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FAST TRACK

Roles of p300, Pocket Proteins, and hTBP in E1A-Mediated Transcriptional Regulation and Inhibition of p53 Transactivation Activity

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Abstract The conserved region 1 and the extreme N-terminus of adenoviral oncoprotein E1A are essential for transforming activity. They also play roles in the interaction of E1A with p300/CBP and pRb and are involved in both transactivation and repression of host gene expression. It was reported recently that p53-mediated transactivation is specifically repressed by E1A and that p53-induced apoptosis can be protected by pRb. In this report, we investigated the roles of pRb and p300 in the N-terminus of E1A-mediated transcriptional regulation. We demonstrate here that p300 and pRb have no effect on DBD.1-70 transactivation and that overexpression of p300 or pRb failed to relieve the repression by E1A. Repression of p53 transactivation requires both the extreme amino terminus and CR1 but not CR2. This repressive activity of E1A specifically correlates with E1A's ability to bind p300 and TBP. On the other hand, E1A inhibited the transactivation activity of a fusion construct containing the DNA binding domain of yeast Gal4 and the transactivation domain of p53. When p53 was cotransfected with E1A, similar inhibition was found in Saos-2 cells that lack endogenous pRb and p53 activity. Introduction of pRb into Saos-2 cells did not affect p53 transcription activity. E1A-mediated repression can be relieved by overexpression of either p300, hTBP, or TFIIB but cannot be released by overexpression of pocket proteins. Our data suggest that p300/CBP and TBP but not the pocket proteins, pRb, p107, and pRb2/p130 are functional targets of E1A in transcriptional regulation and that p53 transactivation requires the function of the p300/TBP/TFIIB complex, thus delineating a new pathway by which E1A may exert its transforming activity. J. Cell. Biochem. 66:277–285, 1997.

Key words: pRb; p107; p130/Rb2; TBP; transcription

The tumor suppressor p53 has been shown to be functionally involved in G1 and G2 arrest during the cell cycle as well as the regulation of differentiation and apoptosis [Lane, 1992; Wu and Levine, 1994; Steinman et al., 1994, Sang et al., 1995]. As a transcription regulator, p53 binds DNA in a sequence-specific manner and regulates gene expression both positively and negatively. p53-transactivated genes include p21/WAF1/cip, Gadd45, and Bax [Fornace, 1992; El-Deiry et al., 1993; Miyashita and Reed, 1995]. p21/WAF1/cip is an inhibitor of cyclin-dependent kinases (Cdk) which play essential roles in G1 arrest and differentiation, at least partially via the regulation of pocket protein function [El-Deiry et al., 1993; Elledge and Harper, 1994; reviewed in Sang et al., 1995; MacLachlan et al., 1995]. Decreased p21/WAF1/cip activity may lead to inactivation of pRb by phosphorylation. Thus, p21 provides an interplay between p53 and pRb, two important tumor suppressors.

Abbreviations: CAT, chloramphenicol acetyl transferase; Cdk, cyclin-dependent kinase; CR, conserved region; ENT, extreme amino terminus; FBS, fetal bovine serum; hTBP, human TATA binding protein; PVDF, polyvinylidene difluoride; Rb, retinoblastoma.

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Gadd45 stimulates the DNA excision repair pathway and also may play an important role in inhibition of the G1/S transition by interacting with PCNA [Zhan et al., 1994]. High levels of expression of Bax promote apoptosis. In addition, p53 downregulates *bcl2,* a protooncogene whose protein product promotes cell survival [Miyashita et al., 1994].

Proteins encoded by the E1A oncogene of human adenoviruses are able to reprogram transcription regulation of the host cells to produce an environment suitable for viral replication. This altered transcription regulation is closely linked to E1A's ability to immortalize and transform rodent cells in cooperation with other oncogenes such as E1B or ras. Several different mechanisms have been reported to be involved in E1A-mediated regulation of host gene expression. All these mechanisms are based on E1A's interaction with cellular proteins via several functional regions. Two of the three conserved regions, CR1 (aa40–80), CR2 (aa120–140), and the extreme N-terminus (ENT) (aa1–40), of E1A have been linked specifically with its ability to stimulate proliferation and to interact with several cellular proteins.

E1A directly targets pRb and the other pRb-related pocket proteins p107 and p130/Rb2 via CR1 and CR2 [reviewed in Sang et al., 1995]. pRb, p107 [Zhu et al., 1993], and p130/Rb2 [Mayol et al., 1993; Claudio et al., 1994; Li et al., 1993; Li et al., 1996] form the pocket protein family that shares the highly conserved pocket domain that is involved in their interaction with E1A. Pocket proteins have been shown to play roles in growth inhibition, differentiation, and development. As transcription regulators, pocket proteins interact with transcriptional factors such as the E2F family, MyoD, and ID2 via the pocket domain. Interactions between E1A and the pocket proteins release the E2F family of transcription factors from the pocket proteins and allow the transactivation of E2F responsive genes such as DNA pol α and c-myb which are required for the G1/S transition [Sala et al., 1994]. CR1 and ENT are required for interactions with p300 and CBP [Moran, 1993; Wang et al., 1993], two closely related coactivators that are involved in the transactivation of E-box genes and c-Jun/c-Fos–mediated transactivation. E1A may repress the expression of these genes by interacting directly with p300/CBP [Offringa et al., 1990; Yuan et al., 1996; Puri et al., 1997; Eckner et al., 1994, 1996]. Recently, we showed that the ENT and CR1 region are also required for functional interaction with hTBP in vivo, and their interaction with hTBP may be involved in both transactivation and repression of host gene expression [Sang and Giordano, 1997; Sang et al., in press].

It was reported recently that E1A inhibits p53 transactivation and abolishes p53-dependent G1 arrest after DNA damage [Steegenga et al., 1996] and that E1A-mediated apoptosis requires p53 function [Debbas and White, 1993; Lowe and Ruley, 1993; Sabbatini et al., 1995]. Evidence has been provided that pRb might overcome p53-directed killing of neoplastic cells [Haas-Kogan et al., 1995]. In order to molecularly dissect the mechanisms of E1A-mediated repression of p53 transcription activity and the interplay between E1A, p53, and the pocket proteins, we investigated the regions of E1A and its cellular targets involved in repression of p53 transcription activity. Our results show 1) both the extreme N-terminus and CR1 are required for E1A's ability to repress p53 transactivation, 2) pRb does not affect p53 transactivation activity and is not essential for E1A-mediated repression, 3) E1A's ability to repress p53 correlates functionally with its ability to bind p300/CBP and TBP, and 4) overexpression of either p300, TBP, or TFIIB can release E1A-mediated repression. These data suggest that the p300/TBP/TFIIB complex is required for p53 transactivation and that disruption of p300/TBP/TFIIB function is the mechanism by which E1A perturbs p53 function.

MATERIALS AND METHODS

Plasmids

All the plasmids encoding E1A, E1A mutants, DBD.VP16, DBD, DBD.E1A, and DBD.E1A mutants, and the pG5.E1B-CAT reporter have been described previously [Sang et al., in press]. p13.CAT is a reporter construct with 13 synthesized p53 binding sites. pCMV.hp53 is a eukaryotic expression plasmid with p53 under the control of a CMV promoter. The plasmid pCMV-β-p300 was previously described [Eckner et al., 1994; Yuan et al., 1996]. pcDNA3.hTBP and pcDNA3.TFIIB were created by insertion of the PCR fragment of the corresponding cDNA into the pcDNA3 vector [Sang et al., in press]. The eukaryotic expression plasmids, pCMV.p107, pcDNA3.pRb, and pcDNA3.pRb2 were described previously [Zhu

et al., 1993; Mayol et al., 1993; Li et al., 1993; Claudio et al., 1994]. pDBD.p53TA was generated by inserting the cDNA sequences encoding the p53 transactivation domain (aa1–80) into the pDBD vector which leads to an in-frame fusion of p53TA with the DNA binding domain of yeast Gal4.

Antibodies

Monoclonal anti-pRb antibody XZ77, monoclonal anti-p53 antibody pab421, and polyclonal anti-p300 sera were described elsewhere [Giordano et al., 1989; Sang and Giordano, 1997]. Polyclonal anti-hTBP antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell Culture, Transfection, and CAT Assays

BALB/c3T3 (ATCC (Rockville, MD): CCL163, mouse embryo fibroblast) was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with L-glutamine and 10% calf serum. For comparison purposes, two cell lines derived from human osteosarcoma, U-2OS ($RB^{+/+}$, $TP53^{+/+}$, ATCC: HTB-96) and Saos-2 ($RB^{-/-}$, $TP53^{-/-}$, ATCC: HTB-85), two osteosarcoma cell lines, were cultured and transfected using identical experimental conditions. DMEM supplemented with 15% FBS and L-glutamine was used to culture the cells. Transfectam (Promega, Madison, WI) was used for the transfection, and the serum-free transfection procedures are described in the manufacturer's instructions. All transfections were performed in 60 mm tissue culture dishes, and pON260 was cotransfected to normalize the transfection efficiency. When needed, salmon sperm DNA was used to keep the total amount of DNA equal per dish. Three hours after transfection, fresh medium with serum was added, and the cells then were cultured for 48 h before harvest. The cell lysates were assayed for β-gal activities from cotransfected pON260. A liquid scintillation counter was used to determine CAT activities. The CAT activities then were corrected with the β-gal activities to normalize the transfection efficiency. Relative activity was defined as the ratio of normalized activity obtained from cotransfection of an effector to the normalized activity obtained from the cotransfection of pDBD, which encodes only DBD of yeast Gal4.

Immunoprecipitation and Western Blot

The cultured cells were washed twice with phosphate buffered saline (PBS), and the cells were harvested and lysed on ice by resuspending the cell pellets in lysis buffer (50 mM TrisCl, 0.1% Triton X-100, 5 mM EDTA, 250 mM NaCl, 50 mM NaF, 0.1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 10 μg/ml Leupeptin) and keeping them on ice for 30 min. After the samples were centrifugalized twice in a microcentrifuge at 11,000*g* for 5 min at 4°C, the supernatant was collected. The protein concentration of the lysates was determined by the BioRad (Richmond, CA) method. For immunoprecipitation, the cell lysates were precleared by the addition of normal rabbit serum (NRS) and by subsequent precipitation with pretreated *Staphylococcus aureus* (SAC). Precleared lysates then were incubated with antibodies at 4°C for 1 h. Immunocomplex was precipitated with 40 μl of protein-A sepharose. The precipitates were washed with 1 ml of lysis buffer four times and finally were resuspended in 40 μl of Laemmli sample buffer (LSB) for SDS-PAGE. For Western blot analyses, protein samples resolved by an SDS-PAGE were electrotransferred onto a PVDF membrane (Millipore, Bedford, MA) in CAPS buffer (10 mM CAPS, 20% methanol, pH 11). The membrane then was blocked with 5% milk in TBS-T (2 mM Tris, 13.7 mM NaCl, 0.1% Tween-20, pH 7.6). Antibodies were incubated overnight at 4°C with the membrane in TBS-T containing 2.5% milk. The membrane then was developed by the ECL system (Amersham, Arlington Heights, IL, or Dupont, Wilmington, DE) with horseradish peroxidase coupled rabbit antimouse IgG as the secondary antibody by following the manufacturer's instructions.

RESULTS

Roles of p300 and pRb in ENT- and CR1-Mediated Transactivation and Repression

Previously we showed that the N-terminal transforming region of E1A contains two overlapping but distinct transactivation domains [Sang et al., in press]. Because the ENT and the CR1 regions interact with p300/CBP and the CR1 region is essential for the interaction with pRb, the roles of p300 and pRb in E1A-mediated transactivation and repression were investigated. Figure 1 shows that DBD.1-70 has the ability to transactivate the pG5.E1B-CAT re-

porter in BALB/c3T3 cells. When E1A without a DNA binding domain was used to repress the transactivation, overexpression of either p300 or pRb failed to relieve the repression by E1A, suggesting that neither p300 nor pRb is involved in the transactivation and repression activity of the N-terminus of E1A. As a positive control, overexpression of TBP relieved the repression [Sang et al., in press].

Inhibition of p53 Transactivation Activity by E1A Requires the N-Terminus of E1A to Disturb the Function of the p53 Transactivation Domain

Work from Steegenga et al. [1996] demonstrates that E1A is able to inhibit p53 transactivation and to abolish p53-mediated G1 arrest in response to DNA damage. Previous work from other laboratories shows that E1A-mediated apoptosis requires the stabilization of p53 [Debbas and White, 1993; Lowe and Ruley, 1993; Sabbatini et al., 1995], suggesting a functional interplay between p53 and E1A. In order to understand this interplay, we investigated the effect of E1A on p53 transactivation in the U-2OS cell line using a p13-CAT construct as a reporter gene. As expected, transfection of exogenous p53 increased the reporter gene expression around fivefold (Fig. 2). Cotransfection of wild-type 243R E1A inhibited the transactivation in a dose-effective manner. The E1A point mutant that changes aa47 from tyrosine to histidine (YH47) inhibited the transactivation in a similar manner to wild type E1A. The N-terminal deletion mutants (Δ2-36, Δ15-36, and Δ38-67) failed to inhibit p53 transactivation, suggesting a role for this region in E1A-mediated repression of the p53 reporter. Deletions in the carboxyl terminus (aa223 or aa128 to C-terminus) of E1A showed no effect on E1A's inhibitory function.

As a transcription factor, p53 possesses both an acidic activation domain at its N-terminus and a DNA binding domain [Sang et al., 1995]. Thus, inhibition of p53 transcription activity by E1A may result from interference with either the transactivation domain or the DNA binding domain. To learn which domain is functionally affected by E1A, we fused the transactivation domain of p53 (N-terminal 80 amino acids) with the DNA binding domain of Gal4 (N-terminus 1–147 aa). This construct has a strong transactivation activity with the pG5.E1B-CAT reporter. While coexpression of E1A only inhibits the transactivation activity of Gal4.VP16 less than 50%, it inhibits the transactivation activity of Gal4.p53 more significantly (Fig. 3). The effects of different E1A mutants in the Gal4.p53/pG5.E1B-CAT system generally were consistent with those in the p53/p13.CAT system. Because E1A does not affect the DNA binding ability of Gal4, it is suggested that E1A inhibits p53 transactivation by interfering with the p53 transactivation domain.

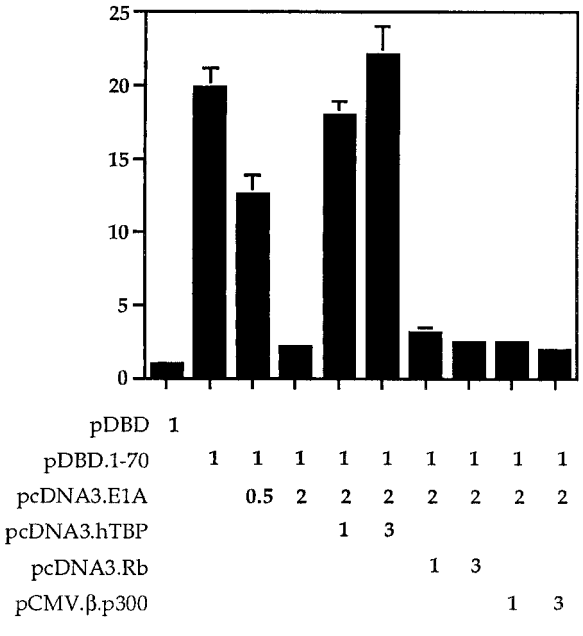

Fig. 1. p300 and pRb have no effect on DBD-1-70 transactivation. Indicated amounts (in micrograms) of plasmids were cotransfected into BALB/c3T3 cells, and CAT activity resulting from the pG5.E1B-CAT reporter gene was assayed by triplicate LSC.

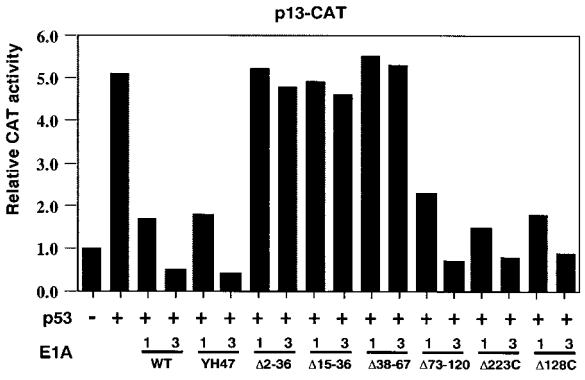

Fig. 2. E1A-caused inhibition of p53 transactivation activity requires the ENT and CR1. pcDNA3.p53 (2 μg) was cotransfected into U-2OS cells together with 2 μg of the p13-CAT report gene in the presence or absence of wild-type and various E1A mutants. The nature and amounts (in micrograms) of the transfect plasmis are indicated in the figure.

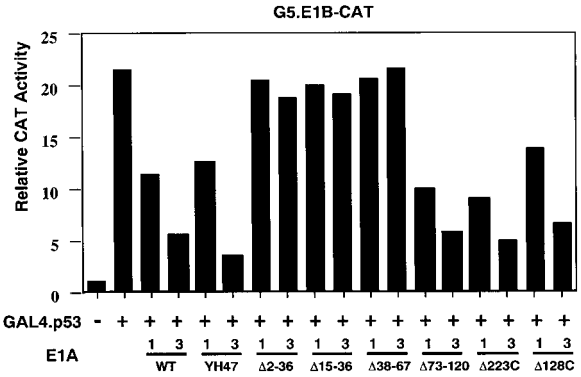

Fig. 3. E1A exerts inhibitory effects via the transactivation domain of p53. The N-terminal transactivation domain of p53 was fused with the DNA binding domain of Gal4 to generate pDBD.p53TA. pG5.E1B-CAT (2 μg), which contains upstream Gal4 binding sites at the promoter region, was cotransfected with 2 μg of pDBD.p53TA to U-2OS cells. 243R E1A and various mutants were coexpressed to test their effects on the CAT activity.

Role of pRb in p53 Transactivation and E1A Repression

The ENT and the CR1 of E1A have been shown to be involved in binding to p300/CBP, pRb, and hTBP. However, both Δ128C, which is defective in binding to the pocket protein family, and YH47, which is defective in binding to pRb, retained an inhibitory effect on p53 transactivation in U-2OS, suggesting that the pocket proteins may be eliminated from being involved in this function. To determine the role of pRb in E1A-mediated inhibition of p53 and to exclude the influence of endogenous p53, we used the Saos-2 cell line, which also is a human osteosarcoma cell line but lacks functional p53, for additional studies. Saos-2 cells express only a truncated form of pRb which is neither functional nor nuclear localized. When exogenous p53 was expressed, a seventeenfold transactivation of the reporter was observed (Fig. 4A). The higher fold transactivation in the Saos-2 cell line compared with the U-2OS cell line might be due to the lack of functional p53 in the Saos-2 cells; thus, a lower basal level of transactivation occurs in Saos-2 cells. In the absence of functional pRb, both wild-type E1A and the E1A mutants that do not interact with pRb inhibited p53 transactivation in a similar manner (Fig. 4A, lanes 3–8). Expression of exogenous, wild-type pRb had no effect on p53 transactivation (Fig. 4A, lanes 9,10). Cotransfection of wild-type pRb with E1A showed that pRb neither enhanced nor released the E1A-mediated repression of p53 transactivation (Fig. 4A, lanes 11–14). The expression of exogenous wild-type pRb was confirmed by Western blot analysis with an anti-pRb antibody XZ77, which does not recognize the endogenous, truncated pRb in Saos-2 cells (Fig. 4B). The E1A binding property of the expressed exogenous pRb was confirmed by coimmunoprecipitation and subsequent Western blot assays (Fig. 4C). We concluded that pRb is not participating in either p53 transactivation or E1A-mediated repression of p53 transactivation.

Role of p300 and hTBP in E1A-Mediated Repression of p53 Transactivation

To examine the possible involvement of p300 and TBP in the E1A-mediated inhibition of p53 transactivation, plasmids encoding p300 or hTBP were cotransfected with E1A. Data presented in Figure 5A show that coexpression of p300 or hTBP released the E1A-mediated inhibition. Interestingly, coexpression of TFIIB, which does not interact with E1A, also released the inhibition. In the absence of p53, overexpression of p300, hTBP, and TFIIB had no or very little effect on p13-CAT activity (Fig. 5B), suggesting that p300, hTBP, and TFIIB are involved in p53-dependent transactivation and that their functions are disrupted by E1A. Like pRb, pocket proteins p107 and pRb2/p130 had no effect on E1A-mediated inhibition of p53 transactivation.

To test if the inhibition rendered by E1A resulted from the disruption of the interactions between p300 and hTBP, E1A-transfected Saos-2 cells were lysed, and protein interactions were examined by immunoprecipitation and Western blot. Figure 5C,D shows that p300 and hTBP coimmunoprecipitated with each other, suggesting that p300 can interact with hTBP in the presence of E1A. Therefore, E1A perturbs the function of p300 and hTBP but does not disrupt the complex formation.

DISCUSSION

The importance of the ENT, CR1, and CR2 regions of E1A in immortalization and transformation has been well documented previously. While the essential role of CR1 and CR2 is to inactivate the pocket proteins, the involvement of the ENT is not clear. The observation that both the ENT and CR1 of E1A are involved in the inhibition of p53 transactivation activity delineates a novel function to the N-terminus

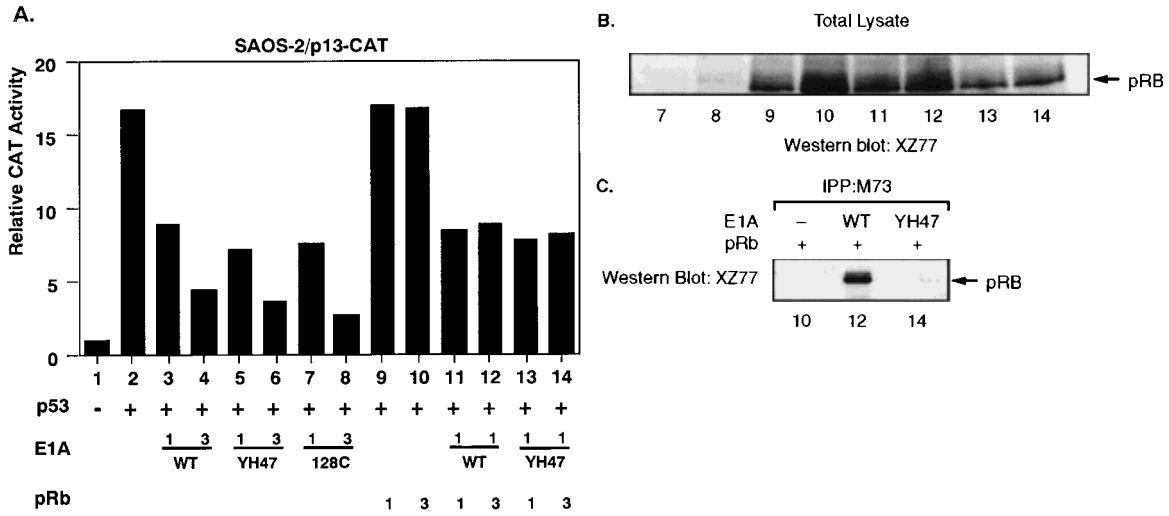

Fig. 4. Inhibition of p53 activity does not require E1A's ability to interact with pRb family proteins. **A:** p53 (1 μg) (+) and 1 μg of p13-CAT (every transfection) were transfected into Saos-2 cells which have an origin similar to U-2OS cells but lack endogenous p53 and pRb. The effects of pRb, E1A, and E1A mutants on p53 activity were tested. The amounts (in micrograms) of E1A and pRb plasmids used for each transfection are indicated in the figure. **B:** Expression of pRb was detected by Western blot analysis. Additional transfected cells identical to samples 7–14 were lysed. Total lysate (20 μg) was loaded onto 6% SDS-PAGE followed by transferring onto PVDF membrane and detection by the anti-pRb antibody XZ77, which does not recognize the truncated, endogenous pRb in Saos-2. **C:** In vivo interaction between E1A and pRb. The portion of cell lysate used in B was immunoprecipitated with the monoclonal anti-E1A antibody M73. Western blot was performed with antibody XZ77 as in B.

that has an obvious implication in cell growth regulation. While suppressing the function of the pocket proteins by direct protein-protein interactions, E1A does not bind p53 directly. The repressive effect of E1A is mediated by hTBP and p300, both of which can physically interact with E1A. These findings also suggest that p300 may function as a cofactor for p53 transactivation, probably by forming a complex with hTBP and TFIIB and by facilitating the communication between p53 and the basic transcription machinery. In support of this model, an earlier report showed that p21/WAF1/cip1 is induced through a p300-dependent mechanism during nerve growth factor (NGF)–mediated neuronal differentiation [Billon et al., 1996]. Because p300 and hTBP are able to form a complex in the presence of E1A, the possible mechanisms by which E1A inhibits p53 transactivation include impairing the p300-p53 interaction and disturbing TATA-hTBP binding.

It is reported that overexpression of pRb is able to prevent both p53-dependent and p53-independent apoptosis. Our data show that the pocket proteins have no effect on p53 transactivation, suggesting that modulation of p53 transactivation is unlikely to be a mechanism by which pRb protects cells from apoptosis. p53 plays a role in both G1 arrest and apoptosis. In response to DNA damage, p53 induces G1 arrest to provide time for cells to repair damaged DNA. If the DNA damage is too severe to be successfully repaired, p53 will cause apoptosis. However, how p53 chooses between G1 arrest and apoptosis remains unclear. Data presented here and from other laboratories suggest that, on one hand, E1A inhibits p53 transactivation, abolishes p53-dependent G1 arrest and DNA repair, and promotes the G1/S transition to facilitate viral replication. On the other hand, p53 triggers the apoptosis pathway to dispose cells with DNA damage and deregulated cell cycle progression. Therefore, E1A switches p53 function from inducing G1 arrest and DNA repair to triggering apoptosis. The protective effect of pRb may extend from the restoration of G1 arrest. Implicated in differentiation, G1 arrest, and apoptosis, p21/WAF1/cip1, one of the transcriptional target genes of p53, may play a key role in the interplay among p53, E1A, and pocket proteins.

Analysis of the previous reports and the data presented in this report reveals that E1A-mediated repression can be classified into two classes: one is the universal repression, which does not require consensus DNA sequences,

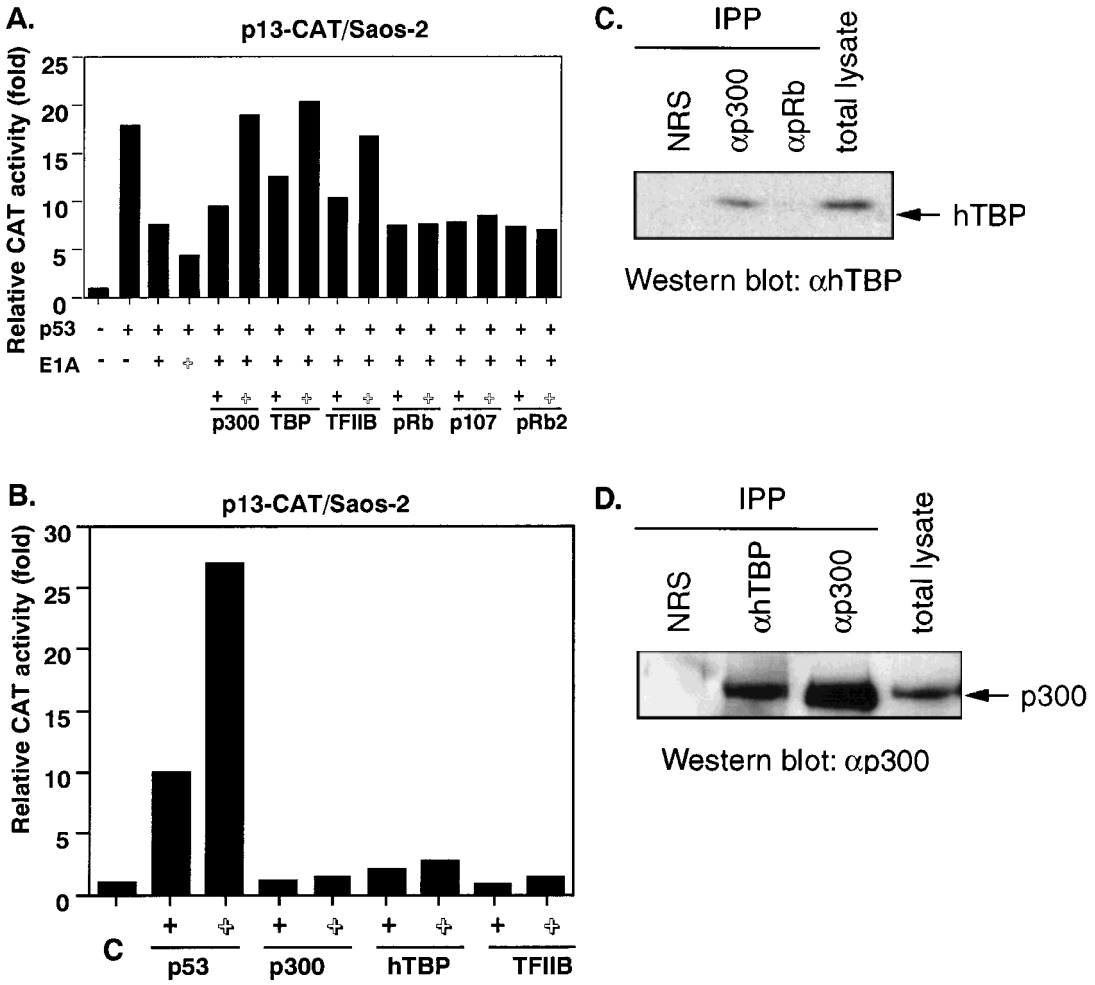

Fig. 5. E1A functionally targets the p300/TBP/TFIIB complex to repress p53 transactivation. **A:** Overexpression of p300, TBP, and TFIIB but not pRb, p130/Rb2, or p107 released the E1A-mediated repression in Saos-2 cells. p53 (1 μg) and 1 μg of p13-CAT were transfected into Saos-2 cells. In some dishes wild-type E1A plasmids (+, 1 μg, *open plus sign,* 3 μg) were transfected as an inhibitor. One microgram (+) or 3 μg (open plus sign) of p300, hTBP, TFIIB, pRb, p107, and pRb2 was transfected into cells in different dishes. **B:** p300, hTBP, and TFIIB do not transactivate p13-CAT directly. One-half microgram (indicated by +) and 2 μg (open plus sign) of p53, p300, hTBP, and TFIIB was cotransfected with 1 μg of p13-CAT into Saos-2 cells in 60 mm dishes in the absence of E1A. C, control; 2 μg of pcDNA3 vector was cotransfected with 1 μg p13-CAT. **C,D:** E1A-transfected Saos-2 cells were lysed and subjected to immunoprecipitation with normal rabbit serum, anti-p300, anti-pRb, and anti-hTBP. Total lysate and the immunoprecipitates were resolved through 6% SDS-PAGE and blotted with polyclonal anti-TBP (C) and antibody polyclonal anti-p300 (D).

and the other is promoter-specific repression, which requires one of the E1A-responsive elements [Caruso et al., 1993; Datta and Bagchi, 1994]. In the promiscuous repression, it was proposed that the factor(s) required for transactivation is (are) bound by E1A without specific promoter targeting, thus leading to a squelching effect. The inhibition of DBD.VP16 and DBD.E1A transactivation requires the same region of E1A as that required for transactivation. As mentioned above, the overexpression of hTBP could release the E1A-mediated repression, whereas overexpression of TFIIB failed to release the repression. These data suggest that, when provided with a promoter targeting device, the N-terminus of E1A may effectively target and recruit hTBP to TATA or TATA-like elements and transactivate. If E1A lacks a promoter targeting device, E1A may titrate out the hTBP and prevent hTBP from binding to TATA or TATA-like elements as proposed in the squelching model. In agreement with this model, the overexpression of E1A resulted in repression of both DBD.VP16 and DBD.E1A activity. Previous reports that TATA elements are required for 243R E1A-mediated repression

[Tang et al., 1995; Tsang et al., 1996] also support the theory that a common mechanism underlies both transactivation and promiscuous repression mediated by 243R E1A.

Like the promoter-specific transactivation, the promoter-specific repression by 243R E1A requires E1A-responsive elements that do not bind E1A directly. Several such responsive elements have been identified [Datta and Bagchi, 1994; Offringa et al., 1990], and some of them are elements for specific transcriptional factors which use p300/CBP as coactivators [Offringa et al., 1990; Yuan et al., 1996; Puri et al., 1997; Eckner et al., 1996; Lee et al., 1996; Bannister and Kouzarides, 1995]. Recently, a p300/CBP-associated factor (P/CAF) which competes with the adenoviral oncoprotein E1A was identified and characterized as a histone acetyl transferase [Yang et al., 1996]. It is reported that both p300 and CBP also have histone acetyl transferase activities [Ogryzko et al., 1996]. Disturbing these histone acetyl transferase activities is a possible molecular mechanism for p300/E1A-mediated repression.

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