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An interplay between TATA box-binding protein and transcription factors IIE and IIA modulates DNA binding and transcription

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ABSTRACT **The basal transcription factor IIE (TFIIE) is thought to be one of the last factors to be assembled into a preinitiation complex (PIC) at eukaryotic promoters after RNA polymerase II and TFIIF have been incorporated. It was shown that a primary function of TFIIE is to recruit and cooperate with TFIIH in promoter melting. Here, we show that the large subunit of TFIIE (E56) can directly stimulate TBP binding to the promoter in the absence of other basal factors. The zinc-finger domain of E56, required for transcriptional activity, is critical for this function. In addition, the small subunit of TFIIE (E34) directly contacts DNA and TFIIA and thus providing a second mechanism for TFIIE to help binding of a TBP/IIA complex to the promoter, the first critical step in the PIC assembly. These studies suggest an alternative PIC assembly pathway in which TFIIE affects both TBP and TFIIH functions during initiation of RNA synthesis.**

Studies over the last two decades have identified a highly elaborate molecular machinery that has evolved to accommodate and direct the proper timing of transcription in animal cells. In the case of gene-specific production of mRNA by RNA polymerase II (pol II), it has become evident that multiple transcription complexes work in concert to regulate gene expression. These include the general machinery composed of RNA pol II and its associated factors (TFIIA, IIB, IID, IIE, IIF, and IIH), a host of gene-specific DNA-binding factors as well as various cofactors that are required to direct the regulation of activated transcription in animal cells (see refs. 1–3). The focus in recent years therefore has shifted from merely identifying the molecular players in the complex process of transcription toward dissecting the functional and mechanistic relationships between these various components of the initiation complex.

During the formation of a fully functional preinitiation complex (PIC) that requires an assemblage of basal transcription factors, TFIIA, B, D, E, F, H, and RNA pol II, the recruitment of the TATA-binding protein (TBP) to the promoter has emerged as a key step (see refs. 2, 4). *In vivo*, TBP most likely operates in concert with a tightly associated set of transcription factors called TBP-associated factors (TAFs), that together with TBP form the holo-TFIID complex (5; see refs. 2, 4, 6, 7). *In vitro*, it has been demonstrated that one possible pathway of assembly begins with the formation of a TBP/TFIIA/TFIIB (TAB) complex at the promoter, followed by the recruitment of RNA pol II /TFIIF, and ending with the incorporation of TFIIE which in turn recruits TFIIH (8, 9). TFIIA and TFIIB are thought to play an important role early during PIC formation by helping to recruit or stabilize the binding of TFIID or TBP to the core promoter. In contrast, TFIIE was suggested to participate in promoter-melting together with TFIIH during the transition from a stable PIC to subsequent promoter clearance and elongation stages of the transcription cycle (see refs. 2, 4, 10, 11).

The TFIIE tetramer comprised of two 56-kDa and two 34-kDa subunits is capable of interacting with multiple components of the PIC including TBP, TFIIF, TFIIH, and unphosphorylated RNA pol II *in vitro* (12). A specific mutation in the zinc-finger domain of the E56 subunit renders TFIIE transcriptionally incompetent, whereas its ability to interact with other protein factors and recruit TFIIH remains intact (13, 14). Thus, the zinc-finger domain of E56 is responsible for functions distinct from TFIIH recruitment. In particular, we have been intrigued by the possibility that TFIIE, through the zinc-finger domain of the large subunit, may contribute to protein–DNA interactions of the basal machinery. Here, we report that TFIIE directly interacts with TBP as well as TFIID *in vitro* (12), suggesting that TFIIE also may participate in an early step of PIC assembly that affects the formation of the T(D)AB complex. Our results suggest that this function of TFIIE is distinct from its ability to recruit TFIIH at later stages of PIC formation.

MATERIALS AND METHODS

Purification of Basal Factors. The basal factors were expressed in *Escherichia coli* (BL21 DE3) except TFIIF, which was expressed by using the baculovirus expression system largely as described (18). *Drosophila* TBP and TFIIA were expressed and purified as described (15–17). Human TBP, E34, E56, and the zinc-finger mutant of E56, which contains a substitution of cysteine 154 with alanine (13), were expressed as histidine-tagged proteins allowing them to be purified with Ni-NTA agarose resin (Quiagen). The human TBP, TFIIB, and E34 were purified further by SP Sepharose column (Pharmacia). E56 was purified on a Mono Q column (Pharmacia). The TFIIF RAP30/74 heterotetramer was purified by phospho-cellulose and Mono Q chromatography. RNA pol II A/O, a generous gift from J.-L. Chen (Tularik, Inc.), was purified from HeLa cell nuclear pellet as described (19) and is free from other basal factors (data not shown).

***In Vitro* Transcription.** *In vitro* transcription and primer extension were performed as described (17). In brief, purified recombinant basal factors (TBP, TFIIA, TFIIB, TFIIE, and TFIIF) and partially purified RNA pol II were incubated with 100 ng of template (G6TI or E4) for 30 min at 30°C. rNTPs were then added and incubated for an additional 15 min. The transcription products were detected by primer extension with

Abbreviations: Pol II, polymerase II; TBP, TATA box-binding protein; TFIIE, transcription factor IIE; TFIIA, transcription factor IIA; TFIIF, transcription factor IIF; TFIIH, transcription factor IIH; PIC, preinitiation complex; AdML, adenovirus major-late; TAF, TBP-associated factors.

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^{32}P-labeled specific downstream primers. The amounts of recombinant factors were typically 2–5 ng of TBP, TFIIA, TFIIB and 15 ng of TFIIF per reaction.

DNase I and Hydroxyl Radical Footprinting. DNase I footprinting was performed as described (16). AdML (adenovirus major-late) 2 promoter (*Xba*I/*Bam*HI) and E4 promoter (G5E4T) (*Hin*dIII/*Eco*RI) were used. Unless indicated, the DNase I footprinting analyses were performed on the AdML 2 promoter with the 5′ end of the nontranscribed strand labeled. Typically, ≈5–7.5 ng of DNA was used in the reaction containing 0.75 μg of BSA, 5 ng of poly-dGTP/dCTP, and 0.025% Nonidet P-40 (Calbiochem, La Jolla, CA). The amounts of recombinant proteins are indicated in figure legends. Binding reactions were carried out at 30°C for 30 min and digested with DNase I. Samples were analyzed by 6% urea–acrylamide gel electrophoresis followed by autoradiography. For the hydroxyl radical footprinting, binding reactions were similar to that for DNase I footprinting except glycerol was omitted from the buffer. To a 50 μl reaction, 3 μl each of freshly prepared 0.2 mM $(NH_3)_2Fe(SO_4)_2$ containing 0.4 mM EDTA, 6% H_2O_2, and 6.7 mM Na ascorbate were added. After a 2-min incubation at 25°C, reactions were stopped by the addition of 30 μl of 0.2 M thiourea. Samples were processed and analyzed similar to the DNase I reactions. NIH image was used for quantitation. The nucleotide positions were determined by a G+A ladder.

UV-Crosslinking Assays. The AdML2 promoter was used as a template with the primers spanning the promoter regions from −72 to −53 and −11 to +8 for a PCR containing 25 mM BrdUTP, unlabeled dCTP and dGTP, and 200 μCi of [^{32}P]dATP. Protein concentration of E34, E56, and TBP used were 8 ng, 8 ng, and 20 ng, respectively. The procedure is essentially described (16). In brief, after 30 min of binding at 30°C, the mixtures were UV-irradiated and mixed with affinity-purified antibodies against E34 coupled to protein A beads. After 3-hr incubation at 4°C, DNase I and Micrococcal nuclease were then added together with $MgCl_2$ and $CaCl_2$ to the beads, and the reaction mixtures were incubated for 20 min at 37°C. The radio-labeled proteins were analyzed by SDS/PAGE followed by autoradiography.

***In Vitro* Protein-Binding Assays.** Both human and *Drosophila* TFIIA large subunit were fused to glutathione *S*-transferase (GST) in pGEX, expressed in *E. coli* and bound to glutathione Sepharose beads. Flag-tagged E34 in pAR3038 was expressed in *E. coli*, and the soluble protein was bound to anti-flag M2 beads (Kodak). ^{35}S-labeled *in vitro* translated proteins [E34, E56, TFIIB, hTFIIAα β (20), dTFIIA-L and cotranslated two subunits of dTFIIA] were incubated for 3 hr at 4°C with bait proteins bound to beads. Beads were then washed with buffer containing 0.1 M KCl and 0.1% Nonidet P-40. The binding of proteins was analyzed by SDS/PAGE and visualized by autoradiography.

RESULTS

TFIIE Stimulates Basal Transcription in the Absence of TFIIH in a E56 Zinc-Finger-Dependent Manner. To examine potential activities of TFIIE other than interaction with TFIIH, we carried out *in vitro* transcription in a TFIIH-independent transcription system, consisting of recombinant TBP, TFIIA, TFIIB, TFIIE, and TFIIF and purified RNA polymerase II (Fig. 1*A*). Using super-coiled templates, very weak transcription was observed in the absence of TFIIE from both the G6TI promoter containing the AdML TATA box (21) (lane 1) and E4 promoter (lane 3). Similar to published observations with the AdML promoter (22, 23), we found that TFIIE stimulates transcription from the G6TI promoter in the absence of TFIIH (lane 2). In contrast to a previously published result (23), we also observed a stimulatory effect of TFIIE on transcription from the E4 promoter (lane 4). To demonstrate that this stimulation of transcription was dependent on TFIIE activity, we used recombinant TFIIE containing a zinc-finger mutant of the large subunit of TFIIE (E56) (13). This zinc-finger mutant, previously shown to be inactive in a transcription system containing TFIIH (13), is also inactive for transcription in the absence of TFIIH (lane 5). These experiments further suggest that TFIIE plays a role that is independent of TFIIH and that the zinc-finger domain of E56 is important for this activity.

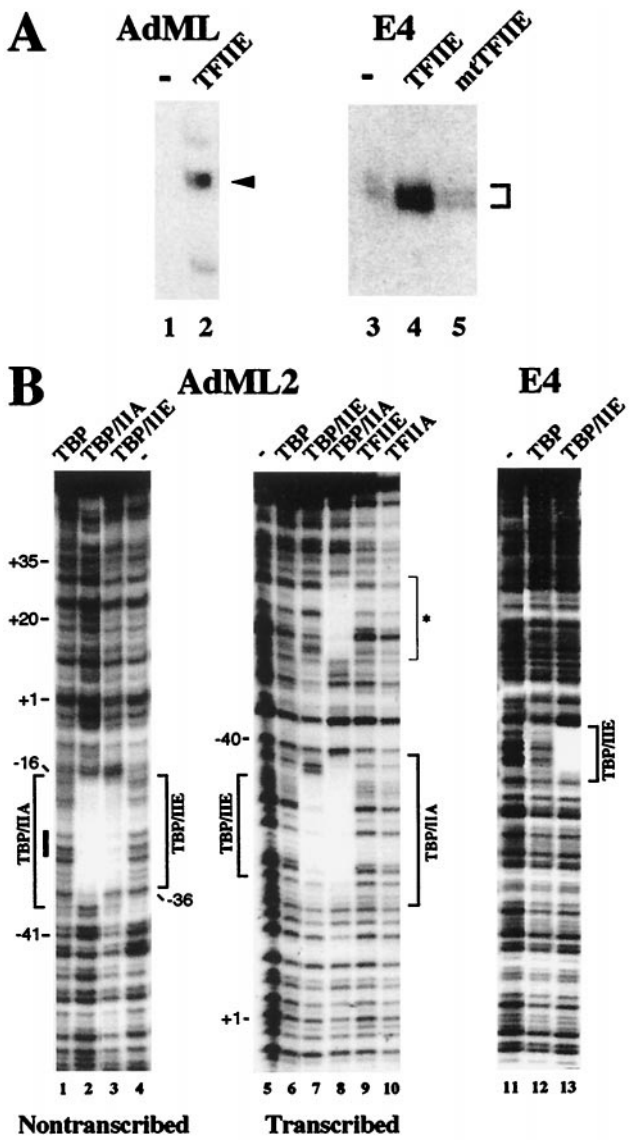

FIG. 1. The TFIIH-independent function of TFIIE. (*A*) The effect of TFIIE in basal transcription was determined in the absence of TFIIH as described in *Materials and Methods*, by using the templates indicated. Transcription was in the absence (lanes 1 and 3) or presence of recombinant TFIIE (lane 2; 2 ng, and lane 4; 4 ng each of E56/E34) or mutant (mt) TFIIE (lane 5; 4 ng each of E34 and mt E56; ref. 13). Primer extension products of the transcripts are indicated by an arrowhead (G6TI) and a bracket (E4). (*B*) The effect of TFIIE on TBP binding to AdML 2 and E4 promoter DNA in DNase I-footprinting analyses on the nontranscribed DNA strand (lanes 1–4 and 11–13) and the transcribed DNA strand (lanes 5–10). Proteins in the binding reactions are indicated on the top, including "no protein" (lanes 4, 5, and 11). Ten nanograms each of TBP and TFIIA and 8 ng each of E56 and E34 were used. Nucleotide positions on the promoter relative to the start site of transcription of the AdML 2 promoter are numbered, and protected regions against DNase I digestion over the TATA box are indicated by brackets. The protection indicated by an asterisk in lane 8 corresponds to the cryptic TATA sequence further upstream of the TATA box. TATA box ("TATAAAA") is indicated by a bar.

TFIIE Directly Recruits TBP to the TATA Box. Because zinc-finger motifs often play a role in DNA binding, we tested the DNA-binding activity of TFIIE by using DNase I-footprinting assays (Fig. 1*B*). We used the E4 promoter and the AdML 2 promoter as a template because it is one of the best studied promoters for TBP binding and was shown to be stimulated by TFIIE in the absence of TFIIH (Fig. 1*A*) (22, 23). In these experiments, a low concentration of TBP was used that is insufficient to give high occupancy of the TATA box (Fig. 1*B*, lanes 1, 6, and 12). Although TFIIE by itself does not bind and protect a specific region at the promoter (Fig.1*B*, lane 9), we found that TFIIE substantially enhances TBP binding to the TATA box region in the absence of other basal factors (Fig. 1*B*, lanes 3, 7, and 13). The ability of TFIIE to increase or stabilize the binding of TBP to promoter DNA is not restricted to the AdML promoter but also can be seen with the E4 promoter, consistent with the transcription result (Fig. 1*A*). This activity of TFIIE is reminiscent of the ability of TFIIA to enhance and stabilize the binding of TBP to DNA (Fig. 1*B*, lanes 2 and 8). However, unlike TFIIA, which can promote TBP binding to AT-rich sequences that mimic weak or cryptic TATA elements (Fig. 1*B*, lane 8, indicated by an asterisk), the enhancing effect of TFIIE on TBP binding was restricted to the authentic TATA box (Fig. 1*B*, cf. lanes 7 and 8). This indicates that the recruitment effect of TFIIE is highly specific for the core promoter. Moreover, unlike TFIIA, which extends protection to sequences upstream of the TATA box (17), TFIIE does not change the boundaries of the DNase-protected region of the canonical TBP footprint (compare the protections indicated by brackets in Fig. 1*B*). Given these properties of TFIIE, it is not difficult to see why enhancement of TBP/DNA binding may have been overlooked because the footprint pattern of TBP is not qualitatively altered in the presence of TFIIE. Furthermore, despite the striking effect of TFIIE on DNase I footprinting, no TBP/TFIIE complex bound to DNA can be detectable in gel-shift assays (data not shown). Together these results reveal that TFIIE can function in a manner analogous to, but somewhat distinct from, TFIIA in influencing the binding of TBP to DNA and suggests that TFIIE can participate in an early step during PIC assembly even before RNA pol II/TFIIF or TFIIH come into play.

E56 but not the Zinc-Finger Mutant Subunit Enhances TBP Binding to the Promoter. To dissect this identified activity of TFIIE, the two subunits of TFIIE (E56 and E34) were tested separately in DNase I-footprinting assays (Fig. 2*A*). In the presence of a limiting amount of TBP, E56 is sufficient to stimulate TBP binding to the TATA box, resulting in full protection (cf., lanes 1, 2, and 4). By contrast, E34 had no effect on TBP binding (lane 3). Thus, the E56 subunit appears to be primarily responsible for this activity of TFIIE. In UV-crosslinking experiments, we failed to detect any evidence for direct E56/DNA contacts, although anti-E56 antibodies precipitated TBP crosslinked to DNA in the presence of E56 (data not shown).

Because the E56 subunit of TFIIE alone is sufficient to mediate TBP recruitment (Fig. 2*A*), we tested whether the zinc-finger motif was required for this activity (Fig. 2*B*). Equivalent amounts of purified wild-type and mutant E56 proteins were added to DNase I-footprinting assays in the presence of TBP. As expected, wild-type E56 enhanced the binding of TBP to the TATA element of the AdML 2 promoter (Fig. 2*B*, lanes 3 and 4). By contrast, the zinc-finger mutant E56 failed to promote efficient TBP binding to the TATA box (Fig. 2*B*, lanes 5 and 6). Serial titration assays indicate that the activity of mutant E56 is ≈10–20% of wild type (data not shown). These results suggest that the zinc-finger domain is important for TBP recruitment and could account for the transcriptional defect ascribed to the zinc-finger mutant.

To analyze the binding of E56 in more detail, hydroxyl radical footprinting was performed (Fig. 2*C*). TBP induces a

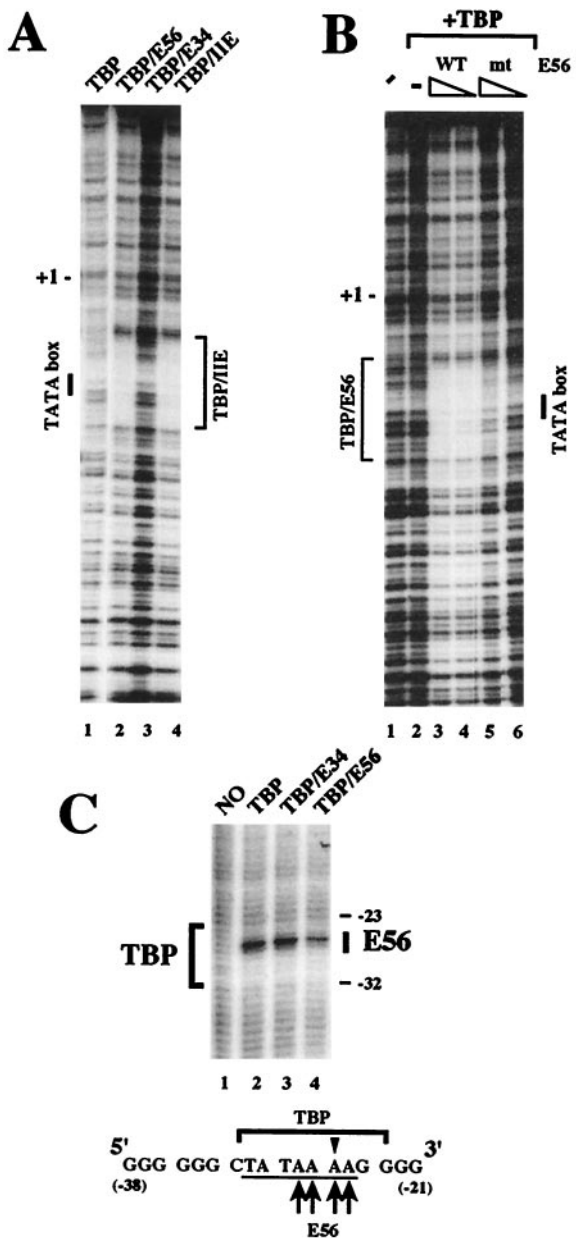

FIG. 2. E56 promotes cooperative binding of TBP to the TATA element. (*A*) Analyses of the effect of the individual subunits of TFIIE on TBP binding to the AdML promoter by DNase I-footprinting assays. TBP (10 ng), E56 (8 ng), and E34 (8 ng) were used in the binding reactions as indicated on the top. The protection over TATA box is indicated by a bracket. The transcription start site is +1. (*B*) DNase I-footprinting analysis of the effect of a zinc-finger mutant of E56 on TBP binding to the promoter region. The assays were carried out in the presence of a constant amount of TBP (6 ng) (lanes 2–6) with 8 ng and 4 ng of either the wild-type E56 (lanes 3 and 4) or the zinc-finger mutant E56 (lanes 5 and 6). Lane 1 is in the absence of any protein. The protected region is indicated by a bracket. (*C*) Analysis of E56 and TBP binding to the AdML promoter in hydroxyl radical-footprinting assays. DNA was incubated with no protein (lane 1) or TBP (10 ng, lanes 2–4) in the absence of other proteins (lane 2), or in the presence of E34 (8 ng, lane 3) or E56 (8 ng, lane 4) before treatment with hydroxyl radicals. The area over the TATA box protected by TBP is indicated by a bracket (nucleotide position −23 to −32), and further protection caused by the presence of E56 is indicated by a bar (lane 4, −25 to −28). [These experiments were repeated four times.] Corresponding sequences are shown underneath, indicating the TBP protection (a bracket) and the change of protection by E56 (arrows). The hypersensitive band created by TBP also is indicated by an arrowhead (nucleotide −26). The TATA box is underlined.

hypersensitive site in the middle of the TATA box that corresponds to the kink in the DNA generated upon TBP binding (2, 4) (lanes 2–4, indicated by the arrowhead on the sequence). Upon addition of E56, there was a reproducible 15–20% reduction in the hypersensitive site created by TBP as well as minor changes in the overall pattern of protection surrounding it (lane 4, indicated by a bar, corresponding sequences indicated by arrows underneath). E56 by itself in the absence of TBP had no effect (data not shown) similar to the DNase I-footprinting result (Fig. 1*B*, lane 9). Consistent with the DNase I footprinting (Fig. 2*A*), E34 had no affect on TBP (Fig. 2*C*, lane 3). Taken together, these results suggest that E56 causes some changes in TBP binding to the TATA box either directly by contacting DNA within the TATA sequence when complexed with TBP and/or indirectly by inducing some conformational changes in TBP.

E34 Helps E56 and TFIIA Promote TBP Binding to the TATA Box. Interestingly, when a suboptimal amount of E56 was present in the DNase I-footprinting assay, E34 was found to help E56/TBP recruitment to the promoter (Fig. 3*A*, cf. lanes 3 and 4).

The ability of TFIIE to enhance TBP binding to DNA raises the possibility that TFIIE may be able to participate in an early step of PIC assembly. We therefore decided to test whether TFIIE also could influence the activities of TFIIA and TFIIB to promote TBP binding to the promoter (Fig. 3*B*). TFIIE significantly enhances cooperative DNA binding of TFIIA/TBP to the AdML 2 promoter (lane 4). A similar effect also was observed with the E4 promoter (data not shown). The enhancement of TBP binding to the promoter by TFIIA and TFIIE is highly synergistic, whereas a minor cooperative effect was observed in the case of TFIIB and TFIIE (cf. lanes 6, 7, and 8). Interestingly, the E34 subunit of TFIIE (without E56) stimulated cooperative DNA binding of a TBP/TFIIA complex (lane 5), reminiscent of its effect on the binding of a TBP/E56 complex to the promoter (Fig. 3*A*). Similarly, E34 enhanced the TBP/TFIIA binding to DNA but not TBP/TFIIB in the gel-shift assay (data not shown). The slight changes of the protection patterns were observed at the upstream side of the TBP footprint in the presence of all three factors TBP, TFIIA, and TFIIE compared with TBP/TFIIA or TBP/TFIIE as indicated by the open arrowheads (Fig. 3*B*, cf. lanes 4 and 5; also see Fig. 1*B*, lane 2). These changes probably reflect the interplay of three factors, TBP, TFIIA, and TFIIE occurring on the DNA at the upstream side of the TATA box.

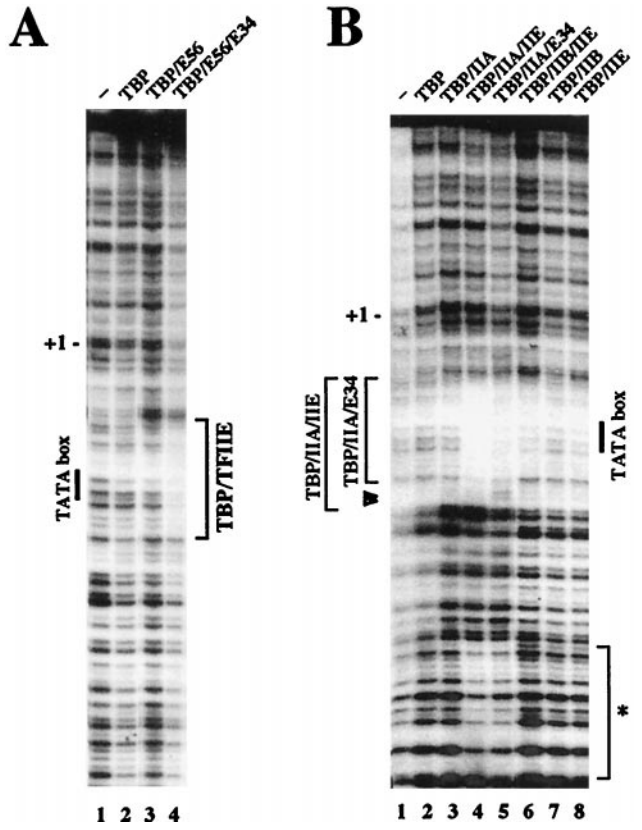

FIG. 3. The effect of E34 on TBP/E56 and TBP/TFIIA binding to the promoter. (*A*) Analysis of the effect of E34 on promoter binding by E56 and TBP by DNase I-footprinting assays. DNA was incubated with either no protein (lane 1) or 6 ng of TBP alone (lane 2–4) in the presence of 3 ng of E56 (lane 3) or 3 ng each of E56 and E34 (lane 4). The protection is indicated by a bracket. (*B*) The effect of TFIIE and E34 on TBP/TFIIA and TBP/TFIIB binding to the promoter in DNase I-footprinting analysis in the presence of limiting amounts of TBP (5 ng), TFIIA (3 ng), TFIIB (3 ng), and TFIIE (2 ng of E56 and E34). Lane 1 is no protein. Proteins in the binding reactions are indicated on the top. Open arrowheads indicate two bands that are present in the presence of TBP/TFIIA/E34 (lane 5; also see Fig. 1*B*, lane 2) but disappeared upon the addition of E56 (lane 4). The TFIIA/TBP-dependent protection of a cryptic TATA sequence is indicated by a bracket with an asterisk (lanes 4 and 5).

E34 Has a Nonspecific DNA-Binding Activity and Directly Interacts with TFIIA. Using chemical-crosslinking reagents, Robert *et al.* (24) recently reported that, in the context of a complete PIC, E34 can be specifically crosslinked to the −14- to −2 region of the AdML promoter. Taking this data together with the result obtained above (Fig. 3), we decided to investigate the potential DNA-binding properties of E34 (Fig. 4*A*). UV-crosslinking and immunoprecipitation experiments by using the AdML 2 (−72 to +8) promoter fragment revealed that E34 alone has DNA-binding activity (Fig. 4*A*, lane 1). Because the labeled DNA fragment was gel-purified before the assay, E34 most likely binds to double-stranded DNA. This result is in contrast to the recent report of the single-stranded DNA-binding activity of the yeast small TFIIE subunit (25). Competition experiments with the TATA box sequences did not affect E34 DNA binding, suggesting that E34 binding is TATA box-independent (data not shown). Furthermore, there were no apparent differences in the efficiency of E34 binding to sequences with and without the −14 to −2 region at which E34 was found to be crosslinked in a complete PIC (24) (data not shown). Taken together with our footprinting studies, these results suggest that E34 is a DNA-binding protein of low sequence specificity.

Because E34 further stimulated the ability of TFIIA to recruit TBP to DNA, we tested whether E34 directly contacts TFIIA (Fig. 4*B*). *In vitro* protein interaction assays revealed that the large subunit of TFIIA (TFIIAα β) fused to GST specifically interacts with E34 but not, for example, TFIIB (Fig. 4*B*, lanes 1–6). No significant interactions between TFIIAα β and E56 could be detected in our assays (Fig. 4*B*, lanes 7–9). The reciprocal experiment by using flag-tagged E34 confirmed that E34 interacts with the large subunit of TFIIA (Fig. 4*B*, lanes 10–15). The interaction between E34 and TFIIA appears to be as efficient as the interaction between E34 and E56 under our assay conditions. Similar results were obtained with *Drosophila* TFIIA (data not shown), indicating that the interaction domains between E34 and TFIIA are conserved. Furthermore, E34 is capable of interacting with the holo-TFIIA complex (Fig. 4*B*, lanes 16–18). Taken together, these data indicate that E34 stimulates the promoter binding of E56/TBP and TFIIA/TBP complexes through direct protein–protein and protein–DNA interactions.

DISCUSSION

Previous studies provided strong evidence for a role of TFIIE at a late step of PIC assembly in the recruitment of TFIIH and its subsequent involvement in promoter melting and clearance. We report here a previously undetected activity of TFIIE that

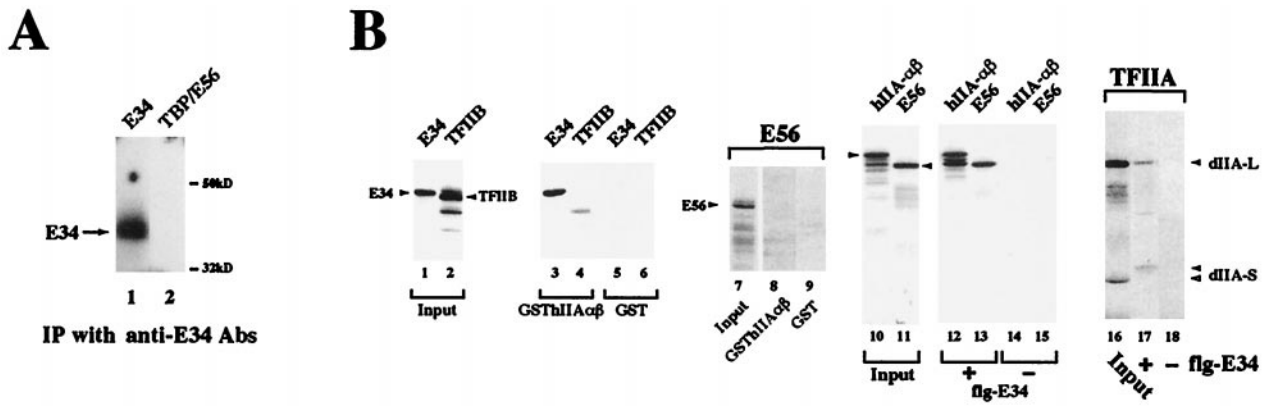

FIG. 4. Detection of E34 interactions with DNA and TFIIA. (*A*) Detection of E34 and DNA interactions by UV-crosslinking experiments by using AdML 2-promoter region (−72 to +8) as a template. E34 protein alone (lane 1) and TBP/E56 (lane 2) were subjected to crosslinking DNA and immunoprecipitated by anti-E34 antibodies. (*B*) Detection of the direct interaction of E34 with the large subunit of TFIIA *in vitro* protein interaction assays. Five percent of the input ^{35}S-labeled proteins were shown. In lanes 1–9, proteins indicated on top of the lanes were mixed with GST-hTFIIA$\alpha\beta$ [(lanes 3, 4, and 8) or GST protein (lanes 5, 6, and 9)] on glutathione Sepharose beads. In lanes 10–18, *in vitro* translated ^{35}S-labeled proteins indicated on top of the lanes were incubated with either flag-tagged E34 protein bound to M2 beads (lanes 12, 13, and 17) or M2 beads alone (lanes 14, 15, and 18). Full-length proteins are indicated by arrowheads. The 14-kDa subunit of TFIIA (indicated by a closed arrowhead) migrates aberrantly (indicated by an open arrowhead) in the input because of the comigrating hemoglobin in the rabbit reticulocyte lysate.

directly influences the promoter DNA binding of TBP and TBP/TFIIA, the first step of PIC assembly, in the absence of other basal factors. Importantly, the zinc-finger domain of the E56 subunit is critical for this activity, and E34 indirectly helps TBP binding to the promoter by stabilizing the complex on DNA via specific protein–protein interactions between E34 and TFIIA as well as nonspecific binding of E34 to DNA.

Alternative PIC Assembly Pathways. Based on these observation, we propose that TFIIE may participate as an integral component of the PIC at an early step (Fig. 5*A*). It also is possible that the interactions between TFIIE and TBP on DNA may either enhance the stability of the PIC or induce conformational changes of the TBP/DNA complex affecting later steps of transcription. Consistent with the first possibility, the zinc-finger mutant failed to incorporate into a stable PIC in the presence of TFIIF and Pol II (13). Crosslinking of E34 adjacent to the transcription start site in the context of a complete PIC (24) suggests that upon assembly of a complete PIC, there may be dynamic changes in the interaction between E56, E34, or TBP and DNA, allowing E34 to make an additional contact with the downstream region (e.g., DNA bending induced by the PIC formation; Fig. 5*B*). Alternatively, the TBP/IIE association may occur only transiently at the beginning of PIC formation and then dissociate upon assembly of the entire complex. Although we failed to obtain evidence for direct DNA binding by E56, the opposite face of the TATA-binding sequence could be involved in additional protein–DNA interactions.

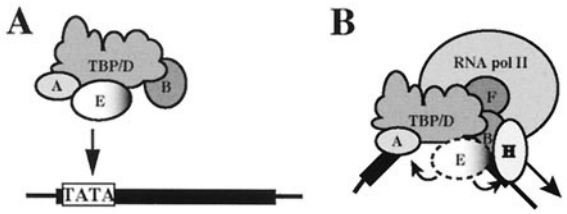

FIG. 5. An alternative model for the assembly of a PIC. These models are different from the conventional stepwise assembly pathway in which TFIIE together with TFIIH are the last basal factors to enter the PIC. (*A*) TFIIE participates in the initial binding of TBP/TFIID to the promoter before the recruitment of the RNA pol II/TFIIF complex and TFIIH. It is pertinent to note that a role of TFIIE during the first steps of PIC assembly does not preclude a role during TFIIH recruitment (see *Discussion*). (*B*) To recruit TFIIH, TFIIE may either shift to alternate positions within the PIC or may contact both TBP/TFIIA and TFIIH at the same time, possibly accompanied by conformational changes of DNA upon PIC assembly (e.g., DNA bending by TBP/TFIID). This model illustrates the potential dual role of TFIIE in PIC formation and does not attempt to accommodate distinct RNA pol II complexes that have been described.

TFIIH-Dependent and Independent Activities of TFIIE. In addition to the recruitment of TFIIH, it was reported that TFIIE and TFIIH are involved in promoter melting and clearance after PIC assembly (23, 26), and TFIIE can stimulate the CTD-kinase and ATPase activities of TFIIH, suggesting that TFIIE and TFIIH are close functional partners in transcription (27, 28). However, recent evidence suggests that TFIIE also may have a transcriptional activity independent of TFIIH. First, the stimulatory effect of TFIIE on transcription is significant even in the absence of TFIIH under certain conditions (Fig. 1*A*) (23). Second, the minimal functional domain of E56 was mapped to the N-terminal half that contains a zinc-finger but lacks the putative TFIIH-binding domain (14, 25, 29), suggesting that the interaction with TFIIH is not sufficient and may not be necessary for TFIIE function under certain conditions. It is interesting to note that the characterization of RNA pol II transcription factors from rat liver revealed that coincubation of TBP and TFIIE is required for efficient template commitment (30). It also was reported in yeast that some mutations in E56 (TFA1) that affect TATA-dependent transcription, do not affect promoters lacking a typical TATA sequence, suggesting a TATA box-dependency of TFIIE function (29). Taken together, these studies are consistent with our finding that TFIIE has an ability to specifically help TBP binding to the TATA box in a zinc-finger-dependent manner. This activity of TFIIE, however, is most likely not mutually exclusive with the cooperative interaction between TFIIE and TFIIH during promoter melting and clearance. By isolating conditional mutants, Kuldell and Buratowski (25) identified two genetically distinct functional domains in yeast E56 (TFA1), a zinc-finger region and the C-terminal region, correlating with the two distinct TBP and TFIIH recruitment activities of TFIIE, respectively. Therefore, there appears to be at least two activities carried out by TFIIE during PIC formation, and both activities are likely to be important for transcription of certain genes.

Role of TFIIE in Transcriptional Activation. The binding of TBP/IID to the promoter is thought to be a critical and in some cases a rate limiting step in PIC assembly. One potential mechanism for transcriptional activation is to increase the

efficiency of this step (2, 4, 7, 31). Many factors influence TBP-promoter interactions, including TFIIA (17, 32, 33), TFIIB (32), TAFs (see refs. 2, 4, 6), and TFIIE (data not shown). In addition to interactions between TFIIE, TBP. and TFIIA, an integral subunit of TFIID ($hTAF_{II}80$, a homolog of $dTAF_{II}60$) was recently shown to interact with E56 (34). E56 also was identified as a potential substrate for the $TAF_{II}250$ kinase (35). Together with the previous observations that TFIIE can interact with both TBP and the holo-TFIID complex *in vitro* (12), these findings suggest that TFIIE is likely to make multiple contacts with components of the TFIID complex and, therefore, may actively participate in transcriptional activation through interactions with TFIID. In fact, in the presence of activators, the cooperation of TFIIA and TFIIE in directing transcription is even more pronounced (K.Y. and R.T., unpublished data). Although TBP/TFIID has been identified as a major target of certain activators, several studies revealed that other basal factors also may serve as targets for certain activators, including TFIIA, IIB, IIF, and IIH (36–42). Similarly, TFIIE was reported to be a direct target for Jun/Fos (38), the homeodomain proteins Abd-B (Abdominal-B) and Antp (antennapedia) (43), EBNA2 (Epstein–Barr virus nuclear antigen 2) cofactor P100 (44), and a repressor, Krüppel (40). In light of the results reported here, it will be interesting to determine how TFIIE contributes to the activation or repression of transcription initiation by enhancer binding factors.

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