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Direct interaction between adenovirus E1A protein and the TATA box binding transcription factor IID

(transcription/regulation/adaptors/protein–protein interactions)

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ABSTRACT **Adenovirus E1A has long been known to activate/repress cellular and viral transcription. The transcriptional activity of nuclear extracts was depleted after chromatography on immobilized E1A protein columns that specifically retained the transcription factor (TF) IID. Stronger direct interactions between E1A and human TFIID than between E1A and yeast TFIID suggest that the unique sequences of the human protein may be involved. We have demonstrated that this interaction occurs directly between bacterially produced E1A and bacterially produced human TFIID in a protein blot assay. We propose that E1A protein may transduce regulatory signals from upstream activators to basal elements of the transcriptional machinery by contacting TFIID.**

Most genes transcribed by RNA polymerase II contain an A+T-rich sequence, the TATA box, located 25–30 nucleotides upstream of the transcription start site. This sequence is an important element that determines the efficiency of promoter utilization and participates in locating the start site of transcription. A general transcription factor, transcription factor IID (TFIID), that binds to the TATA box is required for transcription of all RNA polymerase II genes tested (see refs. 1 and 2 for reviews) and commits a template to transcription (3, 4). Cooperative interactions of TFIID with factors bound at upstream sequences [like major late promoter transcription factor, adenovirus transcription factor (ATF), pseudorabies immediate early, and GAL4] stimulate transcription, presumably by increasing the stability of the committed preinitiation complex (5–8). TFIID bound to the promoter sequences precludes the transcriptional inhibition caused by chromatin assembly on the DNA template (9).

The gene encoding TFIID has been recently cloned from several sources (10–17). The human TFIID protein contains a highly conserved carboxyl terminus core region essential for DNA binding and basal transcription (14) and other regions required for the response to stimulation of transcription by upstream factors (18). Human TFIID produced in bacteria is unable to replace TFIID purified from HeLa cells in a system responding to stimulation by upstream factors, suggesting the need of additional (coactivator or adaptor) proteins that connect the basal transcriptional machinery and upstream activators (see ref. 19 for review). Previous difficulties in obtaining homogeneous preparations of TFIID might be due to copurification with these adaptors (18) or other bound proteins.

The adenovirus E1A gene encodes two polypeptides, 289 and 243 amino acids long, that are expressed early after infection. These differ by a Zn^{2+} finger domain important for transactivation and present only in the larger protein (see refs. 20 and 21 for reviews). E1A regions 1 and 2, common to both proteins, are essential for transformation and they participate in interactions with the retinoblastoma (Rb) 105-kDa protein, a 300-kDa protein, cyclin A protein, and other as yet unidentified polypeptides (22–25). Since no single target DNA sequence for E1A action has been identified, it has been suggested that the E1A protein influences the activities of cellular transcription factors that interact with E1A responsive promoters.

Some promoters responding to E1A transactivation contain either ATF, activator protein 1 (AP1), or early 2 factor (E2F) sequences upstream of their basal promoters (20, 26). Mutations in these elements affect both uninduced and E1A-induced activity. Previous studies (ref. 27 and refs. therein) suggest that a member of the ATF family mediates E1A response by bringing E1A in close proximity to the transcriptional machinery, where it acts as a bridge between the regulatory and as yet unidentified basal elements to influence the rate of initiation of RNA polymerase II transcription.

Other promoters require a particular TATA box not only for basal transcription but also for E1A responsiveness (see refs. 28 and 29 and refs. therein). Elevated levels of TFIID activity in adenovirus-infected cells suggest that E1A-dependent activation of some genes occurs by increases in activities and/or amounts of the TFIID factor (30).

As a first step to elucidate the E1A regulatory mechanism, we show here direct protein–protein interactions between E1A and one of the basal elements of the transcriptional machinery, TFIID.

MATERIALS AND METHODS

E1A Affinity Column Chromatography. E1A protein was purified to apparent homogeneity from *Escherichia coli* AR120 transformed by pAS1-E1A 410 as described (31). E1A and lactoglobulin proteins (1 mg/ml) linked to Sepharose were used for affinity chromatography as described (32). Briefly, HeLa cell nuclear extract (33) in buffer D [20 mM Hepes, pH 7.9/0.1 M KCl/0.2 mM EDTA/0.2 mM EGTA/2 mM dithiothreitol/20% (vol/vol) glycerol] was loaded at 4°C (2.4 mg of protein per ml of column) on E1A or lactoglobulin Sepharose and allowed to stand at 4°C for 1 hr. Columns were washed and eluted with buffer D supplemented with 0.5 mg of bovine serum albumin per ml, 10 μM $ZnCl_2$, and 0.2 or 1.0 M KCl. Fractions were concentrated by Centricon 10 (Amicon) and dialyzed against buffer D.

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Abbreviations: TFIID, transcription factor IID; Rb, retinoblastoma; ATF, adenovirus transcription factor.

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***In Vitro* Transcription.** Transcriptional activity on the adenovirus major late promoter template was assayed by primer extension as described (34).

Purification of Transcription Factors. HeLa cell nuclear extracts were fractionated on phosphocellulose as described (35). TFIIA was purified from HeLa cell nuclear extracts by chromatography on phosphocellulose (0.1 M KCl eluate), DEAE-Sephacel, Q Sepharose, heparin/agarose, single-strand DNA agarose, S-200, and 5PW-Sepharose. TFIIB, TFIIE, and TFIIF were purified from HeLa nuclear extracts as described (34, 36). Yeast and human TFIID were purified as described (34). RNA polymerase II was solubilized from HeLa cell nuclear pellets and purified to apparent homogeneity by chromatography on DEAE-cellulose, phosphocellulose, heparin/agarose, and TSK-phenyl Superose (H. Lu, O. Flores, R.W., and D.R., unpublished data). *In vitro* transcription reaction mixtures contained 3 μg of TFIIA, 2.5 μg of TFIIB, 3 μg of TFIIE and TFIIF, 1.3 μg of TFIID, and 3 μg of RNA polymerase II in a 40-μl vol.

Immunological Detection of TFIID. HeLa cell nuclear extract proteins were separated on 10% SDS/polyacrylamide gel, transferred to poly(vinylidene difluoride) membranes (Millipore), and reacted with rabbit antiserum (a generous gift of A. J. Berk, University of California) directed against human TFIID produced in bacteria (16) or preimmune serum of the same rabbit. Reactive proteins were detected after incubation with biotinylated goat anti-rabbit antibodies (Cappel Laboratories) followed by incubation with avidin and biotinylated alkaline phosphatase complex as suggested by the supplier (Boehringer Mannheim).

Protein–Protein Blot Assay. Plasmid pEThIID (16) contains an insert of human TFIID cDNA in the pET-3b vector (37). Rb protein cDNA (24) was cloned into the pET-3 vector to create pET-Rb. The induction of *E. coli* strain BL21(DE3) carrying pEThIID, pET-Rb, or pET-3 was performed as described (37). Bacterial cell lysates were separated on SDS/9% polyacrylamide gel and transferred onto nitrocellulose filters. After a denaturation–renaturation cycle in 6 and 3 M guanidinium hydrochloride, the filters were blocked with binding buffer (20 mM Hepes, pH 7.5/5 mM $MgCl_2$/1 mM KCl/10 μM $ZnCl_2$/5 mM dithiothreitol) containing 5% dry milk for 1 hr at 4°C (38) and incubated at 37°C overnight with E1A protein [labeled with ^{32}P using calf thymus casein kinase II purified to apparent homogeneity (39)] in binding buffer with 1% milk. After washing with buffer (20 mM Hepes, pH 7.9/100 mM KCl/0.1 mM EDTA/10 μM $ZnCl_2$/1 mM dithiothreitol/10% glycerol), the filter was exposed to x-ray film.

RESULTS

Depletion of Transcriptional Activity After E1A Affinity Chromatography. Adenovirus E1A protein (289 amino acids) expressed in *E. coli* and purified to homogeneity was covalently linked to Sepharose and used for chromatography of transcriptionally active nuclear extracts. The HeLa cell nuclear extract was depleted of transcriptional activity when chromatographed on an E1A column, but not on a lactoglobulin column [a protein of the same isoelectric point and similar molecular weight (in dimer) as E1A], as analyzed by using the adenovirus major late promoter template, which responds to E1A *in vitro* (refs. 40 and 41; Figs. 1 and 2). The lack of transcriptional activity in the E1A chromatographed extracts was not due to inactivation of the proteins, since addition of the fraction retained (and eluted with 1 M KCl) by the E1A column (but not by the lactoglobulin column) to the depleted extract resulted in transcription (Figs. 1 and 2 *B* and *C*). The transcriptional activity was dependent on both the depleted extract and the eluate from the E1A column, as 1 M KCl eluates were inactive by themselves (Fig. 1). Moreover,

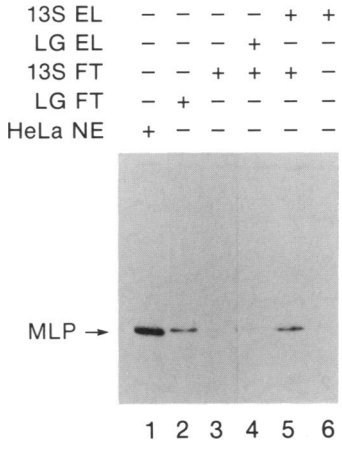

FIG. 1. Depletion and reconstitution of transcriptional activity after E1A affinity chromatography. *In vitro* transcription reactions were monitored by primer-extension assays. Each reaction mixture contains HeLa cell nuclear extract (NE) (lane 1), lactoglobulin (LG) column flow-through (FT; lane 2), E1A column flow-through (lane 3), E1A column flow-through supplemented with lactoglobulin column 1.0 M KCl eluate (lane 4), E1A column flow-through supplemented with E1A column 1.0 M KCl eluate (EL) (lane 5), and E1A column 1.0 M KCl eluate alone (lane 6). Equivalent amounts of fractions were used in these reactions, except for lane 1 where 4 times higher amounts of unfractionated nuclear extract gave higher transcription levels. The 215-nucleotide primer-extension product corresponding to the adenovirus major late promoter (MLP) transcript is indicated.

<1% of the total protein and no detectable RNA polymerase II activity was found in the 1 M KCl eluate fraction (data not shown). We conclude that protein(s) required for transcriptional activity from the adenovirus major late promoter (Fig. 1), as well as from the human ε-globin, adenovirus E3 and E4 promoters (results not shown), were retained by the E1A but not by the lactoglobulin affinity columns.

TFIID Restored Transcription of E1A-Binding Protein(s)-Depleted Extracts. A thermolabile protein in phosphocellulose fraction D restored activity to the E1A-depleted extracts (Fig. 2*A*). Other phosphocellulose fractions were unable to restore the transcriptional activity to the E1A-depleted extracts (data not shown). These results suggest that a factor(s) present in the phosphocellulose fraction D was sufficient to reconstitute transcription and might be the same as the protein(s) retained on the E1A column.

The TFIID activity, which binds to the TATA box, was purified from phosphocellulose fraction D. To distinguish whether TFIID or another transcription factor(s) was depleted by the E1A column, we tested purified preparations of TFIIA, TFIID, TFIIB, TFIIE, TFIIF, and RNA polymerase II (Fig. 2*B*) for their ability to complement the transcriptional activity of the E1A-depleted extracts. Each one of these fractions was purified through at least four chromatographic steps, including gel filtration in high salt concentration. The system responded linearly to exogenous factors. Transcription was efficiently reconstituted by purified TFIID, reconstituted only slightly by TFIIE and TFIIF, and not at all by TFIIA, TFIIB, or RNA polymerase II. The low levels of transcription detected in the presence of all the factors together (Fig. 2*B*, lane 9) were probably due to the absence of the upstream major late promoter transcription factor in the purified reconstituted system. The results presented here strongly suggest that TFIID may be similar to the activities removed from the nuclear extract by E1A affinity chromatography. The low activity in the TFIIE and TFIIF fractions could be due to some residual TFIID in these fractions or to partial depletion of TFIIE and TFIIF by E1A chromatography.

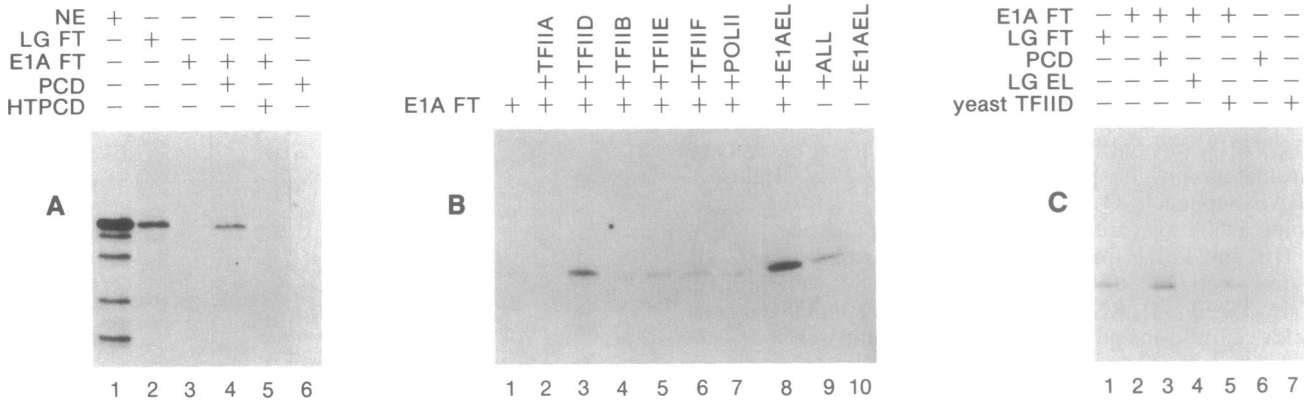

FIG. 2. TFIID activities were able to reconstitute transcription in the flow-through fraction after E1A affinity column chromatography. (*A*) Transcriptional activity of extracts after E1A affinity chromatography was reconstituted with phosphocellulose fraction D of HeLa cell nuclear extract (8 μl; lane 4) but not with heat-inactivated (47°C; 15 min) phosphocellulose fraction D (8 μl; lane 5). (*B*) Highly purified TFIID was able to reconstitute transcriptional activity of extracts passed through E1A affinity column. Supplementation activities of highly purified basal transcriptional factors (lanes 2–7) and 1.0 M KCl eluate from E1A column (lane 8) were assayed as described. Reconstituted activity using only highly purified factors is shown in lane 9. (*C*) Yeast TFIID produced in *E. coli* is able to reconstitute transcriptional activity of extracts after E1A affinity chromatography. Flow-through fractions were supplemented with phosphocellulose fraction D (lane 3), 1.0 M KCl eluate from lactoglobulin column (lane 4), or purified yeast TFIID produced in bacteria, and transcriptional activities were assayed. All reaction mixtures contain supplements of buffer D to adjust for sample volume differences. PCD, phosphocellulose fraction D; POLII, RNA polymerase II; all other abbreviations are the same as in Fig. 1.

Purified Recombinant Yeast TFIID Is Sufficient to Reconstitute Transcription from E1A-Depleted Extracts. Although transcription factor TFIID has not yet been purified to homogeneity from mammalian cells, both the human and yeast TFIID cDNAs have now been isolated and characterized (10–17). Since yeast TFIID can replace the human TFIID in basal transcription, we analyzed whether yeast TFIID produced in bacteria and purified to apparent homogeneity (14, 34) could complement the E1A-depleted extracts. The results presented in Fig. 2*C* demonstrate that recombinant yeast TFIID was able to reconstitute transcription of the E1A-depleted extract, albeit less efficiently than the crude phosphocellulose fraction D. The yeast TFIID preparation may be less efficient (*i*) because it was produced in bacteria and it neither contains "adaptors" nor is it modified or (*ii*) because it lacks the unique amino-terminal domain present in the human protein and/or displays other sequence differences. In summary, both highly purified human and recombinant yeast TFIID are able to restore the transcriptional activity of nuclear extracts depleted by E1A affinity chromatography.

TFIID Protein Bound to the E1A Column. To analyze whether and how efficiently TFIID protein was retained by the E1A column, we used anti-TFIID antibodies. This antiserum is able to detect purified human TFIID from HeLa cells, human TFIID produced in bacteria, crude or purified, as well as *in vitro* translated human TFIID. A specific polypeptide band detected by anti-TFIID but not by preimmune serum represents human TFIID in the nuclear extracts, as shown by comparison to purified TFIID and by comigration with *in vitro* translated human TFIID (Fig. 3*A*; results not shown). When extracts chromatographed on the E1A column were analyzed (Fig. 3*B*), the 37-kDa polypeptide corresponding to TFIID was clearly present in the eluates of the E1A column. The anti-TFIID reactive protein was absent from the corresponding lactoglobulin fractions (Fig. 3*B*).

Comparison of the bands suggested that approximately 20% and 1–4% of the total TFIID polypeptide was eluted from the E1A column with 0.2 M KCl and 1.0 M KCl, respectively. Since partially denatured proteins might bind inefficiently, we assayed the transcriptional activity of protein(s) bound to the E1A column by using an extract heat depleted of TFIID (42). The addition of the fractions chromatographed on the E1A column to the heat-inactivated nuclear extract resulted in recovery of transcriptional activity (Fig. 3*C*). When the amount of bound TFIID polypeptide (Fig. 3*B*) was compared to the TFIID activity (Fig. 3*C*), it became apparent that the three fractions had different TFIID specific activities. The 20–24% of the TFIID polypeptide retained by the E1A column represented most of the TFIID active in transcription. Furthermore, the TFIID protein in 1.0 M KCl eluates contained <1% of the total protein but represented a significant part of the TFIID activity, suggesting some heterogeneity in the population of TFIID molecules. Since only a single gene encoding TFIID has been detected, the heterogeneity could be due to different modified forms of TFIID, or to different cellular proteins associating with TFIID, or to different amounts of transcription inhibitors in these fractions.

The same fractions were also tested for TFIIA activity (a polypeptide that copurifies through some steps with TFIID) by transcription reconstitution with purified TFIIB, TFIID, TFIIE, and TFIIF factors and RNA polymerase II. No TFIIA activity was detectable in the 0.2 or 1.0 M KCl eluates, providing further evidence that the binding of TFIID to E1A columns was specific (results not shown).

TFIID Interacted with E1A. Cloned human and yeast TFIID were translated *in vitro* [a convenient source of transcriptionally active radiolabeled protein (15, 16)] and chromatographed on E1A or lactoglobulin columns. Most of the yeast TFIID bound to the E1A column was eluted at 0.2 M KCl (Fig. 4), suggesting that interactions were weak. The E1A column efficiently retained human TFIID, most of which was eluted with 1.0 M KCl, indicating much stronger interactions (Fig. 4). Both yeast and human TFIID were found in the 0.1 M KCl flow-through of lactoglobulin columns.

These results suggest that TFIID interacted with E1A in the absence of nuclear extract proteins and possibly directly. The nonconserved amino-terminal domain unique to the human TFIID, or other nonconserved sequences, play an important role in determining the strength of interaction with E1A. Both HeLa cell and *in vitro* translated human TFIID bound to the E1A column with low efficiency, probably due to incorrect folding or partial denaturation of this thermolabile protein. However, the more efficient binding of *in vitro* translated human TFIID, compared to the small percentage of TFIID retained from HeLa nuclear extracts, led us to

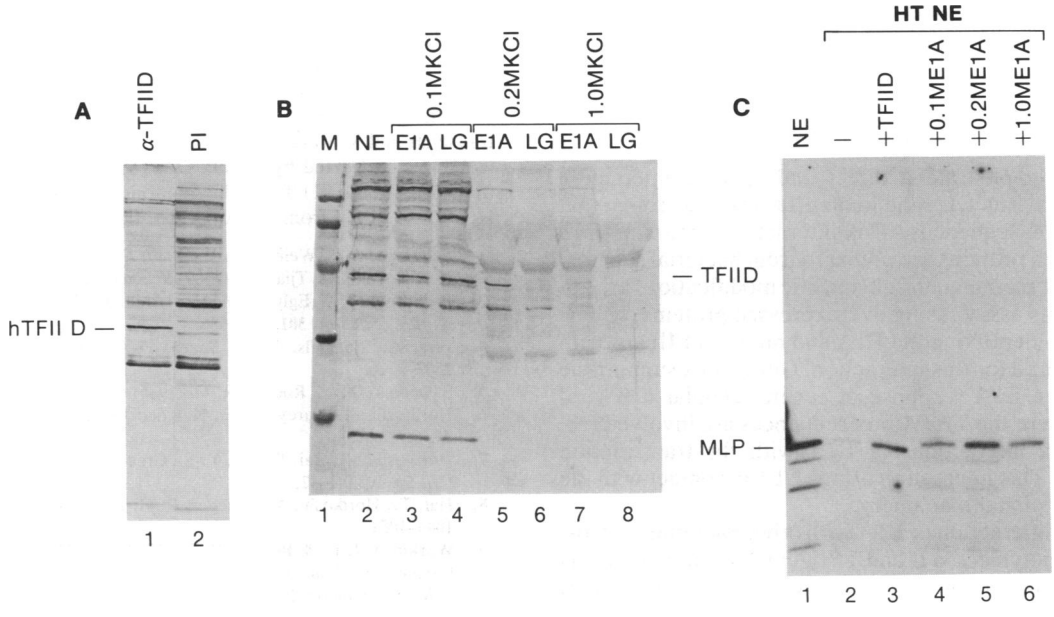

FIG. 3. Efficiency of TFIID interaction with adenovirus E1A protein. (*A*) Human (h) TFIID protein present in HeLa cell nuclear extract was detected with anti-human TFIID antiserum (lane 1) or preimmune (PI) serum (lane 2). (*B*) Human TFIID protein was detected in both flow-through (0.1 M KCl) and eluted (0.2 M; 1.0 M KCl) fractions from the E1A affinity column. Flow-through and eluted fractions (5 times the equivalent amounts) from E1A (lanes 3, 5, and 7) or lactoglobulin (lanes 4, 6, and 8) affinity columns, or nuclear extract before fractionation (lane 2) were analyzed with anti-human TFIID antiserum. Lane 1 contains protein molecular standards of 90, 67, 43, 31, and 21 kDa. (*C*) Measurement of TFIID activity of nuclear extracts fractionated on E1A affinity column. Heat-inactivated (47°C; 15 min) HeLa cell nuclear extract (HT NE) was supplemented with buffer D (lane 2), highly purified human TFIID (lane 3), 0.1 M KCl E1A flow-through fraction (lane 4), 0.2 M KCl eluate (lane 5), or 1.0 M KCl eluate (lane 6). Transcriptional activity of equivalent aliquots of untreated nuclear extract is shown in lane 1. Dilution experiments show that transcriptional activity is proportional to the amount of TFIID-containing fraction added. MLP, major late promoter; all other abbreviations are the same as in Fig. 1.

speculate that in the latter, accessory proteins bound to TFIID might either modulate its affinity to E1A or occupy the sites of E1A interaction.

TFIID Interacted Directly with E1A. To address whether other proteins in the reticulocyte lysate may mediate the TFIID–E1A interaction, we assayed binding between bacterially produced E1A and bacterially produced TFIID in a protein blot assay (Fig. 5). As a control, we included bacterial extracts containing a protein known to interact with E1A (24), a truncated version of Rb (Fig. 5). The E1A protein bound to both the 60-kDa Rb truncated protein and the 37-kDa TFIID (lanes 5 and 4, respectively). The interaction between E1A and TFIID was highly specific since only TFIID, among all other bacterial proteins, was able to react with E1A. These results indicate that the interaction between the two proteins is direct. The phosphorylation of E1A by casein kinase II is not required for this interaction, since similar results were obtained with biotinylated E1A detected by an alkaline phosphatase assay (results not shown). Moreover, these data indicate that mammalian cell-specific post-

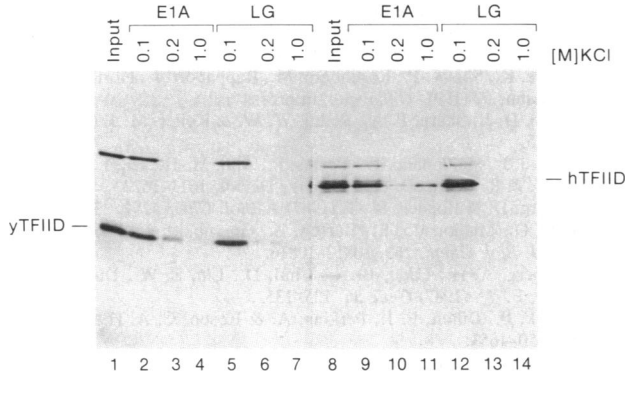

FIG. 4. Interactions between *in vitro* translated human (h) or yeast (y) TFIID and E1A affinity columns. [^{35}S]Methionine *in vitro* labeled yeast (lanes 1–7) and human (lanes 8–14) TFIID were applied on E1A (lanes 2–4 and 9–11) or lactoglobulin (LG) (lanes 5–7 and 12–14) affinity columns and eluted with the indicated concentrations of KCl. Equivalent aliquots of the input amounts of yeast (lane 1) and human (lane 8) TFIID are shown. The TFIID in each fraction was detected by autoradiography after SDS/PAGE.

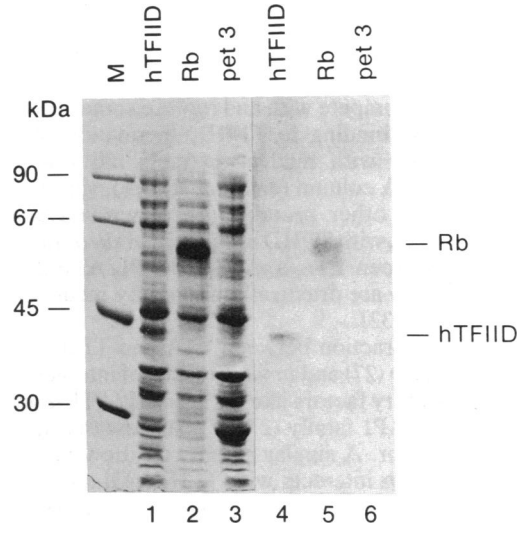

FIG. 5. Direct interaction between purified E1A produced in bacteria and human (h) TFIID overexpressed in bacteria. Whole cell lysates from bacteria were analyzed by SDS/PAGE followed by either Coomassie blue staining (lanes 1–3) or transferred to nitrocellulose filters and incubated with ^{32}P-labeled E1A (lanes 4–6). Lanes: 1 and 4, bacteria overexpressing human TFIID; 2 and 5, overexpressing truncated Rb protein; 3 and 6, expression vector alone, without insert. Protein standards (lane M) are indicated on the left.

translational modifications of TFIID and E1A are not essential for this interaction, although they may affect its strength or stability.

DISCUSSION

Our results demonstrate a direct and specific interaction between adenovirus E1A and human TFIID, the protein that binds to TATA sequences. This interaction occurs *in vitro* even when both proteins are prepared from bacterial sources, suggesting that mammalian cell-specific modifications are not necessary. This assay, using overexpressed proteins, will be very useful in identifying the E1A and human TFIID protein domains required for this interaction. Our results support the notion that the E1A response of several cellular and viral promoters where the TATA box sequences are involved (28, 29) occurs via interactions of E1A with the transcription factor TFIID. This interaction brings E1A in contact with the basal transcriptional machinery.

The lack of interaction of E1A with other bacterial proteins or with RNA polymerase II and TFIIA in nuclear extracts, as well as the low amount of total protein (<1% of the input) compared to the high proportion of TFIID activity bound to the E1A column, suggests that this TFIID–E1A interaction has a high degree of specificity.

In vitro translated human and yeast TFIID were bound to the E1A column with different affinities. The region most conserved between human and yeast TFIID is the 180-amino acid carboxyl-terminal core involved mostly in DNA binding and required for basal transcription (18, 19). The selective strong interaction between E1A and human TFIID may require the unique amino-terminal portion of the protein. The amino terminus is also indispensable for transcription activation by upstream factors, possibly through adaptor intermediates (18, 19). Since a single gene appears to exist for human TFIID (15), previously postulated functional TFIID heterogeneity (29) might be due to the existence of multiple protein factors binding to TFIID. This TFIID/adaptor heterogeneity suggests how promoters containing specific subsets of TATA box sequences may respond to E1A *in vivo* or *in vitro* (28, 29). The difference in E1A binding efficiency of TFIID from nuclear extracts and *in vitro* translated TFIID suggests that the TFIID in the cell could be complexed with these proteins (both positive and/or negative regulatory adaptors) and thus be unavailable for E1A interactions. E1A might be able to compete with and replace some but not other adaptor proteins binding to TFIID. Incubation of *in vitro* translated TFIID with nuclear extracts did not alter its binding to the E1A column (data not shown), suggesting that free adaptors or other proteins are either unavailable or unable to complex with TFIID efficiently *in vitro*. In contrast, interactions between E1A and CREB-BP1/ATF-2 or c-fos translated *in vitro* are drastically altered by incubation with nuclear extracts (32).

This direct interaction between E1A and TFIID, coupled with recent *in vivo* (27) and *in vitro* results of interactions with upstream regulatory factors like CREB-BP1/ATF-2 and other members of the AP1 family (27, 32), suggest that E1A functions as an adaptor. A similar result with the viral transactivator VP16, which interacts with TFIID (43) and with octanucleotide binding protein (44), suggests that this strategy for redirecting the host transcriptional machinery after viral infection might be widespread. Whether this E1A/TFIID interaction results in transactivation or repression and whether it affects all E1A-regulated promoters or only those that respond through the TATA box remains to be determined.

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