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Factors involved in specific transcription by mammalian RNA polymerase II: Purification and characterization of general transcription factor TFIIE

(*in vitro* transcription/transcription initiation/promoter/basal-level transcription/subunit structure)

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ABSTRACT **Human transcription factor TFIIE, a ubiquitous factor required for transcription initiation by RNA polymerase II, was purified to homogeneity by a combination of conventional and HPLC steps. The purified TFIIE contained equimolar amounts of 57-kDa (TFIIE-α) and 34-kDa (TFIIE-β) polypeptides that were judged to be functional subunits on the basis of their copurification with transcriptional activity and the recovery of activity following renaturation of polypeptides separated by reverse-phase HPLC. TFIIE-α had an independent TFIIE activity whereas TFIIE-β had no activity alone but enhanced the activity of TFIIE-α. In conjunction with gel filtration studies, which indicated a molecular mass of ≈180 kDa for the native protein, these results suggested that TFIIE is a heterotetramer containing two α and two β polypeptides. Functional studies with the purified TFIIE demonstrated that it is a general initiation factor, required for all of the genes tested, but it failed to show any DNA-dependent ATPase activity.**

In mammalian cells, transcription initiation by RNA polymerase II is mediated by ubiquitous general factors (reviewed in refs. 1 and 2) that act through core promoter elements (usually a TATA box and associated initiation-site sequences) and regulated by gene- or tissue-specific factors (reviewed in ref. 3) that usually act from distal sites. The initial fractionations of HeLa cell nuclear extracts revealed a requirement for the ubiquitous factors TFIIA, TFIIB, TFIID, and TFIIE (4–8), although the requirement for TFIIA has been variable (for references and discussion, see refs. 2 and 9). More recently, the original TFIIE fraction (5, 6) has been resolved into TFIIE and TFIIF activities (10) and TFIIF or its RNA polymerase-binding equivalent (RAP30/74) has been purified to homogeneity (11–13). The preceding paper (9) described the identification of another human general initiation factor, TFIIG, which appears to obviate the TFIIA requirement. Mammalian factors equivalent to many or all of these factors have been described by alternative fractionation procedures for both HeLa cells (14–16) and rat liver (17–20), and some of these have been purified to apparent homogeneity (15, 17, 18).

Studies of the transcription initiation mechanism by the general mammalian factors have described a number of steps and corresponding complexes in the overall pathway. In the first step, TFIID binds to the promoter via the TATA element (21, 22), a process that may be facilitated by TFIIA (8, 23, 24). This interaction mediates the subsequent stepwise assembly of RNA polymerase II and the other general initiation factors into a functional preinitiation complex (23–28). Although past studies have provided important insights into this problem, the exact order of assembly and precise functions and mechanisms of action of the various factors are not yet clear. Apparent discrepancies (e.g., in the order of assembly) in the various studies may reflect the distinct assay systems employed, the existence of alternative pathways, or the use of impure transcription factors. A definitive analysis of initiation mechanisms, as well as structure–function studies within the individual factors, awaits their complete purification and further analysis.

Various studies with a partially purified TFIIE have indicated that it interacts with RNA polymerase II (8, 10), that it acts at a late stage in preinitiation-complex assembly and function (8, 28, 29), and that it may possess an ATPase activity (7, 19, 30, 31). However, the lack of progress in the purification of this factor has impaired studies of its function. Here we report the complete purification and initial characterization of this factor.

MATERIALS AND METHODS

Purification of TFIIE. HeLa cell nuclear extracts (5) were dialyzed against buffer A [20 mM Tris·HCl, pH 7.9 (at 4°C)/10 mM 2-mercaptoethanol/20% (vol/vol) glycerol/0.2 mM EDTA/0.5 mM phenylmethylsulfonyl fluoride] containing 0.1 M KCl. The dialyzed sample (3.8 g of protein) was loaded onto a phosphocellulose (Whatman P11) column (3.6 × 11 cm) equilibrated with buffer A containing 0.1 M KCl. The column was washed with the same buffer and eluted successively with 0.3 M, 0.5 M, and 0.85 M KCl in buffer A. TFIIE/IIF activity was eluted with 0.5 M KCl and active fractions were dialyzed to 0.1 M KCl and loaded onto a DEAE-cellulose (Whatman DE52) column (2.1 × 7.6 cm) equilibrated with buffer A containing 0.1 M KCl. TFIIE/IIF activity was eluted with buffer A containing 0.3 M KCl, dialyzed against the same buffer containing 0.5 M KCl, and subjected to chromatography on a Sephacryl S-300 (Pharmacia) gel filtration column (2.5 × 73 cm) equilibrated with the same solution. TFIIE and TFIIF activities were partially separated at this step. Active TFIIE fractions were precipitated by 65% saturated ammonium sulfate, dissolved in buffer B [20 mM Tris·HCl (HPLC grade), pH 7.8 (at 20°C)/10 mM 2-mercaptoethanol/10% (vol/vol) glycerol/0.5 mM EDTA, 0.01% (vol/vol) Nonidet P-40] containing 0.095 M KCl and dialyzed against the same solution. After the KCl concentration was adjusted to 0.1 M, the sample was loaded onto a Bio-Gel DEAE-5PW HPLC column (21.5 × 150 mm; Bio-Rad) equilibrated with buffer B containing 0.1 M KCl and eluted with a linear gradient of 0.1–0.4 M KCl in buffer B. All HPLC columns were run at room temperature. TFIIE activity was eluted at 0.3 M KCl and was completely separated from TFIIF activity (eluted at 0.2 M KCl). Active fractions were precipitated by 65% saturated ammonium sulfate, dissolved in buffer C [20 mM Hepes, pH 7.9/10 mM 2-mercap-

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Abbreviations: Ad2, adenovirus type 2; ML, major late.

toethanol/10% (vol/vol) glycerol/0.5 mM EDTA] containing 0.095 M KCl, and dialyzed against the same buffer to 0.1 M KCl. The sample was loaded onto a Bio-Gel SP-5PW HPLC column (7.5 × 75 mm; Bio-Rad) equilibrated with buffer C containing 0.1 M KCl. This column was subsequently washed with the same solution and eluted with a linear gradient of 0.1–0.5 M KCl in buffer C. TFIIE activity was eluted at ≈0.28 M KCl. Active fractions were mixed and diluted to 0.1 M KCl with buffer D [20 mM Tris·HCl (HPLC grade), pH 7.8 (at 20°C)/10 mM 2-mercaptoethanol/10% (vol/vol) glycerol/0.5 mM EDTA/0.1% (vol/vol) Tween 40] and loaded onto a TSK heparin-5PW HPLC column (7.5 × 75 mm; Supelco) equilibrated with buffer D containing 0.1 M KCl. The column was washed with the same solution and eluted with a linear gradient of 0.1–0.8 M KCl in buffer D. TFIIE activity was finally eluted at ≈0.37 M KCl.

Preparation of Other General Transcription Factors and RNA Polymerase II. General transcription factors TFIIB, TFIID, TFIIF, and TFIIG were partially purified from HeLa cell nuclear extracts (5), while TFIID was the cloned yeast factor expressed in *Escherichia coli* and purified (32). TFIIB was the single-stranded DNA-agarose fraction (6). TFIIF was a fraction separated completely from TFIIE by chromatography of the Sephacryl S-300 fraction (above) on a Bio-Gel DEAE-5PW HPLC column (H.S. and Y.O., unpublished results). TFIIG, described in the preceding paper (9), was a Bio-Gel SP-5PW HPLC fraction. RNA polymerase II was purified from HeLa cell nuclear pellets by the method of Bitter (33). Activity units for each factor were as described (34), except that CTP replaced UTP as the radiolabeled nucleotide. One unit of activity represents the incorporation of 1 pmol of CMP under standard transcription conditions using pML(C_2AT) as a template.

***In Vitro* Transcription.** Assay conditions were as described (34) and reaction mixtures contained the following amounts of the general factors prepared as described above: 0.8 μg of TFIIB; 1.0 μg of TFIIF; 0.8 μg of TFIIG; 0.05 μg of bacterially expressed yeast TFIID (32), and aliquots of the TFIIE fractions being assayed. TFIIA was omitted since it is not required in the presence of TFIIG (9). Plasmids used as templates contained the guanine-free casette (6) linked downstream of promoter sequences as follows: pML(C_2AT), sequence from −404 to +10 of the adenovirus type 2 (Ad2) major late (ML) promoter (6); p2.5, sequence from −59 to −21 of the histone H2B promoter (35); pH4(C_2AT)Δ−130 and pH4(C_2AT)Δ−50, sequences from −130 or −50 to +8 of the histone H4 promoter (36). Except for the experiments in Fig. 4*A* the template was pML(C_2AT). Specific transcripts were quantitated by the DEAE-paper assay (7) or by autoradiography and liquid scintillation counting of polyacrylamide gel-fractionated transcripts.

Resolution of TFIIE Polypeptides by Reverse-Phase HPLC. Reverse-phase HPLC was carried out on a Hi-Pore RP-304 C_4 column (4.6 × 250 mm; Bio-Rad) for the separation of the two subunits of TFIIE. The elution system consisted of a linear gradient of acetonitrile and 1-propanol (0–100% solvent B over 90 min) with trifluoroacetic acid. Solvent A was acetonitrile/1-propanol/water/trifluoroacetic acid, 2.5:2.5:95:0.1 (by volume). Solvent B was acetonitrile/1-propanol/water/trifluoroacetic acid, 35:35:30:0.1 (by volume). Elution was at room temperature at a flow rate of 1 ml/min and fractions were collected by hand according to peak volume.

HPLC Gel Filtration of Purified TFIIE. The native molecular mass of TFIIE (heparin-5PW HPLC fraction) was examined by gel filtration on a TSK G5000-PW column (7.5 × 600 mm; Beckman) equilibrated with buffer B containing 0.5 M KCl. The elution position of TFIIE, as determined both by silver staining of SDS/polyacrylamide gel and by transcription assays, was compared with the elution profiles of five standard proteins (thyroglobulin, V_e = 16.92 ml; ferritin, V_e = 17.99 ml; β-amylase, V_e = 18.67 ml; aldolase, V_e = 18.80 ml; bovine serum albumin, V_e = 19.23 ml).

ATPase Assay. Reaction mixtures (25 μl) containing 25 mM Tris·HCl (pH 7.9 at 4°C), 8 mM $MgCl_2$, 50 mM KCl, 0.1 mM dithiothreitol, 50 μg of bovine serum albumin per ml, 2% glycerol, and 50 μM [γ-^{32}P]ATP (Amersham) were incubated at 37°C for 1 hr. Single- or double-stranded DNA was also included in some reaction mixtures (see legend to Fig. 4*B*). ATPase activities were measured by polyethylenimine-cellulose TLC (20, 37).

RESULTS

Purified TFIIE Contains 57- and 34-kDa Polypeptides That Copurify with Transcriptional Activity. The purification of TFIIE from HeLa cell nuclear extracts was achieved with six chromatographic steps (including three HPLC steps) and activity was monitored by a TFIIE-dependent complementation assay containing the other general factors and the Ad2 ML promoter as a template. After isolation of the previously designated TFIIE fraction (4–7) by P11 and DE52 chromatography, TFIIE and TFIIF were partially separated by gel filtration; they were eluted at positions corresponding, respectively, to native molecular masses of around 150 kDa and 240 kDa. Complete separation was achieved by subsequent HPLC on DEAE-5PW; TFIIE was eluted at 0.3 M KCl and TFIIF at 0.2 M KCl (data not shown). HPLC of the active TFIIE fraction on heparin-5PW revealed two major polypeptides (57 and 34 kDa), detected on a silver-stained SDS/polyacrylamide gel (Fig. 1*A*), that were coeluted with TFIIE activity (Fig. 1*B*). This coelution was already apparent at the preceding stage of purification (SP-5PW HPLC; data not

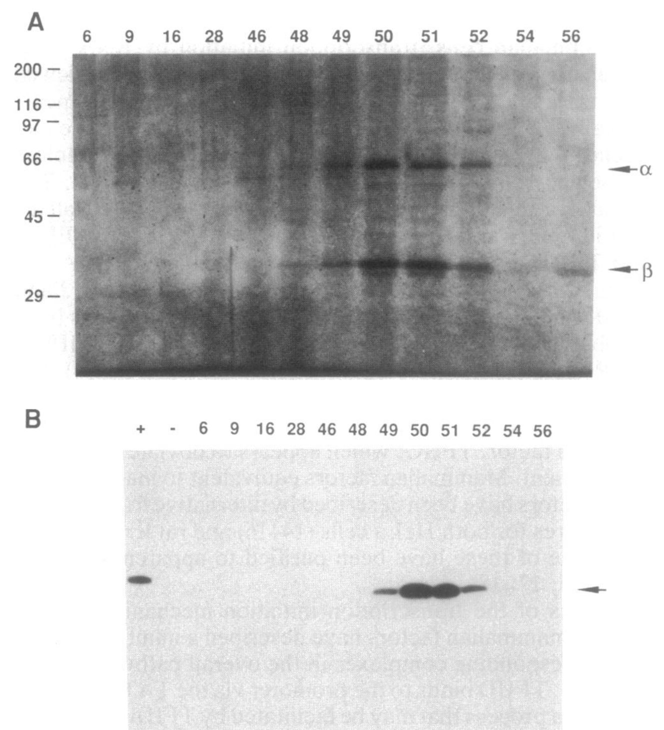

FIG. 1. HPLC of TFIIE on a heparin-5PW column. Active fractions from a cation-exchange (sulfopropyl, SP) HPLC column were subjected to HPLC on a heparin column. (*A*) SDS/10% PAGE of 40-μl aliquots from the indicated column fractions. Proteins were stained with silver (Bio-Rad). Arrows indicate the 57-kDa (α) and 34-kDa (β) bands. Positions of molecular size (kDa) markers are given at left. (*B*) TFIIE transcription assays of 2-μl aliquots of the indicated column fractions. Lanes + and −, control assays with the column input and with no TFIIE, respectively.

shown). These results suggest that TFIIE consists of 57- and 34-kDa polypeptides, designated here as TFIIE-α and TFIIE-β.

Table 1 summarizes the purification and indicates a final yield of 8 μg of TFIIE from 3.8 g of nuclear extract protein. The overall purification was >2000-fold from the P11 column fraction and the recovery of its transcriptional activity was almost 16%. If one assumes there was no loss of activity in the P11 step, the overall purification from the nuclear extract would be ≈60,000-fold.

TFIIE Exists in Solution as a Tetramer. Purified TFIIE (heparin HPLC fraction) was further analyzed by HPLC gel filtration on G5000-PW. In this procedure the α and β polypeptides still comigrated, in a constant ratio, with TFIIE activity (Fig. 2 *A* and *B*). This result supports the previous assumption that TFIIE consists of two distinct polypeptides. Based on the elution position of the TFIIE peak fraction with the elution positions of five other standard proteins, the native TFIIE is ≈180 kDa (Fig. 2*D*).

As protein amounts cannot be accurately estimated from silver-stained SDS/polyacrylamide gels, a Coomassie blue-stained gel was used to quantitate each polypeptide by densitometric scanning (Fig. 2*C*). By taking into account the peak intensities and the relative molecular masses, the molar ratio of the α and β polypeptides was estimated to be about 0.85:1 (Fig. 2*C* legend). Assuming an actual ratio of 1:1, and based on the size estimate (180 kDa) of the native protein, it appears that the TFIIE in solution is a tetramer of two α and two β subunits (calculated molecular mass of 182 kDa).

Renatured TFIIE-α Alone Displays Basal Transcriptional Activity. For the purpose of further characterization of the TFIIE transcriptional activity, TFIIE-α and -β were separated by reverse-phase HPLC (Fig. 3*A*), renatured individually or together (38), and assayed for TFIIE activity (Fig. 3*B*). A control experiment showed that ≈40% of native TFIIE activity was recovered following denaturation and renaturation of the column input. In the analysis of the HPLC fractions, renatured TFIIE-β did not show any independent transcriptional activity. Surprisingly, however, renatured TFIIE-α by itself showed substantial transcriptional activity; this amounted to ≈22% of the total activity when corrected for the recovery of activity, following denaturation and renaturation, in the unfractionated input sample.

Interestingly, when individually renatured subunits were mixed the transcriptional activity was 1.6-fold that observed with renatured TFIIE-α alone. When both subunits were renatured together the activity was almost 5-fold that of TFIIE-α alone, resulting in a quantitative recovery of total activity (Fig. 3*B*). Almost the same increases were observed when a bacterially expressed human TFIID (32) was used in place of yeast TFIID in the TFIIE complementation assay (data not shown). Thus, both TFIIE subunits are necessary for full activity, but TFIIE-α by itself shows partial activity. These renaturation results were different from those observed with TFIIF, which required both subunits for any activity (13).

Purified TFIIE Is Essential for *in Vitro* Transcription by Various Promoters. To prove that the purified TFIIE repre-

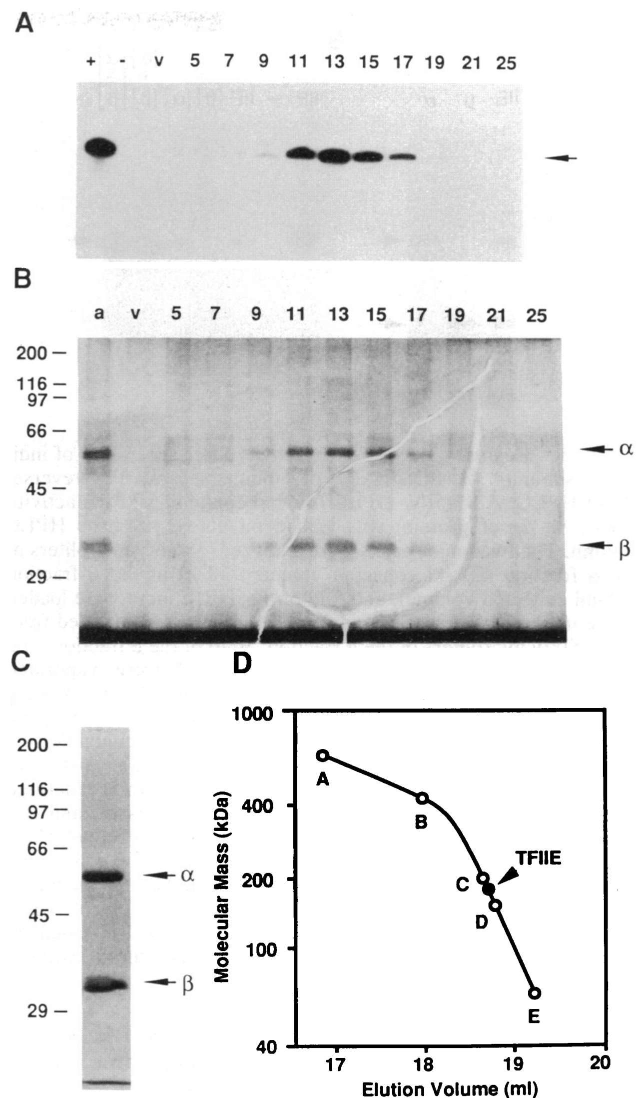

FIG. 2. Gel filtration HPLC of purified TFIIE. A sample of purified TFIIE (heparin HPLC fraction) was subjected to HPLC on a G5000-PW column. (*A*) TFIIE transcription assays of 2-μl aliquots of the indicated column fractions. Other lanes indicate assays of the input fraction (+), buffer (−), and the void fraction (v). The position of the specific transcript is shown by an arrow. (*B*) Silver-stained SDS/polyacrylamide gel. Aliquots (100 μl) of the indicated fractions were analyzed by SDS/10% PAGE. Positions of molecular size (kDa) markers are indicated. (*C*) Coomassie blue-stained SDS/polyacrylamide gel analysis. About 1 μg of purified TFIIE (G5000-PW HPLC fraction) was analyzed. The α/β mass ratio was 1.43:1 as determined with a gel scanner (LKB UltroScan XL, Pharmacia). By taking into account the relative molecular masses, the α/β molar ratio was estimated at about 0.85:1. (*D*) Determination of the native molecular mass of TFIIE by HPLC on a G5000-PW gel filtration column. Molecular mass markers: A, thyroglobulin (669 kDa); B, ferritin (440 kDa); C, β-amylase (200 kDa); D, aldolase (158 kDa); E, bovine serum albumin (66 kDa).

Table 1. Purification of TFIIE

Fraction	Protein, mg	Volume, ml	Activity, units	Specific activity, units/mg	Fold purification	Recovery, %
Nuclear extract	3800	500	—	—	—	—
P11	130	80	14,000	110	1	100
DE52	38	20	7,700	200	1.8	56
Sephacryl S-300	6.2	25	6,100	980	8.9	48
DEAE HPLC	0.33	8.0	4,300	13,000	120	36
SP HPLC	0.040	3.0	3,400	85,000	770	29
Heparin HPLC	0.008	2.5	1,800	230,000	2100	16

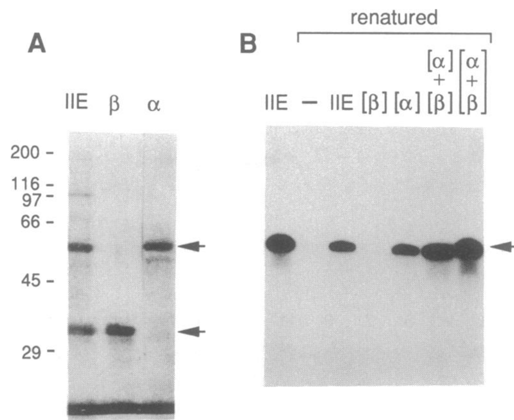

FIG. 3. Renaturation of TFIIE activity after separation of individual subunits. (*A*) SDS/PAGE of subunits separated by reverse-phase HPLC. A SP-5PW HPLC fraction containing TFIIE activity (about 80 μg of protein) was loaded on a reverse-phase HPLC column. The two major peaks were analyzed. Twenty microliters of the α fraction (2.5-ml collected volume), 10 μl of the β fraction (1.5-ml collected volume), and 5 μl of the TFIIE input were loaded in the indicated lanes. (*B*) Transcription activity of renatured fractions. Sixty microliters of the α fraction, 30 μl of the β fraction, and 90 μl of a mixed fraction (60 μl of α and 30 μl of β) were evaporated to dryness in a Speed-Vac concentrator. These fractions were dissolved in 6 μl of 6 M guanidine hydrochloride, incubated at room temperature for 20 min, mixed with 300 μl of buffer A containing 0.1 M KCl, renatured for 12 hr at 4°C, and dialyzed for 3 hr against 1 liter of buffer A containing 0.1 M KCl. A 2-μl aliquot of each renatured fraction was used for the transcription assay. Lanes [α] and [β], analysis of individually renatured α and β subunits, respectively; lane [α]+[β], individually renatured α and β fractions were mixed and added; lane [α+β], subunits were renatured together and analyzed. Untreated TFIIE (0.1 μl) was used as a positive control, while renatured (unfractionated) TFIIE (0.06 μl) was used to calculate recovery of activity. Lane –, control fraction "renatured" without TFIIE.

sents a general transcription factor, various TATA box-containing promoters attached to the guanine-free casette were tested in the TFIIE-dependent complementation assay. TFIIE was essential for accurate transcription initiation not only from the Ad2 ML promoter, but also from the histone H2B and H4 promoters (Fig. 4*A*). The lower bands detected in the assays most likely reflect transcripts specifically initiated downstream of a TATA-like sequence in the guanine-free casettes, and, as expected, histone H4 upstream sequence elements had no noticeable effect on the basal transcription with the H4 core promoter. These results indicate that TFIIE has a general role in initiation from class II promoters by RNA polymerase II.

Purified TFIIE Does Not Possess an Intrinsic DNA-Dependent ATPase Activity. Previous studies have indicated that the hydrolysis of the β–γ bond of ATP (dATP) is required for transcription initiation (30, 31). TFIIE or its equivalent has been reported to contain a DNA-dependent ATPase activity on the basis of assays with partially purified TFIIE fractions (7, 19, 30). To examine this possibility, purified TFIIE and TFIIE fractions at various steps of the purification were monitored for ATPase in the absence or presence of bacteriophage φX174 single-stranded DNA (Fig. 4*B*). None of the HPLC-purified TFIIE preparations exhibited any detectable DNA-dependent or DNA-independent ATPase activity (lanes 5 and 6), whereas a relatively crude DE52 fraction contained a DNA-independent ATPase activity (lanes 11 and 12). Moreover, the purified TFIIE fractions did not show any ATPase activity with two double-stranded DNAs, pUC18 and pML(C_2AT) (data not shown). A control ATPase B purified from mouse FM3A cells (39) was stimulated by both single-stranded DNA (Fig. 4*B*) and double-stranded DNA (data not shown). From these results, we conclude that purified TFIIE does not have an intrinsic DNA-dependent ATPase activity.

DISCUSSION

Along with other general transcription factors from mammalian cells, TFIIE plays an indispensable role in transcription initiation by RNA polymerase II. As a necessary step to elucidate the precise mechanisms of transcription initiation, including the detailed structure–function relationships of the factors, human TFIIE was purified to apparent homogeneity from nuclear extracts, with an overall purification estimated at >60,000-fold. Functional studies with the purified factor

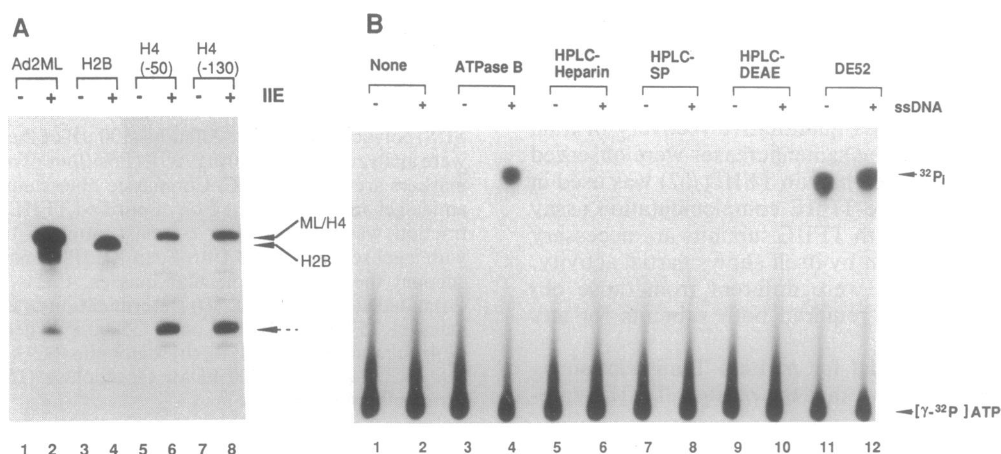

FIG. 4. Functional properties of purified TFIIE. (*A*) Effect of purified TFIIE on transcription of various promoters. Transcription assays were carried out using different promoters (200 fmol) with (+) or without (–) purified TFIIE (heparin HPLC fraction). Ad2ML (lanes 1 and 2), pML(C_2AT); H2B (lanes 3 and 4), p2.5; H4(–50) (lanes 5 and 6), pH4(C_2AT)Δ–50; H4(–130) (lanes 7 and 8), pH4(C_2AT)Δ–130. Positions of specific transcripts (ML/H4 and H2B) are shown by arrows. The specific transcript from the H2B promoter is about 20 nucleotides shorter than the others because of a deletion in the guanine-free cassette. The lower major band (broken arrow) is probably a transcript resulting from a TATA-like sequence in the guanine-free cassette region. (*B*) ATPase assay of TFIIE column fractions. ATPase assays were carried out in the presence (+) or absence (–) of 50 ng of φX174 single-stranded (ss) DNA. Lanes 1 and 2, no protein; lanes 3 and 4, ATPase B; lanes 5 and 6, heparin HPLC fraction; lanes 7 and 8, SP-5PW HPLC fraction; lanes 9 and 10, DEAE-5PW HPLC fraction; lanes 11 and 12, DE52 fraction. Each fraction of TFIIE added (lanes 5–12) was adjusted to about 1 unit of transcriptional activity. [^{32}P]Phosphate ($^{32}P_i$) and [γ-^{32}P]ATP were detected by autoradiography of the thin-layer chromatograms.

supported previous suggestions that TFIIE is a general transcription factor.

Purified native TFIIE appears to be a tetramer composed of two 57-kDa (TFIIE-α) and two 34-kDa (TFIIE-β) subunits on the basis of the following: (*i*) copurification of the 57-kDa and 34-kDa polypeptides, at a constant mass ratio, with transcription activity during chromatography on three HPLC columns (SP, heparin, and gel filtration); (*ii*) a nearly 1:1 stoichiometry of the 57- and 34-kDa polypeptides in purified TFIIE and a native molecular mass estimate of 180 kDa; and (*iii*) nearly quantitative recovery of activity following renaturation of 57- and 34-kDa polypeptides that were separated (under denaturing conditions) by reverse-phase HPLC. The latter analysis also revealed that TFIIE-α has an independent TFIIE activity that is markedly enhanced by TFIIE-β, which alone showed no activity. These results suggest that TFIIE-β might be a regulatory subunit for the essential TFIIE-α subunit (discussed below).

TFIIE is distinct from most other general factors with respect to chromatographic properties, native molecular mass, and subunit structure but resembles TFIIF in many of these properties. TFIIF also exists as a tetramer, with two 78-kDa and two 30-kDa subunits (13). Nonetheless, TFIIE is clearly different: (*i*) the chromatographic properties of TFIIE and TFIIF were distinct on high-resolution HPLC columns (G5000-PW and DEAE-5PW), (*ii*) TFIIE and TFIIF were independently required for transcription, (*iii*) the molecular masses of the composite polypeptides (57 and 34 kDa for TFIIE versus 78 and 30 kDa for TFIIF) are clearly distinct and not compatible with a simple derivation of one factor from the other by proteolysis, and (*iv*) both subunits of TFIIF were required (following denaturation, separation, and renaturation) for activity whereas TFIIE-α clearly showed some TFIIE-β-independent transcriptional activity.

Previous studies demonstrated a TFIIE interaction with RNA polymerase II (10) and suggested the possibility of an associated ATPase (or dATPase) activity (7, 19, 30). The latter observations are consistent with the prior demonstrations of an ATP hydrolysis for initiation (30, 31) and suggestions that TFIIE is involved at a late stage of preinitiation-complex assembly (28, 29). However, those studies were performed with relatively impure fractions of TFIIE, which in most cases also contained TFIIF. Importantly, our initial functional studies with the purified TFIIE have shown that this factor does not possess an intrinsic DNA-dependent (or DNA-independent) ATPase activity, indicating either that this activity resides in a distinct factor or that it is dependent upon interactions with other factors in the preinitiation complex.

The availability of a purified TFIIE now makes possible more definitive studies of the structure and mechanism of action of TFIIE and its composite polypeptides, especially as the other general transcriptional factors become available in purified form. Moreover, although recent studies of the mechanism of action of various regulatory factors have implicated either TFIID (reviewed in ref. 40; see also ref. 41 and references therein) or unidentified coactivators (reviewed in ref. 42) as targets, it is possible that TFIIE might also be a target for some regulatory factors. In light of the demonstration here of an absolute requirement for TFIIE-α for initiation, and a possible modulatory role for TFIIE-β, it will be particularly interesting to assess the TFIIE subunit requirements for the action of regulatory factors.

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