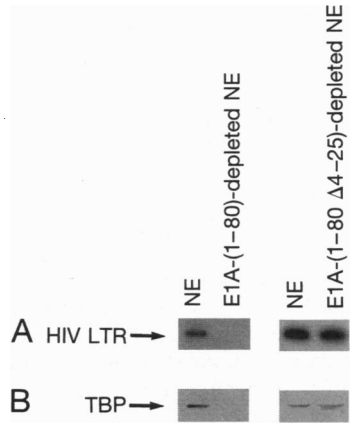

FIG. 4. TBP restores the transcriptional activity of E1A-(1–80) affinity-depleted nuclear extracts. (*A*) Nuclear extracts (NEs) lose the ability to support transcription of HIV LTR *in vitro* after passage through an affinity column containing wild-type E1A-(1–80) protein but not through a column containing repression-defective E1A-(1–80 Δ4–25) protein. The arrow points to the primer extension product. (*B*) TBP is specifically retained by the E1A-(1–80) column but not by the E1A-(1–80 Δ4–25) column, as shown by immunoblot analysis with anti-TBP antibody. These results suggest that the loss of transcriptional activity after passage through an E1A-(1–80) affinity column is due to the depletion of TFIID in the nuclear extract. These experiments were repeated twice with identical results.

TBP Interacts Directly with E1A-(1–80) Protein *in Vitro*. The above results show that E1A-(1–80) protein affinity chromatography removes TFIID from a nuclear transcription extract and that basal transcriptional activity can be restored by supplementation with TBP. These findings could mean that E1A interacts with TBP directly or with a protein intermediate

FIG. 5. TBP can restore transcriptional activity to an E1A-(1–80)-depleted nuclear extract. The transcriptional activity of the original nuclear extract (NE) (lane 1) is repressed by added E1A-(1–80) protein (400 ng) (lane 2). Transcriptional activity is lost by passage through an E1A-(1–80) affinity column (lane 4) but not an E1A-(1–80 Δ4–25) column (lane 3). The addition of TBP (5 ng) (lane 5) but not TFIIB (20 ng) (lane 7) restores transcriptional activity to the E1A-(1–80) affinity-depleted nuclear extract. The activity restored by TBP can be repressed by addition of E1A-(1–80) (400 ng) (lane 6). *In vitro* transcription and primer-extension assays were performed as described in Fig. 1 with pBennCAT as template. These experiments were repeated three times with similar results.

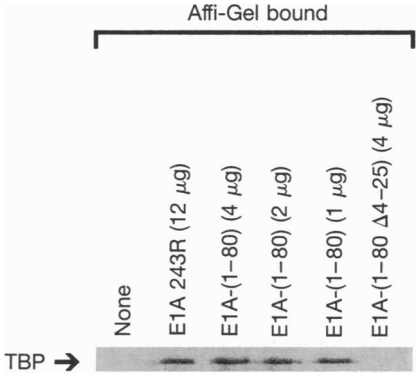

FIG. 6. TBP interacts directly with E1A-(1–80). Protein–protein interaction assays were carried out between ^{35}S-labeled *in vitro*-translated TBP and Affi-Gel 10 alone (none) or Affi-Gel-immobilized recombinant E1A 243R (12 μg), E1A-(1–80) (1, 2, and 4 μg), or E1A-(1–80 Δ4–25) (4 μg). About 20% of input TBP was bound by E1A-(1–80) and E1A 243R (data not shown).

that is associated with TBP, for example, a TAF (TBP-associated factor). To determine whether there is a direct physical interaction between TBP and the E1A N-terminal region, *in vitro* protein–protein interaction studies were performed. As shown in Fig. 6, Affi-Gel-bound E1A-(1–80) (1, 2, and 4 μg) but not Affi-Gel by itself strongly binds TBP. As expected, the full-length E1A 243R protein [12 μg, about the same molar amount as 4 μg of E1A-(1–80)] also binds TBP. Importantly, the transcription-repression-deficient mutant E1A-(1–80 Δ4–25) does not interact *in vitro* with TBP (Fig. 6). This result provides strong presumptive evidence that the interaction with TBP is functionally significant for E1A repression. Identical results were obtained by Western blot analysis using recombinant human TBP as ligand or in solution binding to E1A-(1–80) (unpublished data).

DISCUSSION

Our combined results point to the conclusion that the TBP subunit of TFIID is the direct functional target of E1A repression. (*i*) E1A-(1–80) efficiently represses basal transcription from TATA-containing core promoters *in vitro* (Figs. 1 and 2). (*ii*) TBP reverses completely E1A repression *in vitro* (Fig. 3). (*iii*) TBP restores transcriptional activity to E1A-(1–80)-depleted nuclear extracts (Fig. 5). (*iv*) The N-terminal repression domain of E1A interacts directly with TBP *in vitro* (Fig. 6).

Because TFIID plays a central role in both regulated and basal transcription (13), the targeting of TFIID may provide the simplest explanation for the repression of diverse promoters by E1A. Repressibility of a promoter may be determined by the availability to the E1A repression domain of a specific surface of TBP that could be embedded within a promoter-specific transcription complex. This hypothesis may best explain the repression of diverse promoters by E1A and why some promoters, such as MLP, are insensitive to E1A repression. In addition, it emphasizes the importance of combinatorial regulation of gene expression by communication of non-DNA-specific activators/repressors, promoter-specific transcription regulators, and the general transcription machinery. Several cellular proteins have been reported to interact with the E1A N-terminal region (14–18). The interaction between E1A and TFIID may be subject to regulation by some of these proteins depending upon promoter context and cellular phenotype. For example, the p300/CBP family of transcription factors, which can interact with the E1A N terminus, could possibly serve to block E1A interaction with TBP, i.e., as an antirepressor.

Of interest, several cellular repressors have been reported recently to negatively modulate transcription by direct interaction with TBP and/or TFIID (for review, see ref. 19). By targeting TFIID, these repressors inhibit transcription through diverse mechanisms. Dr2/DNA topoisomerase I-mediated repression of basal transcription can be overcome by activators and/or TFIIA (20). Dr1 can repress both basal and activated transcription by certain activators. Dr1 represses transcription by dissociating the interaction of TBP with TFIIA and/or TFIIB but does not affect binding of TBP to the TATA box element (21). Dr1-mediated repression can be overcome by TBP *in vitro* and *in vivo* (21, 22). NC1 and NC2 also form complexes with TBP and block the association of TFIIA and TFIIB with TFIID (23, 24). On the other hand, MOT1, a member of the Snf2/Swi2 family, represses transcription by preventing TBP binding to the TATA element in an ATP-dependent way (25). Because E1A also represses transcription by directly targeting TFIID, we propose that E1A may be a viral counterpart of this group of cellular repressors. However, our preliminary DNase I footprint analysis shows that E1A can inhibit the binding of TBP to the TATA element (unpublished data). Thus the E1A N-terminal domain appears to repress transcription by a mechanism that differs, at least in part, from that of the various cellular repressors discussed above. Further studies on the mechanism of E1A repression and the interaction of the E1A repression domain with TFIID will be of interest.

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