Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 6583–6588, June 1996
Biochemistry

Separation of the transcriptional coactivator and antirepression functions of transcription factor IIA

DONGMIN MA, IVAN OLAVE, ALEJANDRO MERINO, AND DANNY REINBERG

Howard Hughes Medical Institute, Division of Nucleic Acid Enzymology, Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 663 Hoes Lane, Piscataway, NJ 08854-5635

Communicated by Aaron J. Shatkin, Rutgers, The State University of New Jersey, Piscataway, NJ, March 15, 1996 (received for review December 1, 1995)

ABSTRACT Human transcription factor IIA (TFIIA) is composed of three subunits (α, β, and γ). TFIIA interacts with the TATA-box binding protein and can overcome repression of transcription. TFIIA was found to be necessary for VP16-mediated transcriptional activation through a coactivator function. We have separated the coactivator and antirepression activities of TFIIA. A TFIIA lacking the α subunit was isolated from HeLa cells. This "mini-TFIIA" interacts with the TATA-box binding protein and can overcome repression of transcription, but it is defective in transcriptional coactivator function.

Initiation of transcription at promoters transcribed by RNA polymerase II (RNAPII) is complex. Different families of factors operate on these promoters (for a review, see ref. 1). One family of factors recognizes common promoter elements such as the TATA or the initiator motifs. These proteins are known as the basal, or general, transcription factors (GTFs) and include transcription factor IID (TFIID), TFIIB, TFIIE, TFIIF, and TFIIH (2). TFIID is the only GTF known to exhibit sequence-specific DNA binding activity, with specificity for the TATA element (3, 4). *In vivo*, TFIID exists as a large, multisubunit complex (5, 6), yet its DNA binding activity is intrinsic to only one polypeptide of 38 kDa, namely the TATA-box binding protein, TBP (7–9). The other subunits of TFIID are known as the TBP-associated factors, TAFs (10–12).

A second class of factors that operate at promoters transcribed by RNAPII is composed of regulatory factors (13, 14). One class of regulatory factors recognizes specific sequences located upstream or downstream of the TATA motifs (13–16). These factors can activate or repress transcription of specific genes upon binding to their sites. The molecular mechanisms by which regulatory factors regulate the activity of specific genes are poorly understood. Ultimately, regulatory factors encroach upon the basal transcription machinery to elicit their effect. In fact, some regulators have been shown to directly interact with components of the basal transcription machinery (1).

Another growing family of transcription factors is known as coactivators (2, 17). Coactivators do not directly bind to promoter sequences (18), and they appear to function as "molecular bridges" between the basal transcription machinery and the promoter-bound activator. The prototype coactivators are the collection of TAFs in the TFIID complex (17). However, not all coactivators are subunits of the TFIID complex. Some coactivators are general, whereas others are activator- and tissue-specific. One such example is the B-cell-specific coactivator Oca-B (19–22). Another collection of coactivators acts more generally, and they are required for optimal activation of transcription regardless of the activator type (23, 24). These general coactivators (cofactors) include some of the TAFs (25), PC4 (26–28), Dr2/topoisomerase I (Topo I)/PC3 (24, 29), ACF (24), cofactor A (30), HMG2 (31), and the mediator (32). A growing body of evidence indicates that TFIIA also functions as a cofactor during transcriptional activation (33–38).

Different functions have been ascribed to TFIIA. TFIIA (*i*) interacts with TBP (39, 40) and (*ii*) facilitates the binding of TFIID (or TBP) to the promoter (41, 42). (*iii*) Since the binding of TFIIA to TBP results in the displacement of negative components from the TFIID complex (39, 43, 44), TFIIA acts as an antirepressor. (*iv*) TFIIA acts as a cofactor for activated transcription, a hypothesis strengthened by the finding that TFIIA interacts with certain activators (41, 42) as well as with $TAF_{II}110$ (45). Moreover, in the presence of TFIID and an activator, TFIIA is required to overcome a rate-limiting step during open complex formation (46).

The isolation of cDNA clones encoding the different subunits of yeast and human TFIIA has permitted a careful, detailed analysis of the factor, which has revealed some intriguing features. The yeast TFIIA is composed of two subunits (47) encoded by the *TOA1* and *TOA2* genes (48). In higher eukaryotes, TFIIA is composed of three subunits, α, β, and γ (39, 40). The α and β subunits are encoded by a single gene and are probably produced by protein processing (45, 49, 50). Studies in yeast have demonstrated that TFIIA is essential for viability (48). The essential function of TFIIA is related in part to its interaction with TBP (51, 52). Moreover, the TBP–TFIIA interaction is important for activation of transcription *in vivo* (52). However, whether the different functions ascribed to TFIIA in activation of transcription are a consequence of TFIIA promoting binding of TFIID to the TATA motif or of TFIIA allowing the formation of transcription-competent complexes by removing repressor molecules from TFIID (antirepression) is unknown. Using a highly purified reconstituted transcription system, devoid of repressor molecules, we find that human TFIIA is essential for VP16-mediated activation of transcription. We also observed that the association of TFIIA with the TBP-TATA complex, while sufficient for antirepression, is not sufficient for activation of transcription.

MATERIALS AND METHODS

Purification of Recombinant TFIIA (rTFIIA) ($\alpha + \beta + \gamma$) and Mini-TFIIA (mTFIIA) ($\beta + \gamma$). Plasmid DNAs encoding the γ and the synthetic α and β subunits of TFIIA were as described (33). Recombinant proteins were expressed in bacteria, and the polypeptides were purified on nitrilo-triacetic

Abbreviations: RNAPII, RNA polymerase II; GTF, general transcription factor; TFIIA, transcription factor IIA; TBP, TATA-box binding protein; TAF, TBP-associated factor; Topo I, topoisomerase I; rTFIIA, recombinant TFIIA; mTFIIA, mini-TFIIA; eTFIID, epitope-tagged holo-TFIID; GST, glutathione *S*-transferase; TA complex, TFIIA-TBP-TATA motif complex; mTA complex, mini-TA complex.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

acid agarose under denaturing conditions as described (33). Recombinant polypeptides were mixed in an equimolar ratio and renatured. To avoid precipitation, the protein mixture was diluted with buffer E (8 M guanidine-hydrochloride/0.1 M NaH_2PO_4/0.01 M Tris, adjusted to pH 4.5 with HCl) to a concentration of 0.5 mg/ml and then renatured by extensive dialysis against three changes of 4 liters of buffer C-100 (BC100) (25 mM Tris·HCl, pH 7.9/10% glycerol/0.1 M KCl/0.5 mM EDTA/10 mM 2-mercaptoethanol/0.2 mM phenylmethylsulfonyl fluoride) at 4°C for 20 hr.

Purification of Native TFIIA and mTFIIA. TFIIA was purified from HeLa cell nuclear extracts as described (42) with modifications. Western blotting detecting the α and γ subunits and a gel mobility shift assay were employed during purification of TFIIA. Fractions of the gel filtration step were analyzed in transcription activation as well as in antirepression. Proteins present in single-stranded DNA agarose pool (40 mg/50 ml) were precipitated by the slow addition of solid ammonium sulfate (0.42 mg/ml). Precipitated proteins were collected by centrifugation (35,000 $\times g$ for 30 min) and resuspended in 2 ml of buffer C containing 0.5 M KCl and 0.01% Nonidet-P40. Proteins were loaded onto an FPLC Superdex-200 (120-ml bed volume) gel filtration column. Fractions (1 ml of each fraction) were pooled (see Fig. 1*A*) and dialyzed against BC100. mTFIIA (1.6 mg/9 ml) was further concentrated by chromatography on a DEAE–Sephacel column (200 μl of resin). mTFIIA activity was bound to the column and eluted with buffer C containing 0.35 M KCl. Quantitative Western blotting using antibodies against the γ subunit was performed to determine the concentrations of TFIIA and mTFIIA.

***In Vitro* Transcription Assays.** Transcription assays were performed as previously indicated using the two DNAs described in the text (49). Transcription factors used in the assays were rTFIIA $\alpha + \beta + \gamma$ (0.47 mg/ml), native TFIIA (0.55 mg/ml total protein; TFIIA concentration = 4.46 nM), recombinant mTFIIA $\beta + \gamma$, (0.4 mg/ml), mTFIIA (0.19 mg/ml; mTFIIA concentration = 1.12 nM), rTFIIB (10 ng), epitope-tagged holo-TFIID (eTFIID) (4 ng of TBP determined using quantitative Western blots), rTFIIE (15 ng), rTFIIF (23 ng), HeLa cell-purified TFIIH [1 μl, 26 ng/μl, Mono-S (53)], and anti-RNAPII-carboxyl-terminal domain affinity purified RNA polymerase II [50 ng, 50 ng/μl (30)]. Assays measuring activation of transcription also contained $Gal4_{(1-94)}$-VP16 (1.14 mg/ml) and recombinant PC4 (0.29 μg). Topo I (Dr2) was purified as described (24). Reactions were incubated at 30°C for 1 hr (except for the antirepression assay; see Fig. 2 legend). The RNA products were separated by electrophoresis on denaturing gels. Quantitation was performed using a PhosphorImager (Bio-Rad).

Release of Topo I from the TFIID Complex. The 12CA5 antibody affinity column (2 mg/ml) was prepared as described (33) and equilibrated with BC100. Nuclear extracts prepared from LTR3α cells expressing eTFIID (10) were fractionated by chromatography on phosphocellulose and DEAE-52 columns. The bound material, which was enriched in both eTFIID and Topo I activities, was dialyzed against BC100. The material (1.6 ml) was incubated with 400 μl of 12CA5 resin at 4°C for 8 hr. The column was washed extensively with BC100 until no Topo I activity could be detected. The sample was then divided into four identical columns. Equimolar amounts (22.4 pmol) of glutathione *S*-transferase (GST) or Gal4-VP16, normal or mTFIIA (native and recombinant forms), or 12CA5 (hemaglutinin) peptide were incubated with each column (100 μl) for 10 min at 4°C. Fractions were collected and assayed for Topo I activity using a DNA relaxation assay as described (24).

RESULTS AND DISCUSSION

Isolation of a Form of TFIIA Lacking the α Subunit. TFIIA interacts with TBP and stabilizes the formation of the TBP-TATA complex (41, 42). The resulting DNA protein complex (TFIIA-TBP-TATA motif, TA complex) is stable and can be isolated by electrophoresis on native gels (41, 42). Functionally, TFIIA activity can be measured in a transcription activation assay, as well as by its ability to displace repressors from TBP (or TFIID) to allow transcription. TFIIA activity was purified from HeLa cells as previously indicated using the three assays described above (namely TA complex formation, transcription activation, and antirepression).

Fractionation of TFIIA on a gel filtration column, the sixth chromatographic step of purification, resulted in the separation of TFIIA into two forms. One form was capable of producing a TA complex that comigrated with a complex formed using highly purified transcriptionally active TFIIA (Fig. 1*A*, + lane). The second form yielded a faster-migrating TA complex [mini-TA (mTA); Fig. 1*A*]. Human TFIIA is composed of three subunits, α, β, and γ. Western blot analysis using antibodies directed against the α and γ subunits indicated that fractions yielding TA and mTA complexes contained the γ subunit. However, only fractions forming the slower-migrating TA complex were reactive to the α subunit (Fig. 1*A*).

To further analyze the two different DNA-protein complexes observed, fractions were pooled as indicated in Fig. 1*A*. The addition of increasing amounts of each protein pool to DNA binding reactions resulted in the formation of a complex specific to each fraction; TFIIA produced the TA complex and mTFIIA produced the mTA complex. Formation of each complex depended on TBP, as its omission from the DNA binding assay resulted in no complex (Fig. 1*B*). Antibodies against the different subunits of TFIIA were used to study the composition of TFIIA and mTFIIA (Fig. 1 *C* and *D*). Western blot analysis demonstrates that the TFIIA protein pool contained the three subunits of TFIIA, whereas mTFIIA contained the β and γ polypeptides but was devoid of the α subunit (Fig. 1*C*; see also *A*). Using the gel mobility shift assay, we found that antibodies against the α subunit supershifted the TA complex (Fig. 1*D*, lane 3) but were without effect on the mTA complex (lane 9). Antibodies against the γ subunit supershifted both the TA (lane 4) and the mTA (lane 10) complexes. Antibodies against the β subunit supershifted the TA complex (lane 12) yet inhibited formation of the mTA complex (lane 16). The effect of the antibodies on the TA and mTA complexes was specific and due to the presence of different TFIIA subunits within the complexes. The addition of the TFIIA antibodies to DNA protein complexes formed in the absence of TFIIA, such as the TBP-dependent complexes containing Dr1 (TDr1) (54, 55), was without an effect (Fig. 1*D*). The most likely explanation for these results is that mTFIIA lacks the α subunit. This finding is not inconsistent with studies that demonstrated that the β and γ subunits independently interact with TBP and that the α subunit interacts with the γ subunit (33–35). Moreover, we reported that recombinant β and γ subunits interacted with TBP to produce a DNA protein complex that migrated faster than the complex reconstituted with the three recombinant subunits on native polyacrylamide gels (33). Studies with the yeast-derived factor have shown that the C terminus of *TOA1*, which is homologous to the human β subunit, contains residues required for DNA contact within the TA complex (56). Therefore, the β and γ subunits appear important in establishing a TA complex, and the α subunit appears to be brought into the complex by its interaction with the γ subunit of TFIIA. The simple experiment of adding recombinant α subunit to mTFIIA was without effect (data not shown). This is not surprising since we previously observed that the addition of α to the preformed β-γ subcomplex resulted in no appreciable effect on complex migration (33). Whether a mTFIIA exists *in vivo* is not known; however, the biochemical isolation of this form of TFIIA provided us with an opportunity to assess whether

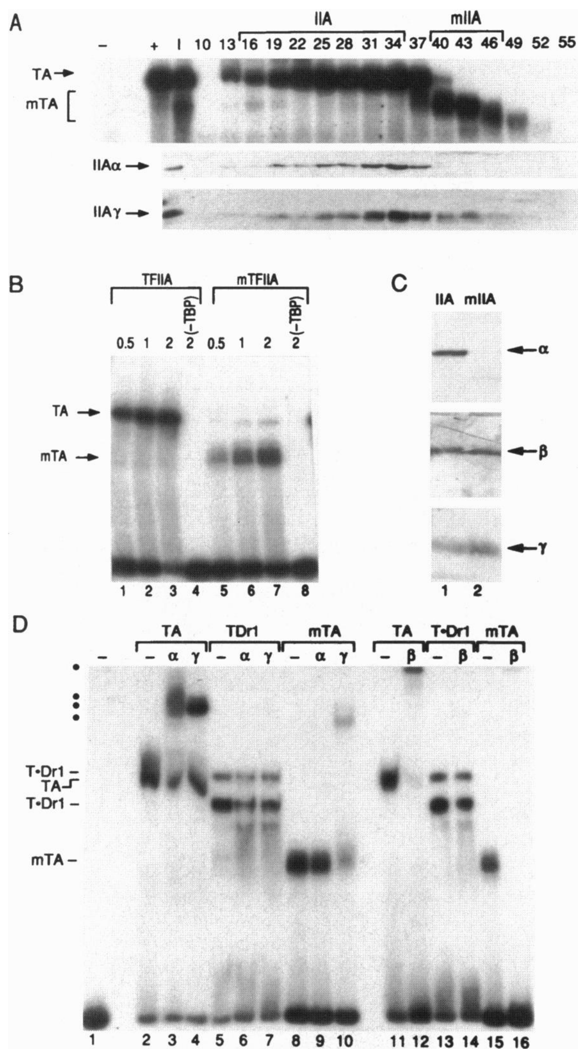

FIG. 1. mTFIIA contains the β and γ subunits and lacks the α subunit. (*A*) Aliquots (2 μl) of the different fractions of a Superdex-200 column were analyzed for their ability to form TBP-dependent complexes using the gel mobility shift assay as described (42). The migration of the normal TA and mTA complexes are indicated on the left of the panel. Aliquots (20 μl) were also analyzed by Western blotting using antibodies against α (*Middle*) or γ (*Bottom*) subunits. The arrow on the left denotes the different polypeptides. (*B*) Aliquots of the TFIIA (0.55 mg/ml) and mTFIIA (1.7 mg/ml) protein pools were analyzed for their ability to produce TBP-dependent DNA-protein complexes using the gel mobility shift assay. Additions (in μl) are indicated on the top of the panel. The migrations of the different complexes are indicated on the left. (*C*) Purified TFIIA (27 μg) and mTFIIA (40 μg) were analyzed for the presence of the different TFIIA subunits using Western blotting as indicated. (*D*) The effect of antibodies against the different subunits of TFIIA on the TA and mTA complexes was analyzed using the gel mobility shift assay. DNA protein complexes were formed as described in *B* and incubated with different antibodies as indicated on the panel. In addition, complexes formed by the association of Dr1 with TBP were used as control. In this case, the association of Dr1 with TBP produced at least two different DNA-protein complexes (54). In lanes 1–10, the DNA-protein complexes were formed first and then reacted with protein A-purified antibodies. Complexes reacted with antibodies against the β subunit were as follows. Because the antibodies are of low titer and do not have any effect on preformed complexes, TFIIA (or mTFIIA) was first incubated with these antibodies and then added to DNA binding assays. Complexes were formed as described (42) on a DNA fragment containing the adenovirus major late promoter TATA motif.

the interaction of TFIIA with TBP is sufficient for activation of transcription as well as antirepression.

mTFIIA Functions in Antirepression of Transcription. Previous studies have shown that TFIIA interacts with both TBP and TFIID (39, 45, 49, 50). TFIIA is without effect on basal transcription directed by TBP but is stimulatory towards reactions directed by TFIID (33, 34, 39). Studies have suggested that one of the functions of TFIIA is to counteract proteins that copurify with TFIID and that negatively affect transcription (39, 44). Since these findings were reported, factors that interact with TFIID and repress transcription have been isolated (23, 24, 43, 44, 54). The activity of some of these factors is counteracted by TFIIA (24, 43, 49). Moreover, their addition to transcription reactions reconstituted with TBP imposes a requirement for TFIIA in basal transcription (24). The yeast MOT1 is one such repressor that is neutralized by TFIIA (43). Another factor is Dr2/PC3, which was isolated independently by two groups seeking activities that enhanced the response of the basal machinery to activators (24, 29). Despite its ability to enhance activation, Dr2/PC3 represses basal transcription and was identified as DNA Topo I (24, 29). These observations were in agreement with genetic studies in the yeast *Saccharomyces cerevisiae*, indicating that Topo I-mediated repression is a primary means through which transcription is halted upon entry into the stationary phase (57). Interestingly, the effect of Topo I in transcription was independent of its DNA relaxation activity. Dr2-mediated repression was found to be specific and not due to the nonspecific DNA binding activity intrinsic to Topo I. Specifically, the repressing effect was observed only on TATA-containing promoters (24). Furthermore, Topo I was found to interact with TBP and was also present in the TFIID complex (30). Topo I-mediated repression of transcription was found to be counteracted by activators and/or TFIIA (24, 49).

To analyze whether the interaction of TFIIA with TBP is sufficient for antirepression, we examined whether mTFIIA could overcome Topo I-mediated repression of transcription. Reactions were reconstituted on the adenovirus major late promoter using TBP and purified GTFs and RNAPII (Fig. 2). In agreement with our previous observations (24), the addition of Topo I to the reconstituted transcription assay resulted in repression of basal transcription (lane 2). Repression could be overcome by the addition of recombinant (lane 5) or native TFIIA (lanes 6 and 7). TFIIA lacking the α subunit (mTFIIA), purified from HeLa cells (lanes 8 and 9), or a recombinant form reconstituted with the β and γ subunits produced in bacteria (lanes 10 and 11), was also active in overcoming Topo I-mediated repression. This effect was specific to TFIIA and mTFIIA, as the addition of excess TFIIE (lane 3) or TFIIF (lane 4) failed to overcome repression.

To further analyze whether TFIIA or mTFIIA overcome repression by physically removing Topo I from the TFIID complex, as previously suggested (24), the following experiment was performed (Fig. 3*A*). TFIID containing epitope-tagged TBP (eTFIID) (11) was partially purified and immobilized on an immunoaffinity resin containing antibodies against the epitope tag. This material was distributed equally into three identical columns, designated A, B, and C. The input TFIID contained high levels of Topo I as measured by DNA relaxation activity (Fig. 3*B*, lanes 1 and 2). Each column was washed until no DNA relaxation activity was detected in the flow through fraction (lanes 3–5). Columns A, B, and C were then treated with TFIIA, GST protein alone, or GST-VP16, respectively, to determine whether either reagent could remove Topo I from the column. Importantly, TFIIA, GST, and GST-VP16 did not contain Topo I activity by themselves (lanes 12–14). Substantial DNA relaxation activity was released from the TFIIA-treated column, indicating displacement of Topo I from the TFIID complex (lane 6). While GST alone did not release Topo I (lane 7), GST-VP16 released barely detectable amounts (lane 8). Subsequent treatment of each column with 0.6 M KCl (or peptide eluting eTFIID, Fig. 3*C*, lane 6) yielded

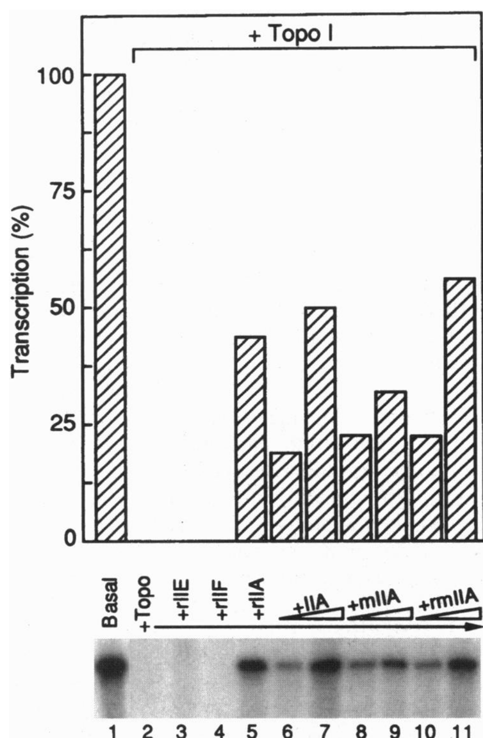

FIG. 2. TFIIA and mTFIIA overcome Topo I-mediated repression. Transcription assays were reconstituted with recombinant human TBP (2.5 ng) and the other GTFs (lane 1). Addition of Topo I (10 ng) resulted in the inhibition of transcription (lane 2). Recombinant TFIIA (0.17 μM, lane 5), native TFIIA (2.23 nM, lane 6; 4.46 nM, lane 7) or native mTFIIA (2.24 nM, lane 8; 4.48 nM, lane 9), or recombinant mTFIIA (0.12 μM, lane 10, 0.25 μM, lane 11) were added to reactions mixtures, as indicated. In this experiment, TBP was preincubated for 30 min at 30°C with DNA with and without TFIIA and mTFIIA, as indicated on the panel, before the addition of Topo I. Excess TFIIE (8.3 nM, lane 3) and TFIIF (11 nM, lane 4) was added in place of TFIIA. (*Upper*) The relative transcription (%) was quantitated using a PhosphorImager (Bio-Rad). (*Lower*) The transcription assay from where the quantitation was derived.

considerable Topo I activity (lanes 9–11), suggesting that the efficiency of removal of Topo I was low. The relaxation activity measured was due to Topo I activity as it was sensitive to the Topo I-specific inhibitor, camptothecin (lane 15).

Having established that TFIIA removes Topo I from the TFIID column, we then analyzed whether mTFIIA could perform the same function. The results presented in Fig. 3*C* demonstrate that mTFIIA (native and recombinant) can remove Topo I from the TFIID complex. The observed result was specific to mTFIIA (or TFIIA) because GST (lane 1) or lysozyme (data not shown) did not release Topo I. This result is in agreement with the functional experiments presented above, demonstrating that mTFIIA could function in antirepression. Together, these results demonstrate that the association of TFIIA (or mTFIIA) with the TBP subunit of TFIID results in displacement of Topo I from TFIID. Thus, we concluded that the α subunit of TFIIA is not required for antirepression.

TFIIA, but Not mTFIIA, Facilitates Gal4-VP16-Mediated Activation of Transcription. To analyze the role of TFIIA and mTFIIA in transcription activation, a transcription assay responsive to Gal4-VP16 was developed. Transcription reactions contained two different DNA templates driven by the adenovirus major late promoter; one with five Gal4 DNA binding sites upstream of the TATA box, directing transcription of a 392-nt G-less cassette (24). The reconstituted transcription activation assay contained the GTFs, RNAPII, Gal4-VP16,

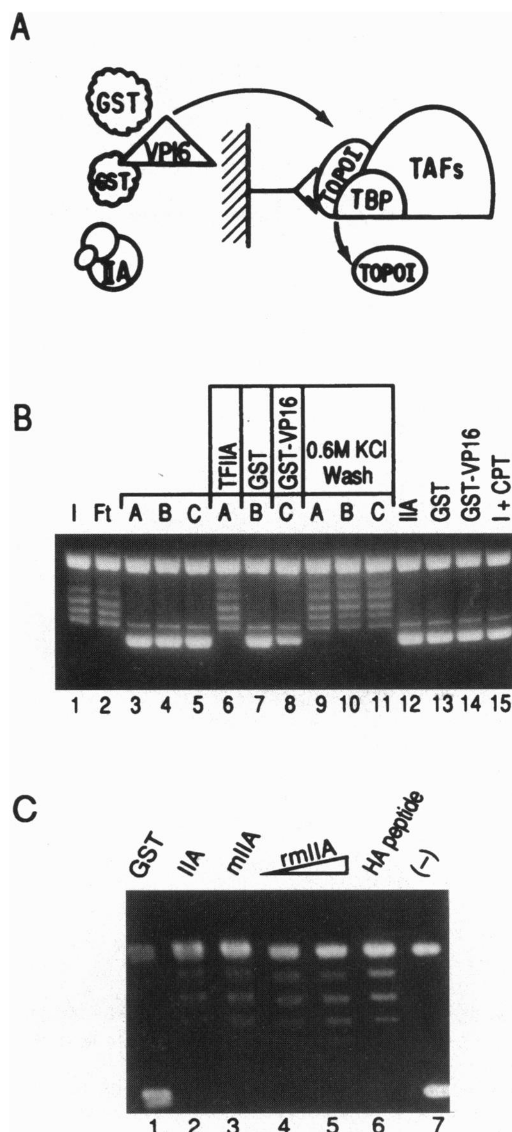

FIG. 3. TFIIA and mTFIIA release Topo I from the TFIID complex. (*A*) Schematic representation modeling the release of Topo I from the TFIID complex by TFIIA. (*B*) TFIIA can release Topo I from the eTFIID complex. eTFIID containing Topo I was loaded onto a 12CA5 affinity column and the fractions were analyzed for Topo I activity using a DNA relaxation assay as described in the text. Three identical columns (A, B, and C) were extensively washed until no Topo I activity was detected (lanes 3–5). Protein fractions (0.02 nmol) free of Topo I (lanes 12–14) containing TFIIA, GST-VP16, and GST were incubated with each column, respectively, at 4°C for 10 min. The different elutes were analyzed for Topo I activity (lanes 6–8). The remaining Topo I associated with the eTFIID was eluted from the column using a 0.6 M KCl wash (lanes 9–11). (*C*) mTFIIA can release Topo I from the eTFIID complex. eTFIID (2 ml of DEAE-cellulose bound material) was loaded onto 500 μl of 12CA5 affinity column. The column was divided into five identical columns and washed extensively with BC100 until no Topo I activity could be detected. Equal molar amounts (0.02 nmol) of GST, TFIIA, mTFIIA, and recombinant mTFIIA were incubated with each column at 4°C for 10 min. The eluates (3 μl of 200 μl, but lane 5 contains 6 μl) were analyzed in a DNA relaxation assay for Topo I activity. Hemaglutinin peptide (3 mg/ml) was used to eluate eTFIID from the column, and the eluate (3 μl of 200 μl) was assayed for Topo I activity (lane 6).

and general cofactors (see *Materials and Methods*). Highly purified eTFIID was used in this analysis. The eTFIID was isolated using affinity chromatography as described (11); however, before elution from the affinity column, the complex was washed extensively with high salt to ensure that repressors

and other molecules that interact with the TFIID complex were washed away. Using highly purified factors, apparently free of repressors, TFIID-dependent basal transcription could be observed in the absence of TFIIA (Fig. 4*A*, lane 1). The addition of TFIIA resulted in a 2- to 3-fold stimulation of transcription (Fig. 4*A*, see transcripts originated from the template lacking the Gal4 DNA binding sites, indicated with −). A different situation was observed when transcription from the DNA responsive to Gal4-VP16 was analyzed. TFIIA dramatically stimulated the response to Gal4-VP16 (Fig. 4*A*). This result suggests that activator-dependent transcription requires TFIIA. To further analyze the involvement of TFIIA in Gal4-VP16-mediated activation of transcription, different amounts of the activator were added to reactions reconstituted in the presence and absence of TFIIA. Consistent with the results presented above (Fig. 4*A*), activation of transcription was observed only in reactions containing TFIIA (Figs. 4*B* and 5 *A* and *B*). The effect observed was specific as it required the presence of Gal4 DNA binding sites (Fig. 4*B*, compare + and −). In addition, the TFIIA-dependent activation of transcription required TFIID, cofactors, and an activation domain (Fig. 5*A*). The observed effect was also specific to TFIIA as a highly purified recombinant factor isolated from bacteria was used (see *Materials and Methods* and ref. 33).

Having established that TFIIA is required for Gal4-VP16-mediated activation of transcription, we analyzed whether mTFIIA could substitute. Despite the fact that mTFIIA could interact with TBP and TFIID, and was active in antirepression of transcription, we found this form of the factor to be deficient in directing activation of transcription (Fig. 5*B*). Moreover, our previous studies have shown that TFIIA can stimulate basal TFIID-dependent transcription approximately 2- to 4-fold (ref. 33; Figs. 4*A* and 5*B*). mTFIIA was also defective in this function (Fig. 5*B*). Therefore, these studies demonstrate that TFIIA is required for both activation of transcription, and enhancement of basal TFIID-dependent transcription. Importantly, the α subunit of TFIIA is necessary for these two functions.

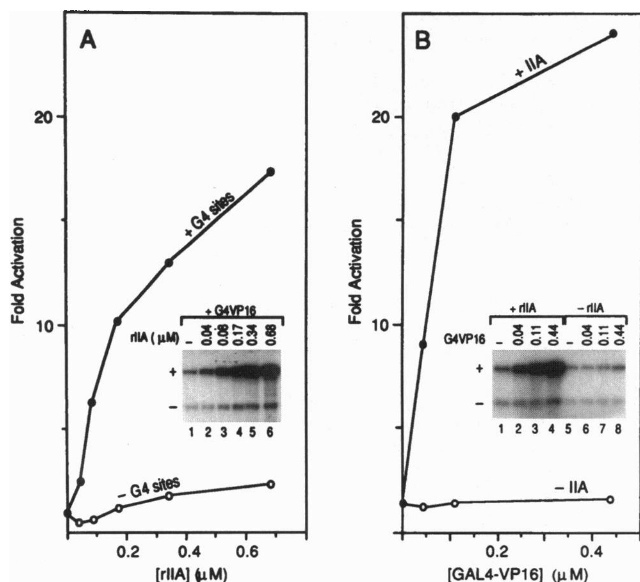

FIG. 4. TFIIA is required for VP16-mediated activation of transcription. Transcription assays were performed using eTFIID. (*A*) Effect of rTFIIA on Gal4-VP16-mediated activation of transcription. The concentration of rTFIIA in each reaction is indicated on the panel. Gal4-VP16 (0.44 μM) was used in this assay. (*B*) Effect of Gal4-VP16 on activation of transcription in the presence (lanes 1–4) or absence (lanes 5–8) of rTFIIA. The concentration of Gal4-VP16 in each reaction is shown on the panel. rTFIIA (0.17 μM) was used in this assay. The transcripts were quantitated on a PhosphorImager (Bio-Rad) and plotted as shown. The *y* axis indicates the fold of activation. Fold activation was estimated as follows: experimental lane (cpm in +) − (cpm in −)/(cpm in +) − (cpm in −) from control lane 1.

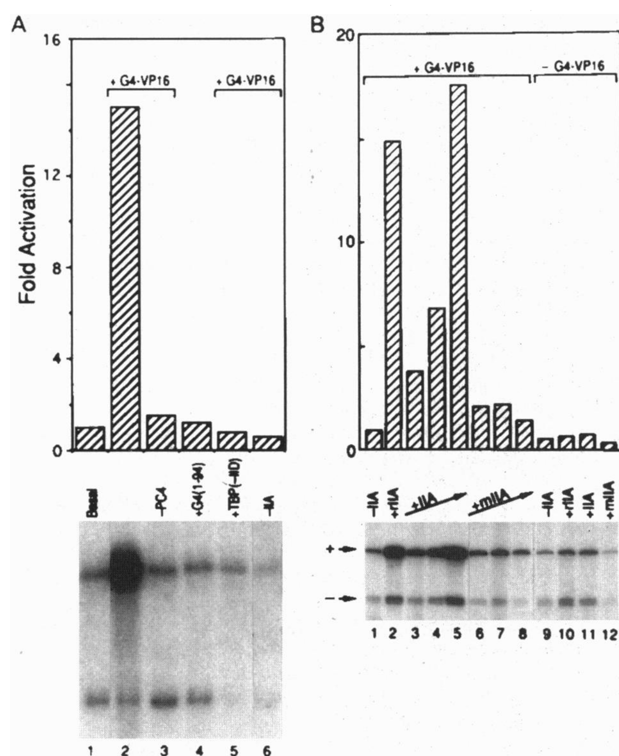

FIG. 5. mTFIIA does not support Gal4-VP16-mediated activation of transcription. Transcription reactions were reconstituted with eTFIID, PC4, GTFs, and G4-VP16 as described in Fig. 4. (*A*) The requirement for different components on activation of transcription was analyzed, as indicated. Omission are indicated on the panel. (*B*) Recombinant TFIIA (0.17 μM, lanes 2 and 10), native TFIIA (1.12 nM, lane 3; 2.24 nM, lane 4; 4.48 nM, lanes 5 and 11), or mTFIIA (1.12 nM, lane 6; 2.24 nM, lane 7; and 4.48 nM, lanes 8 and 12) were added as indicated on the panel. Activator Gal4-VP16 was added as indicated. The transcripts were quantitated using a PhosphorImager, and the activation fold is shown in the upper panels. The lower panel shows the transcription assay from where the quantitation was derived. Fold activation was estimated as described in Fig. 4.

The studies described above demonstrate that TFIIA is required for transcription activation; in its absence, Gal4-VP16 is unable to mediate activation *in vitro*. The involvement of TFIIA in activation is not due to the removal of repressors, since the reconstituted transcription system was devoid of such molecules. However, we cannot rule out the unlikely possibility that one of the subunits of TFIID (TAFs) functions as an activation-specific repressor of transcription that is overcome by TFIIA. Previous studies did not rule out the possibility that the observed effect of TFIIA in activation was due to TFIIA-mediated antirepression. In previous studies, nuclear extracts depleted of TFIIA (35, 50), or a partially purified transcription system in which a HeLa cell-derived fraction containing all GTFs except TFIID and TFIIA (34), were used. Therefore, it is likely that those systems contained repressors. In the present studies, we unequivocally demonstrated that TFIIA is necessary for Gal4-VP16-mediated activation of transcription as a highly reconstituted system was used. Moreover, we found that for activation of transcription, the α subunit of TFIIA is required, whereas for antirepression, this subunit is dispensable.

We thank Drs. George Orphanides, Thierry Lagrange, and Ronny Drapkin for stimulating discussions and reading the manuscript. D.R. is supported by grants from the National Institutes of Health

(GM37120, GM48518, and GM47484) and by the Howard Hughes Medical Institute.

1. Zawel, L. & Reinberg, D. (1995) *Annu. Rev. Biochem.* **64,** 533–561.
2. Zawel, L. & Reinberg, D. (1993) *Progress in Nucleic Acid and Molecular Biology*, eds. Cohn, W. E. & Moldave, K. (Academic, San Diego) **44,** 67–108.
3. Nakajima, N., Horikoshi, M. & Roeder, R. G. (1988) *Mol. Cell. Biol.* **8,** 4028–4033.
4. Parker, C. S. & Topol, J. (1984) *Cell* **36,** 357–369.
5. Pugh, B. F. & Tjian, R. (1991) *Genes Dev.* **5,** 1935–1945.
6. Gill, G. & Tjian, R. (1992) *Curr. Opin. Genet. Dev.* **2,** 236–242.
7. Peterson, M. G., Tanase, N., Pugh, B. F. & Tjian, R. (1990) *Science* **248,** 1625–1630.
8. Kao, C., Lieberman, P., Schmidt, M., Zhou, Q., Pei, R. & Berk, A. (1990) *Science* **248,** 1646–1650.
9. Hoffman, A., Sinn, E., Yamamoto, T., Wang, J., Roy, A., Horikoshi, M. & Roeder, R. G. (1990) *Nature (London)* **346,** 387–390.
10. Dynlacht, B. D., Hoey, T. & Tjian, R. (1991) *Cell* **66,** 563–576.
11. Zhou, Q., Lieberman, P. M., Boeyer, T. G. & Berk, A. J. (1992) *Genes Dev.* **6,** 1964–1974.
12. Tanese, N., Pugh, B. F. & Tjian, R. (1991) *Genes Dev.* **5,** 2212–2224.
13. Ptashne, M. & Gann, A. F. (1990) *Nature (London)* **346,** 329–331.
14. Tjian, R. & Maniatis, T. (1994) *Cell* **77,** 5–8.
15. McKnight, S. & Tjian, R. (1986) *Cell* **46,** 795–805.
16. Johnson, A. D. (1995) *Cell* **81,** 655–658.
17. Lewin, B. (1990) *Cell* **61,** 1161–1164.
18. Pugh, B. F. & Tjian, R. (1990) *Cell* **61,** 1187–1197.
19. Luo, Y., Fujii, H., Gerster, T. & Roeder, R. G. (1992) *Cell* **71,** 231–241.
20. Gstaiger, M., Knoepfel, L., Georgiev, O., Schaffner, W. & Hovens, C. M. (1995) *Nature (London)* **373,** 360–362.
21. Strubin, M., Newell, J. W. & Matthias, P. (1995) *Cell* **80,** 497–506.
22. Luo, Y. & Roeder, R. G. (1995) *Mol. Cell. Biol.* **15,** 4115–4124.
23. Meisterenst, M., Roy, A. L., Lieu, H. M. & Roeder, R. G. (1991) *Cell* **66,** 981–993.
24. Merino, A., Madden, K. R., Lane, W. S., Champoux, J. J. & Reinberg, D. (1993) *Nature (London)* **365,** 227–232.
25. Chen, J.-L., Attardi, L. D., Verrijzer, C. P., Yokomori, K. & Tjian, R. (1994) *Cell* **79,** 93–105.
26. Ge, H. & Roeder, R. G. (1994) *Cell* **78,** 513–523.
27. Ge, H., Zhao, Y., Chait, B. T. & Robert, R. G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12691–12695.
28. Kaiser, K., Stelzer, G. & Meisterernst, M. (1995) *EMBO J.* **144,** 3520–3527.
29. Kretzschmar, M., Meisterernst, M. & Roeder, R. G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11508–11512.
30. Maldonado, E., Shiekhattar, R., Sheldon, M., Cho, H., Drapkin, R., Inostroza, J. A., Rickert, P., Lees, E., Anderson, C. W., Linn, S. & Reinberg, D. (1996) *Nature (London)*, **381,** 86–89.
31. Shykind, B. M., Kim, J. & Sharp, P. A. (1995) *Genes Dev.* **9,** 1354–1365.
32. Kim, Y. J., Bjorklund, S., Li, Y., Sayre, M. & Kornberg, R. (1994) *Cell* **77,** 599–608.
33. Sun, X., Ma, D., Sheldon, M., Yeung, K. & Reinberg, D. (1994) *Genes Dev.* **8,** 2336–2348.
34. Yomomori, K., Zeidler, M. P., Chen, J.-L., Vereijzer, C. P., Mlodzik, M. & Tjian, R. (1994) *Genes Dev.* **8,** 2313–2323.
35. Ozer, J., Moore, P. A., Bolden, A. H., Lee, A., Rosen, C. A. & Lieberman, P. M. (1994) *Genes Dev.* **8,** 2324–2335.
36. Kobayashi, N., Boyer, T. G. & Berk, A. J. (1995) *Mol. Cell. Biol.* **15,** 6465–6473.
37. Lieberman, P. M. & Berk, A. J. (1994) *Genes Dev.* **8,** 995–1006.
38. DeJong, J., Bernstein, R. & Roeder, R. G. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3313–3317.
39. Cortes, P., Flores, O. & Reinberg, D. (1992) *Mol. Cell. Biol.* **12,** 413–421.
40. Coulombe, B., Killeen, M., Liljelund, P., Honda, B., Xiao, H., Ingles, C. J. & Greenblatt, J. (1992) *Gene Exp.* **2,** 99–110.
41. Buratowski, S., Hahn, S., Guarente, L. & Sharp, P. A. (1989) *Cell* **56,** 549–561.
42. Maldonado, E., Ha, I., Cortes, P., Weis, L. & Reinberg, D. (1990) *Mol. Cell. Biol.* **10,** 6335–6347.
43. Auble, D. T., Hansen, K. E., Mueller, C. G. F., Lane, W. S., Thorner, J. & Hahn, S. (1994) *Genes Dev.* **8,** 1920–1934.
44. Meisterenst, M. & Roeder, R. G. (1991) *Cell* **67,** 557–567.
45. Yokomori, K., Admon, A., Goodrich, J. A., Chen, J.-L. & Tjian, R. (1993) *Genes Dev.* **7,** 2335–2345.
46. Wang, W., Gralla, J. D. & Carey, M. (1992) *Genes Dev.* **6,** 1716–1727.
47. Ranish, J. & Hahn, S. (1992) *J. Biol. Chem.* **166,** 19320–19327.
48. Ranish, J., Lane, W. & Hahn, S. (1992) *Science* **255,** 1130–1132.
49. Ma, D. M., Watanabe, H., Mermelstein, F. H., Admon, A., Oguri, K., Sun, X., Wada, T., Imai, T., Shiroya, T., Reinberg, D. & Handa, H. (1993) *Genes Dev.* **7,** 2246–2257.
50. DeJong, J. & Roeder, R. G. (1993) *Genes Dev.* **7,** 2220–2234.
51. Buratowski, S. & Zhou, H. (1992) *Science* **255,** 1130–1132.
52. Stargell, L. A. & Struhl, K. (1995) *Science* **269,** 75–78.
53. Drapkin, R., Reardon, J. T., Ansari, A., Huang, J.-C., Zawel, L., Ahn, K., Sancar, A. & Reinberg, D. (1994) *Nature (London)* **368,** 769–772.
54. Inostroza, J. A., Mermelstein, F. H., Ha, I., Lane, W. S. & Reinberg, D. (1992) *Cell* **70,** 477–489.
55. Yeung, K. C., Inostroza, J. A., Mermelstein, F. H., Kannabiran, C. & Reinberg, D. (1994) *Genes Dev.* **8,** 2097–2109.
56. Kang, J. J., Auble, D. T., Ranish, J. A. & Hahn, S. (1995) *Mol. Cell. Biol.* **15,** 1234–1243.
57. Choder, M. (1991) *Genes Dev.* **5,** 2315–2326.