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Stimulation of the XPB ATP-dependent helicase by the beta subunit of TFIIE

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ABSTRACT

TFIIE and TFIIH are essential for the promoter opening and escape that occurs as RNA polymerase II transits into early elongation. XPB, a subunit of TFIIH, contains an ATP-dependent helicase activity that is used in both of these processes. Here, we show that the smaller beta subunit of TFIIE stimulates the XPB helicase and ATPase activities. The larger alpha subunit can use its known inhibitory activity to moderate the stimulation by the beta subunit. Regions of TFIIE beta required for the helicase stimulation were identified. Mutants were constructed that are defective in stimulating the XPB helicase but still allow intact TFIIE to bind and recruit XPB and TFIIH to form the pre-initiation complex. In a test for the functional significance of the stimulatory effect of TFIIE beta, these mutant forms of TFIIE were shown to be defective in a transcription assay on linear DNA. The data suggest that the beta subunit of TFIIE is an ATPase and helicase co-factor that can assist the XPB subunit of TFIIH during transcription initiation and the transition to early elongation, enhancing the potential diversity of regulatory targets.

INTRODUCTION

Eukaryotic class II transcription requires at least 30 polypeptides for basal transcription and many more are needed for properly regulated transcription. These polypeptides include RNA polymerase II and components of the general transcription factors. TFIID and TFIIB recruit the polymerase and TFIIF to the promoter, and other factors modify the transcription complex to allow it to proceed into early elongation phase (1–3). In the step-wise model of assembly, TFIIE and TFIIH are added immediately after polymerase (4) and are used in the opening of the DNA and in the initial steps of RNA synthesis, including promoter escape (5–10). TFIIE and TFIIH are essential proteins *in vivo* (11,12). *In vitro*, TFIIE and TFIIH are required for these transcription steps, although their function can be partially bypassed on some supercoiled templates (13).

TFIIE is composed of two subunits, a larger 56 kDa subunit TFIIEα and a smaller 34 kDa subunit TFIIEβ (14,15). The TFIIE heterotetramer ($\alpha_2\beta_2$) has no known catalytic activity and recruits TFIIH to complete the assembly pathway (4,8,16,17). TFIIH is a large complex with 10 subunits and several enzymatic activities, including ATP hydrolysis, energy-dependent DNA unwinding and phosphorylation (18–24). Two subunits of TFIIH, XPB and XPD, can accomplish DNA unwinding, although the polarity of the process is different for each (20–22). The XPD ATP-dependent helicase is used to open DNA during repair, and its activity within TFIIH is strong owing to the activation by its partner subunit p44 (25,26). The XPB ATP-dependent helicase is used to open the promoter DNA during transcription and is also used in promoter escape (27–31). Its helicase activity within TFIIH is weaker and its p52 partner subunit does not enhance this activity (32). XPB and XPD have other roles that appear to be independent of helicase action. The ATPase activity of XPB is needed for the activation of the XPG endonuclease during nucleotide excision repair (33). XPD provides structural integrity to TFIIH (25,26), especially to the CAK kinase complex that is involved in the transition from transcription initiation to elongation (16,19,24,26,28,34–36) and other cellular processes (17,37).

Although TFIIE has no catalytic activities (14,15), it can influence those of TFIIH (16,24,36,38). It has been reported to positively regulate the ATPase and kinase activities of TFIIH (19) and negatively regulate its helicase activity (39). The negative regulation is specific to the XPB helicase (39). From these observations, it has been suggested that TFIIE inhibits the ability of TFIIH to open promoter DNA until the complete complement of general transcription factors is assembled, at which time this constraint may be relieved. Overall, TFIIE and TFIIH likely work together to modify the pre-initiation complex so as to allow the RNA polymerase to function in transcription initiation and to transit to early

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elongation. During this critical transition process, the mRNA becomes capped and TFIIH begins to catalyze the staged series of changes in the RNA polymerase CTD that sets the stage for coupled elongation and RNA processing events. Although TFIIE is required for this transition (19,36), its role has not been clearly established. The role of the beta subunit is of particular interest, as its C-terminus is crowded with interaction domains for other general transcription factors (36,38).

Both the alpha and beta subunits of TFIIE can interact with XPB (35) and it has been suggested that the alpha subunit is dominant for the inhibition of the XPB function (39). No specific role has emerged for the interaction of the beta subunit with XPB, although the possibility has been raised that it functions in the transition into early elongation phase (36,38). Because promoter opening and escape require the XPB ATPase and helicase activities (5,28,31,40), we have investigated the possible role of the TFIIE beta subunit in modulating these activities. Below, we show that the isolated beta subunit can stimulate the XPB helicase and ATPase activities and that such stimulation has a functional role in transcription.

MATERIALS AND METHODS

Protein expression and purification

The human *XPB* (*ERCC3*) clone was a gift from the J. -M Egly laboratory. Glutathione *S*-transferase (GST)-tagged XPB was expressed and partially purified as described previously (22,41) with the following modifications. An aliquot of 400 ml of *Escherichia coli* cells were induced with 0.1 mM of isopropyl-β-D-thiogalactopyranoside (IPTG) for 30 min at 37°C. The cell pellets were collected and re-suspended in 10 ml of 1× phosphate-buffered saline (PBS) with 1% Triton™ X-100. An aliquot of 150 µl of Glutathione Sepharose 4B was used in the batch purification protocol as described previously (Amersham Pharmacia Biotech). Each wash used 10 ml of 1× PBS with 1% Triton™ X-100 for a total of four washes. The XPB was eluted with 50 mM Tris–HCl (pH 7.9), 10 mM reduced glutathione and 50% glycerol. The eluted sample was dialyzed against 50 mM Tris–HCl (pH 7.9) and 50% glycerol to remove the reduced glutathione. GST was expressed and purified the same way.

Human TFIIEα and TFIIEβ clones were obtained from Dr K. Yokomori. Human TFIIEβ, its C-terminal truncated mutants (stop codons were placed accordingly using QuikChange™ site-directed mutagenesis kit from Stratagene) and point mutants with His_6 tagged were expressed in 4–500 ml of *E.coli* BL21(DE3) cells in 0.3 mM IPTG, 2% glucose and Luria–Bertani for 1 h at 37°C after OD_{600} nm reached 0.5. Ni-based resin (Qiagen and Active Motive) was used for purification (see Qiagen's manual for purification procedures and solutions). Proteins were analyzed on 12% Tris/Glycine SDS–PAGE with the correct molecular weights and were >95% pure. The proteins contained low but detectable helicase activity.

Human TFIIEα with a His_6 tag was a gift from Hao Duong (UCLA, Department of Chemistry and Biochemistry). Human TFIIEα and N-terminal truncated mutants with an N-terminal FLAG tag were cloned into pET11a (Novagen). They were expressed the same way as TFIIEβ (see above) and were purified using ANTI-FLAG® M2-Agarose (Sigma, see the manufacturer's purification procedures and solutions). The proteins were eluted with FLAG peptide. They were analyzed on 10% Tris/Glycine SDS–PAGE and were >95% pure.

Human TFIIH was purchased from Protein One (http://www.proteinone.com).

Helicase and ATPase assays

Helicase assays were as described previously (21,22,39). Briefly, 20 ng of ^{32}P-labeled oligonucleotide (5′-AA-ACGACGGCCAGTGAATTCGAGCTCGGTACCCGG-3′) complementary to M13mp19$^+$ was annealed to 1 µg of the single-stranded plasmid DNA in 25 mM NaCl and 2.5 mM $MgCl_2$. The duplex substrate was then purified in a Sephadex 200 size exclusion column. An aliquot of 400–500 ng of duplex was recovered free of un-annealed labeled oligonucleotide. Helicase assays for XPB and TFIIEβ or its mutants were incubated for 30 min at 37°C in the presence of 4 mM $MgCl_2$, 4 mM ATP, 50 µg/ml acetylated BSA, 1 mM DTT, 20 mM Tris–HCl (pH 7.9) and 2–3 ng of DNA substrate in 25 µl of reaction volume. For TFIIH, a 3′–5′ XPB-specific substrate was used. This substrate was generated from a ^{32}P-labeled 19 nt long complementary oligonucleotide annealed to a 49 nt long oligonucleotide, resulting in a 5′ blunt end (with respect to the longer oligonucleotide) partial duplex DNA. The reactions were stopped and run on 12% PAGE in 1× TBE.

ATPase assays were as described previously (19,22). The conditions were similar to the helicase assay above, except that 1 µM of [γ-^{32}P]ATP was added instead of 4 mM ATP, reaction volume was 12.5 µl, and the reaction times reduced to 20 min. For the XPB and TFIIH ATPase assay, 10 ng of single-stranded DNA and 50 ng of plasmid DNA were added, respectively, unless otherwise specified. The reactions were stopped and run on PEI-TLC plates in 0.75 M potassium phosphate (pH 4.0). All signals were detected by PhosphorImager and analyzed by ImageQuant 5.2 software.

Co-immunoprecipitation (CoIP) and template pull-down assays

An aliquot of 200 µl of bacterial lysate (~2 µg total protein/µl) containing expressed TFIIEα or its N-terminal truncation mutants was incubated with 200 µl of bacterial lysate (~2 µg total protein/µl) containing expressed TFIIEβ for 45 min at 4°C. An aliquot of 20 µl of Ni-NTA resin was added to the mixture to bind TFIIEβ for an additional 45 min. Resins were washed twice with 400 µl of 20 mM Tris–HCl (pH 7.9) and 100 mM NaCl and eluted with 2× SDS buffer [100 mM Tris–HCl (pH 6.8), 200 mM DTT, 4% SDS, 0.2% bromophenol blue and 20% glycerol]. The samples were resolved by 10% Tris/Glycine SDS–PAGE and detected through western blot using Anti-FLAG M2 peroxidase conjugate (Sigma).

For TFIIEα and TFIIEβ CoIP, 130 µg of FLAG tagged TFIIEα non-expressed or expressed bacterial lysate were mixed with 25 µl of anti-FLAG resin for 45 min at 4°C. Resin was washed as above. An aliquot of 200 µl of 20 mM Tris–HCl (pH 7.9) and 100 mM NaCl and 1 µg of purified TFIIEβ or its mutants were added to the spun down resin and further mixed for 45 min at 4°C. Resins were washed and eluted with 2× SDS buffer. The samples were resolved by

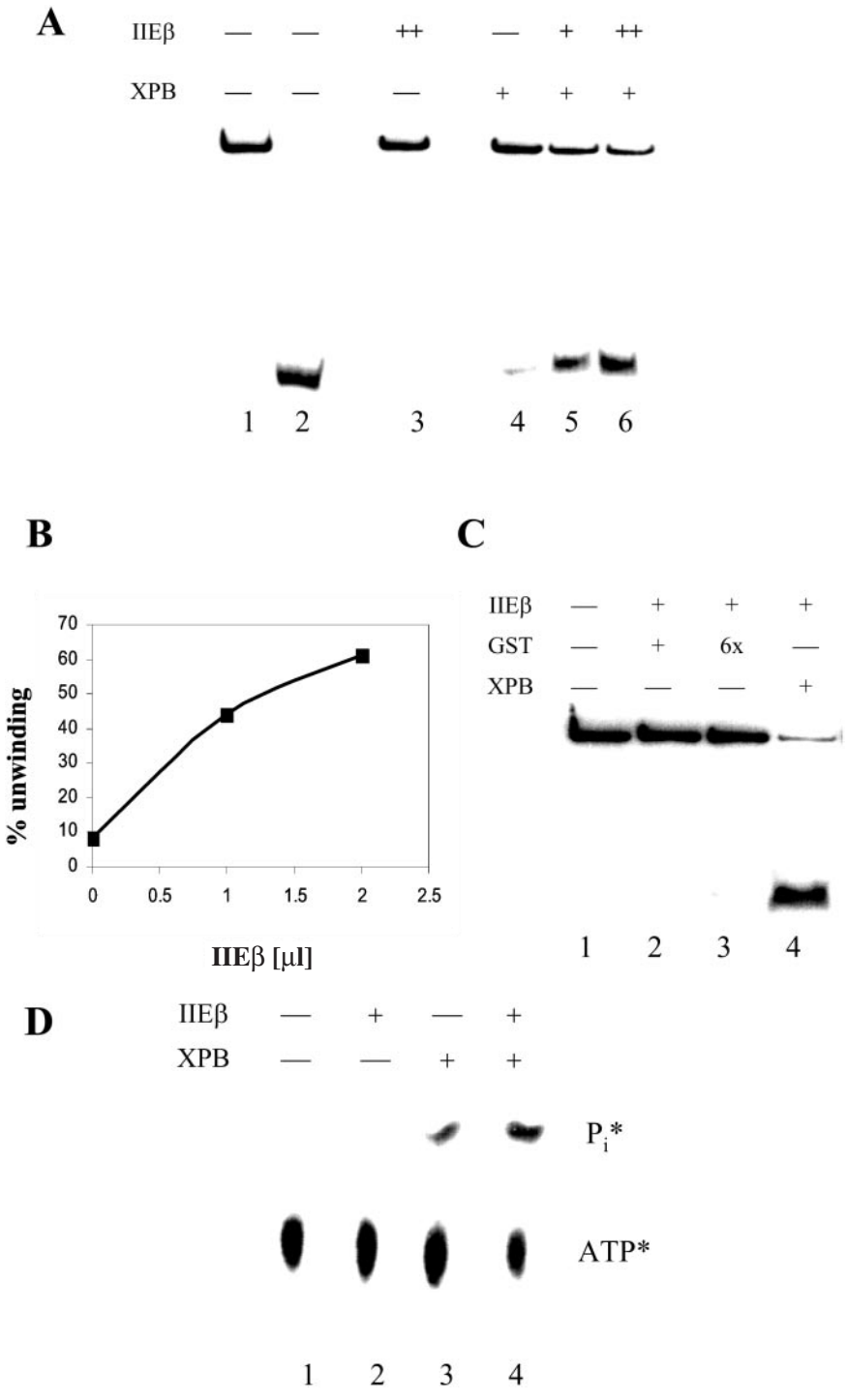

Figure 1. TFIIEβ stimulates XPB helicase activity. (**A**) Helicase assay. Lane 1, substrate alone; lane 2, substrate heated for 2 min to unanneal radioactively labeled oligonucleotide; lane 3, 0.8 μg TFIIEβ was added; lane 4, 2.4 μg of XPB was added; lanes 5 and 6, 0.4 and 0.8 μg of TFIIE were added to 2.4 μg of XPB, respectively. (**B**) A graphical representation of lane 4–6 in (A). (**C**) Helicase assay. Lane 1, substrate alone; lane 2, 0.8 μg of TFIIEβ and 2.4 μg of GST were added; lane 3, 0.8 μg of TFIIEβ and 11 μg of GST were added; lane 4, 0.8 μg of TFIIEβ were added to 2.4 μg of XPB. (**D**) ATPase assay. Lane 1, ATP alone; lane 2, 0.13 μg of TFIIEβ was added; lane 3, 0.8 μg of XPB was added; lane 4 contained both 0.13 μg of TFIIEβ and 0.8 μg of XPB. The ATPase stimulation was 1.8 ± 0.4-fold.

10% Tris/Glycine SDS–PAGE and detected through western blot using Anti-TFIIEβ (Santa Cruz Biotechnology). For FLAG-tagged TFIIEβ and His_6 tagged TFIIEβ CoIP, Anti-His peroxidase conjugate (Qiagen) was used in western blot detection.

For XPB and TFIIEβ CoIP, 8 μg of GST or XPB were pre-incubated with 2 μg of TFIIEβ or its mutants on ice for 45 min. Subsequently, 50 μl of glutathione resin (Amersham) and 400 μl of 20 mM Tris–HCl (pH 7.9) and 100 mM NaCl were mixed with the pre-incubated proteins for 45 min at 4°C.

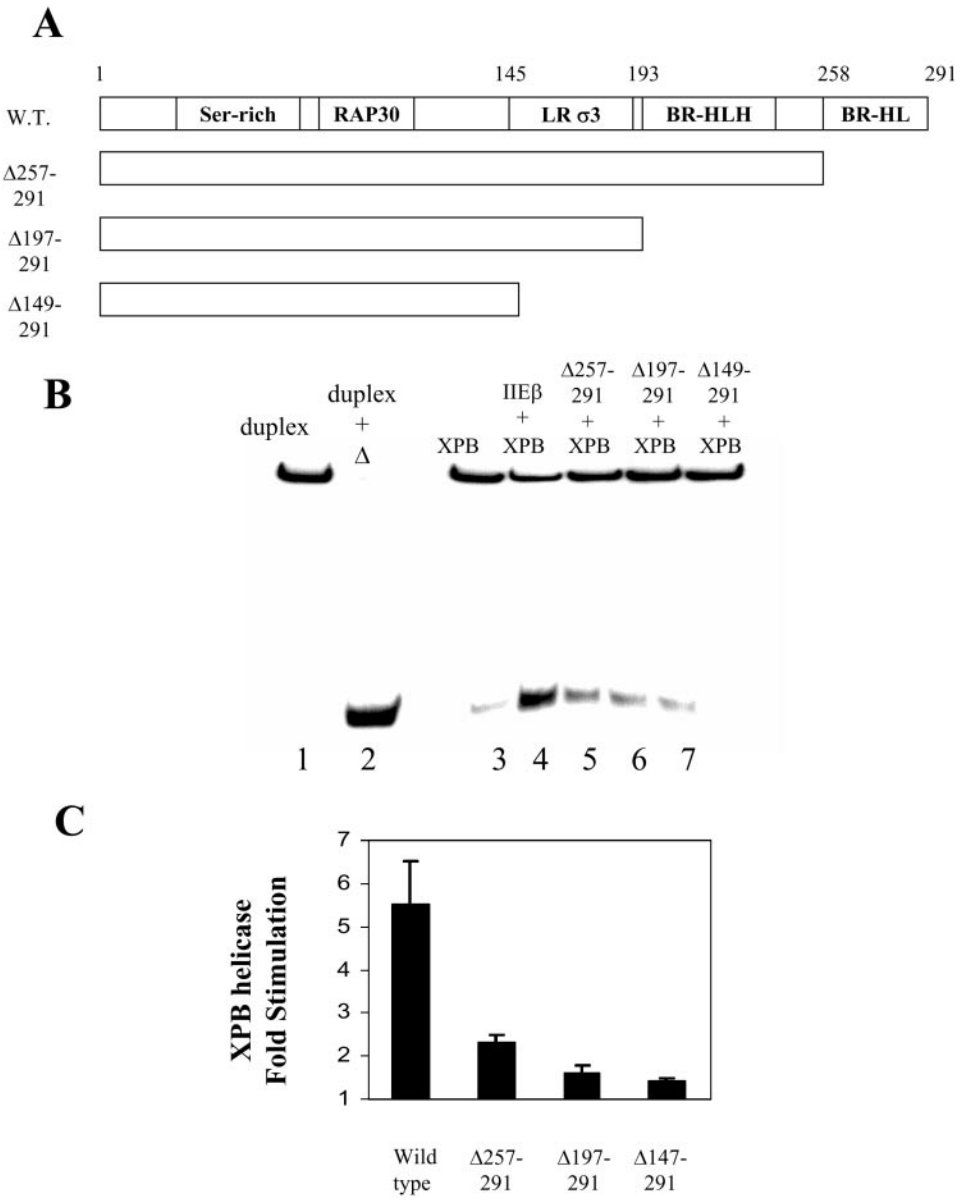

Figure 2. The C-terminus of TFIIEβ is important for the stimulation of XPB helicase activity. (**A**) A diagram of TFIIEβ functional domains (36) and the C-terminal truncation mutants of IIEβ were depicted. (**B**) Helicase assay, conditions as in Figure 1A. Lane 1, substrate alone; lane 2, substrate heated for 2 min; lane 3, XPB was added; lane 4, as in lane 3, except wild-type TFIIEβ was added; lane 5, equimolar of Δ257–291 as in lane 4 TFIIEβ was added to XPB; lane 6, Δ197–291 was added to XPB; lane 7, Δ149–291 was added to XPB. (**C**) A graphical representation of XPB helicase stimulation by wild-type and various TFIIEβ mutants.

Resins were washed and eluted with 2× SDS buffer. The samples were resolved by 10% Tris/Glycine SDS–PAGE and detected through western blot using Anti-TFIIEβ (Santa Cruz Biotechnology).

The pre-initiation complex pull-down assay was performed similarly as described previously (42). Briefly, the 100 µl reaction contained 400 µg of TFIIEβ depleted extract, 7.5 mM $MgCl_2$, 0.05% NP-40, 1 mg/ml of acetylated bovine albumin, 1 µg of pGEM, 20 ng of TFIIEβ or its mutants, 15 ng of TFIIEα, 250 U Gal4-AH (ProteinOne) and the magnetic beads (Dynal) with the biotinylated G9E4 or promoter-less DNA (320 nt long). An aliquot of 0.2 pmol of E4 fragment was also added to each reaction. Incubations were at room temperature for 30 min. The beads were washed twice with 100 µl of Buffer D with 0.5% of NP-40.

In vitro transcription

HeLa nuclear extract (43) was immunodepleted with TFIIE using Protein A-agarose pre-bound with anti-IIE beta antibody (IIEβ C-21 from Santa Cruz Biotechnology). The immunoprecipitation protocol was as described previously (44). *In vitro* transcription was performed as described previously (43). Briefly, the 25 µl reaction contained ~70 µg of immunodepleted extract, 4 mM $MgCl_2$, ~200 ng of linearized AdML fragment with 5 Gal sites and a G-less cassette or 400 ng of linearized E4 fragment with 9 Gal sites, 200 ng of Gal4-VP16 (Protein One), 0.4 mM of ATP, 0.4 mM of CTP and 0.016 mM of [α-^{32}P]UTP (Perkin-Elmer) (and 0.4 mM of GTP was included for E4 transcription). An aliquot of 90 ng of IIEα and 60 ng of wild-type IIEβ or its mutants were allowed to

incubate for 10 min before the addition of other components in the reaction. The reaction was stopped after 60 min at 30°C, phenol–chloroform extracted and ethanol precipitated. Formamide dye was added to the pellet, loaded on to a 6% urea polyacrylamide gel and run for 80 min at 32 W. The signals were detected by PhosphorImager and analyzed by ImageQuant 5.2 software.

RESULTS

TFIIEβ stimulates XPB helicase and ATPase activities

We first tested the effect of the isolated TFIIEβ subunit on the enzymatic activities of the isolated XPB subunit of TFIIH. The results of helicase assays using purified human XPB and TFIIEβ are shown in the autoradiogram of Figure 1. Electrophoresis was used to follow the release of a radioactively labeled oligonucleotide (of length 35 nt) from a partial DNA duplex. The partial duplex is shown in lane 1, and the labeled oligonucleotide, released artificially by heat, is shown in lane 2. The lack of release in lane 3 confirms that TFIIEβ has low intrinsic helicase activity. When XPB is assayed (lane 4), the known weak helicase activity is represented by the low amount of released substrate. When TFIIEβ is added to XPB, there is more released substrate (lanes 5 and 6) showing that TFIIEβ has stimulated the XPB helicase activity. The stoichiometries of TFIIEβ and XPB are 1:2 in lane 5 and 1:1 in lane 6. In Figure 1C, the stimulation of XPB helicase by TFIIEβ is confirmed and shown to be unrelated to the GST tag attached to the recombinant protein.

A titration was used to follow the quantitative stimulation (Figure 1B), which increases with increasing amounts of TFIIEβ. When the amount is raised further, the helicase activity falls (data not shown). This may be related to the prevalence of beta dimers at the higher concentration, as TFIIEβ exists in monomeric and dimeric states (11). At the optimal TFIIEβ concentration the stimulation of XPB helicase activity is 4- to 5-fold under these conditions.

The effect of the isolated beta subunit on the XPB ATPase activity in the presence of DNA was also tested. This activity is needed for helicase action but also may act independently in some circumstances (33,45). The ATPase is assayed by observing the release of radioactive phosphate from gamma-labeled ATP, and Figure 1D shows that XPB (lane 3) but not TFIIEβ (lane 2) has ATPase activity, as expected. When TFIIEβ is added to XPB, its ATPase activity increases ~2-fold (lane 4). We conclude that TFIIEβ can stimulate the helicase and ATPase activities of the isolated XPB subunit of TFIIH.

Domain determinants of helicase stimulation

We used a series of C-terminal deletion mutants (Figure 2A) to learn the region(s) of TFIIEβ that is important for the XPB helicase stimulation. C-terminal deletions were used because the small C-terminal basic region–helix–loop sequence is crowded with potential functions and is needed for transcription (36,38). When this small 35 amino acid region is removed, there is much less stimulation (Figure 2B, lane 5 versus lane 4). The residual helicase stimulation is removed upon further deletion, which also includes the leucine-rich sigma 3 region (lane 7 versus lane 3; summarized in Figure 2C). It appears that a large region of the C-terminus is involved in stimulation.

XPB is in a different context when incorporated into the multi-subunit TFIIH, which contains nine other polypeptides, including its binding partner p52 and another helicase, XPD. We wished to learn whether TFIIEβ would still stimulate XPB helicase activity in this context. To avoid a signal from the XPD helicase activity, an XPB-specific 3′–5′ helicase substrate was used (21,22).

Figure 3A shows that the isolated beta subunits retain the ability to stimulate the XPB helicase in the context of intact TFIIH (compare lane 8 with lane 7 and compare lane 6 with lane 5), although the stimulation is less using this assay (~2-fold). Figure 3B shows that TFIIEβ also stimulates the ATPase activity of TFIIH (lane 4 versus lane 3), as would be expected based on the property of isolated XPB (see above). We conclude that TFIIEβ stimulates the enzymatic activities of XPB, both in isolated form and in the context of TFIIH.

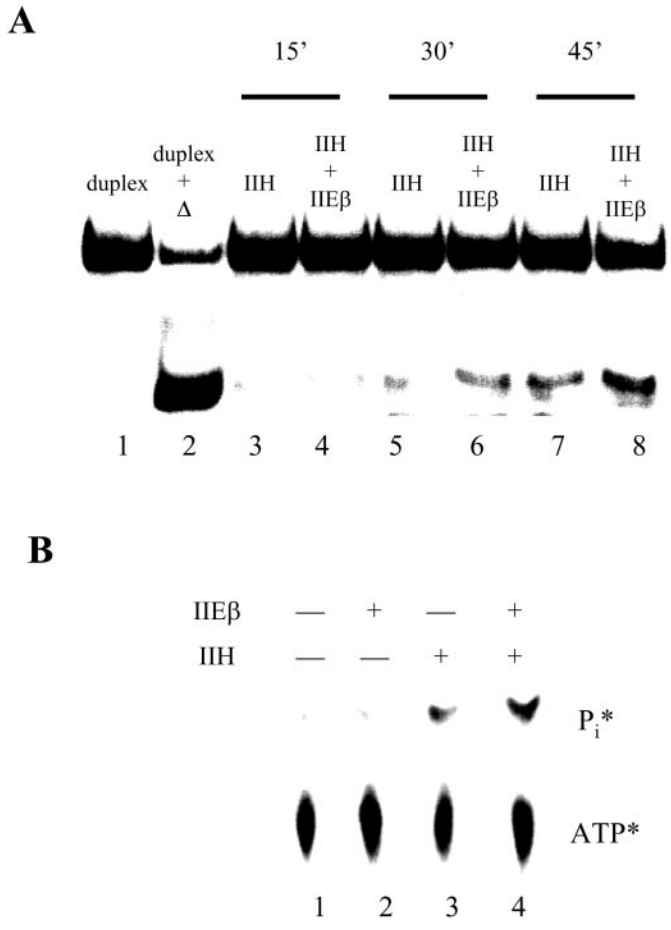

Figure 3. TFIIEβ stimulates TFIIH helicase and ATPase activity. (**A**) Helicase assay. Lane 1, XPB 3′–5′ helicase specific substrate; lane 2, substrate was heated for 1 min; lane 3, 5 and 7 contained 30 ng of TFIIH and the reaction times were 15, 30 and 45 min, respectively; lane 4, 6 and 8 contained 80 ng of TFIIEβ and 30 ng of TFIIH and the reaction times are 15, 30 and 45 min, respectively. (**B**) ATPase assay. Lane 1, ATP alone; lane 2, 80 ng of TFIIEβ was added; lane 3, 60 ng of TFIIH was added; lane 4 contained 80 ng of TFIIEβ and 60 ng of TFIIH. The ATPase stimulation was 2.2 ± 0.4-fold.

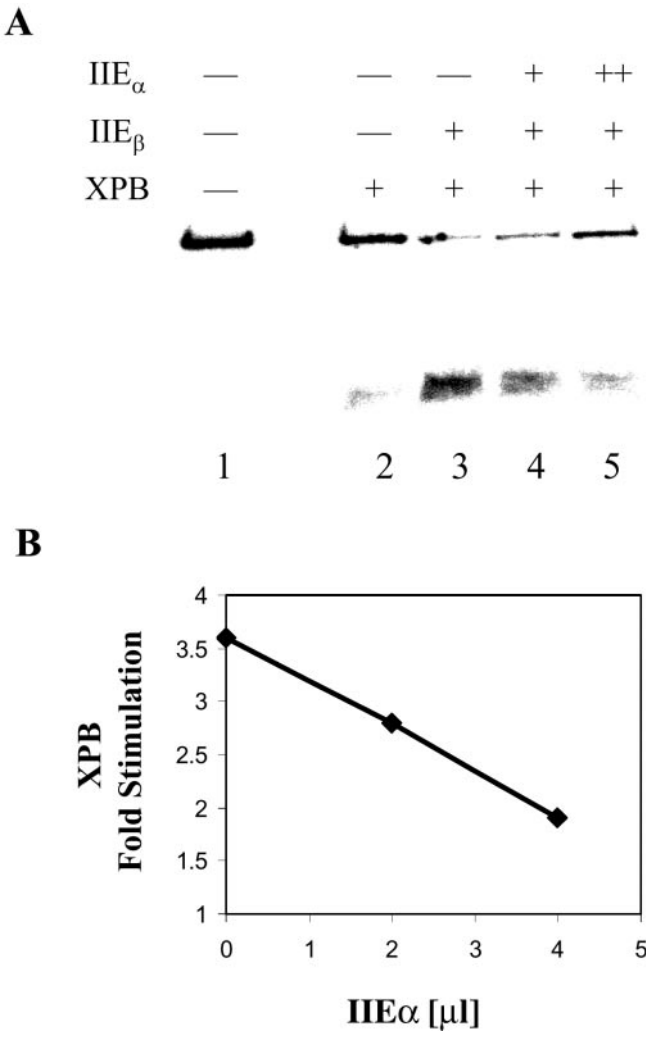

Figure 4. TFIIEα prevents TFIIEβ from stimulating XPB. (**A**) Helicase assay. Lane 1, substrate alone; lane 2, XPB was added; lane 3, 0.8 μg of TFIIEβ and XPB were included. For lanes 4 and 5, 1.6 and 3.2 μg, respectively, of IIEα were incubated with 0.8 μg of TFIIEβ for 15 min before the addition of XPB and the substrate. (**B**) A graphical representation of the helicase activities of samples in lanes 3–5.

TFIIEα counteracts TFIIEβ stimulation of the XPB helicase

In contrast to the above results, the TFIIE complex, consisting of an $\alpha_2\beta_2$ tetramer, is known to have the capacity to negatively regulate the helicase activity of XPB and TFIIH (14,39). This negative regulation is lessened in the presence of an antibody to the alpha subunit (39). These observations raise the possibility that the beta subunit has stimulation potential but in the context of intact TFIIE it can be moderated by the inhibitory potential of the alpha subunit. To test directly whether alpha can inhibit the stimulatory effect of beta, TFIIEα was allowed to form a complex with TFIIEβ before the addition of XPB and its helicase substrate. As alpha is titrated against beta, the stimulatory activity of beta falls progressively (Figure 4A, lanes 3–5 and summarized in Figure 4B), confirming the moderation effect of the alpha subunit of the helicase stimulation by the beta subunit.

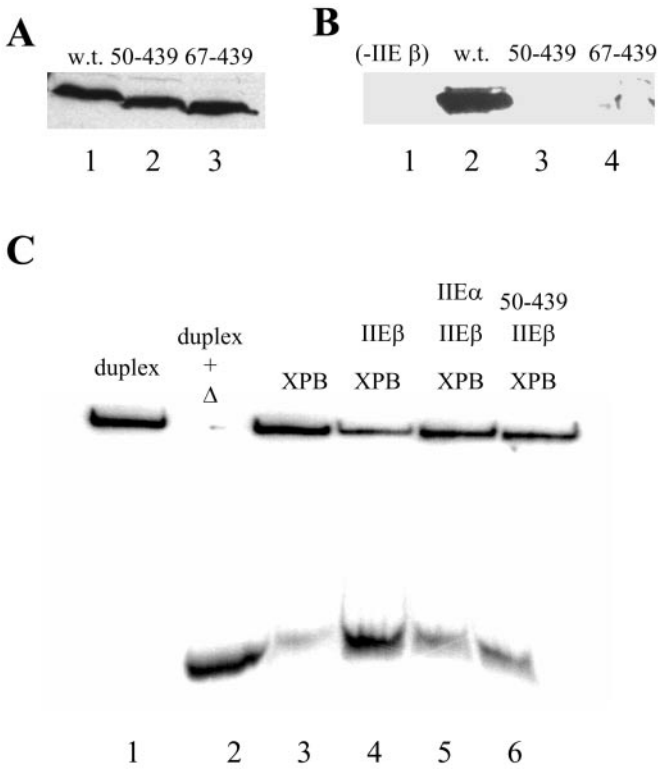

Figure 5. TFIIEα binds to XPB to counteract TFIIEβ stimulation. (**A**) Western blot of wild-type TFIIEα and its mutants. (**B**) CoIP of TFIIEβ with wild-type TFIIEα and its mutants. (**C**) Helicase assay. Lane 3, XPB was added; lane 4, TFIIEβ and XPB were added (70% of substrate was unwound); lanes 5 and 6 as in lane 4, except 2.4 μg of TFIIEα or 50–439 were added, respectively (~30% of substrate were unwound for both).

TFIIEα inhibits the XPB helicase directly, rather than working through TFIIEβ

We tested the possibility that TFIIEα moderates the TFIIEβ helicase stimulation through its interaction with TFIIEβ. Two N-terminal deletion mutants of TFIIEα were constructed (Figure 5A) that would be expected to prevent the formation of a complex with TFIIEβ (16). The CoIP experiment in Figure 5B confirms that the wild-type TFIIEα binds TFIIEβ (lane 2) but that N-terminal truncations of amino acids 1–49 (lane 3) or 1–66 (lane 4) prevent the association of TFIIEα with TFIIEβ.

The non-interacting mutant 50–439 was then tested for the ability to inhibit TFIIEβ-stimulated XPB helicase activity. Figure 5C shows that the inhibition is still intact, even though the mutant TFIIEα can no longer bind TFIIEβ. The inhibition by the non-interacting alpha mutant is comparable with that of wild-type TFIIEα (compare lanes 5 and 6 with the stimulated control in lane 4). We conclude that the ability of TFIIEα to inhibit stimulation of XPB helicase activity does not depend on its association with TFIIEβ. Apparently, TFIIEβ and TFIIEα can act independently directly on XPB, one to stimulate and the other to inhibit.

TFIIEβ mutants defective in the stimulation of the XPB helicase are also defective in transcription

To examine the physiological relevance of the IIE beta stimulation of the XPB helicase, we attempted to identify and test

transcription of IIE beta mutants that would be specifically defective in helicase stimulation. The data of Figure 2 showed that the deletion of the C-terminal BR-HL region lessens helicase stimulation and further deletion through the LR sigma 3 region reduces the residual activity, implicating multiple regions. The C-terminal BR-HLH is dense with overlapping functions (38). These include the IIE–alpha interaction (36,38) that is presumed to be important for recruitment. Therefore, mutants in this region would be expected to alter multiple interactions, not just stimulation of the XPB helicase. In contrast, no functions have yet been ascribed to the LR sigma 3 region, although it is known that its deletion results in a loss of basal transcription activity on a linearized template (36).

Several mutants were made in this region. These changed highly conserved hydrophobic residues to the hydrophilic serine (I171S and I189S). The extract was immunodepleted so as to make transcription depend on added wild-type TFIIE (44); this was reproduced with wild-type TFIIE (Figure 6A, depleted lane 1 versus lane 2 with TFIIE added back). In contrast, transcription was strongly (Figure 6A, lane 3 versus lane 2) or partially (lane 4 versus lane 2) defective when the mutant forms of TFIIE were added back. These two mutants were also found to be associated with defects in stimulated XPB helicase activity (Figure 6B, lanes 3 and 4 versus the wild-type in lane 2). Defects in XPB helicase activity are known to be led to defects in transcription (5,28), so the transcription defect associated with these TFIIE mutants is most likely due to lower XPB helicase activity (Figure 6A and B), although other contributions cannot be excluded.

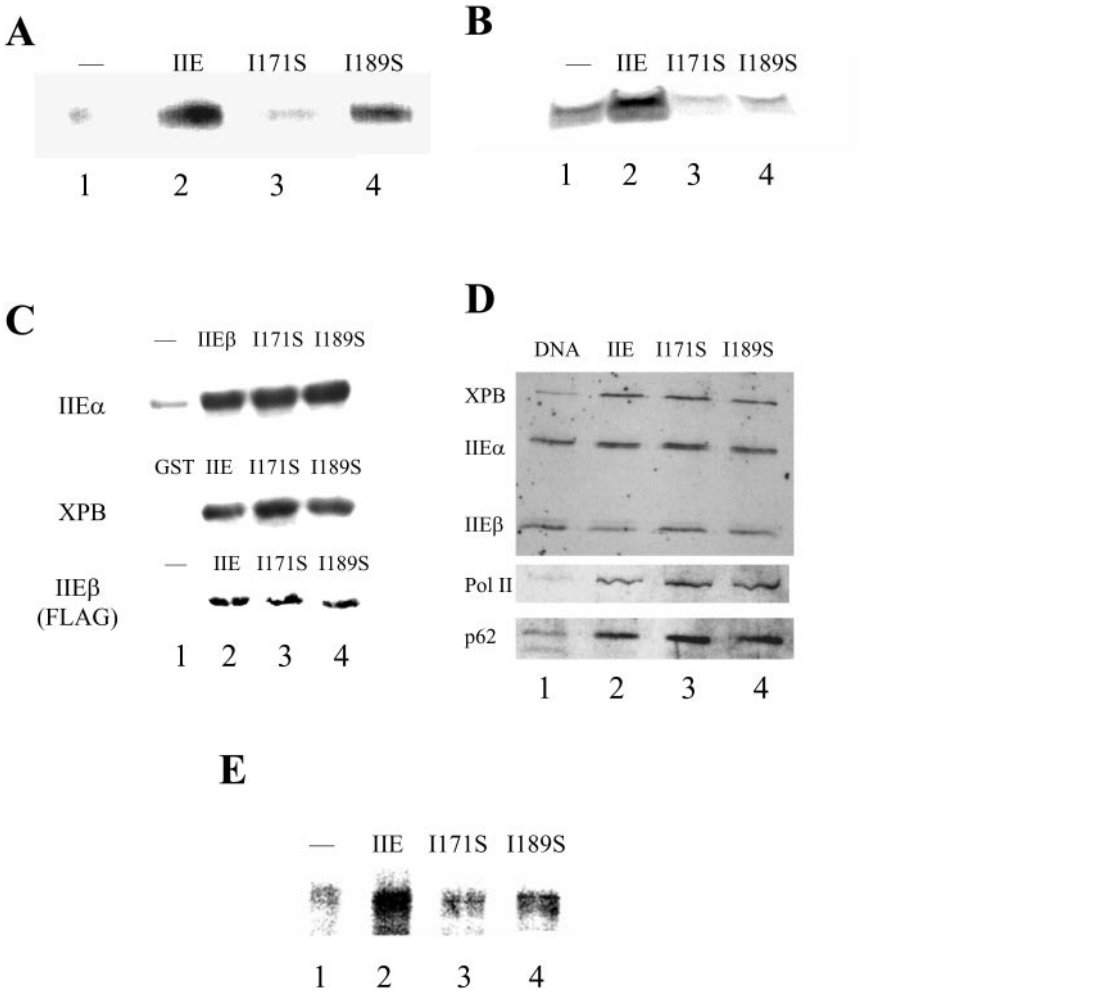

Figure 6. Mutants that fail to stimulate XPB helicase activity also have transcription defects. (**A**) *In vitro* transcription at the AdML promoter. Lane 1, TFIIE depleted extract; lane 2, complementation of wild-type TFIIEβ and TFIIEα; lane 3, with mutant I171S and TFIIEα (~0% transcription activity compared with wild-type); lane 4, with mutant I189S and TFIIEα (~60% transcription activity compared with wild-type). (**B**) Helicase assay. Lane 1, 2 μg of XPB; lane 2, XPB and 1.2 μg of wild-type TFIIEβ (3-fold stimulation); lane 3, XPB and 1.2 μg of I171S; lane 4, XPB and 1.2 μg of I189S. Only the released oligo is shown. (**C**) CoIP with TFIIEβ and general transcription factors. Top panel: lane 1, anti-FLAG resin plus wild-type TFIIEβ; lanes 2–4 anti-FLAG resin pre-bound with FLAG-tagged TFIIEα plus wild-type TFIIE or its mutants. Middle panel: lane 1, glutathione resin with GST plus wild-type TFIIEβ; lanes 2–4 glutathione resin with GST–XPB plus wild-type TFIIEβ or its mutants. Bottom panel: lane 1, anti-FLAG resin plus wild-type TFIIEβ with His_6 tag; lanes 2–4, anti-FLAG resin pre-bound with FLAG-tagged wild type TFIIEβ 6× His tag or its mutants. (**D**) Pre-initiation complex formation of wild-type TFIIEβ and its mutants. Lane 1, beads with equimolar of the template without the E4 promoter and gal sites are incubated with TFIIEβ depleted extract complement with recombinant wild-type TFIIE. Lanes 2–4, beads bound to G9E4 template are incubated with recombinant wild-type TFIIE, I171S or I189S, respectively. The left panel indicates the transcription factors that are being probed. (**E**) *In vitro* transcription at the G9E4 promoter. Lane 1, TFIIE depleted extract; lane 2, complementation of wild-type TFIIE; lane 3, with mutant I171S and TFIIEα; lane 4, with mutant I189S and TFIIEα.

Several other *in vitro* functions were found to be normal using a CoIP assay (8). First, the two beta mutants bind TFIIEα comparably with wild-type (Figure 6C, top row, compare mutants in lanes 3 and 4 with the wild-type in lane 2). Second, the beta subunits are known to dimerize as part of intact TFIIE (11) and this beta dimerization function is normal (Figure 6C, bottom panel). Third, the binding of beta to XPB is also comparable for the mutants and wild-type (Figure 6C, middle panel).

Although this region would not be expected to affect pre-initiation complex formation, as the C-terminus binds the alpha subunit presumably required for assembly, this was tested directly using an immobilized template pull-down assay with a biotinylated promoter (42). A G9E4 template was chosen over the AdML G-less cassette template used earlier because the G-less cassette appeared to bind a significant amount of factors non-specifically (data not shown). The wild-type transcription complex was shown to be functional as hydrolysable NTPs resulted in polymerase II and TFIIH leaving the complex (data not shown) (42).

Figure 6D shows that transcription factors were recruited to the template, whether the wild-type or the mutant forms of beta were present. These include the RNA polymerase itself (second to bottom row) and two subunits of TFIIH, XPB (top row) and p62 (bottom row); the control in this case was the use of promoter-less DNA (compare this control in column 1 with promoter DNA pull-downs in columns 2–4). Although the wild-type and mutant TFIIE subunits were also pulled down comparably, we could not confirm the promoter specificity of the interactions (again compare with column 1 in this context).

Overall, the data demonstrate that the TFIIEβ mutants recruit TFIIH to the template but are defective in stimulating the TFIIH XPB helicase activity. The associated transcription defect is re-confirmed using the same E4 template used in the template pull-down assay (lanes 3 and 4 versus lane 2 of Figure 6E), which complements the transcription data for the ML template (Figure 6A).

DISCUSSION

The ATP-dependent XPB helicase activity of TFIIH is used in triggering promoter-bound RNA polymerase II to progress through initiation and into early elongation (27,28,30,31,34). The enzyme activity *in vitro* is known to be very low; contributions may include the lack of a binding partner, such as p44, which stimulates the TFIIH XPD helicase (25,26) and inhibition by the alpha subunit of TFIIE (39). In this paper, we show that the ATPase and helicase activities of XPB can be strengthened by its partnering with the beta subunit of TFIIE. This stimulation appears to be functionally significant as point mutations in TFIIEβ that have defects in XPB stimulation are also defective in transcription; in contrast, the mutations do not interfere with the recruitment of XPB to the transcription complex or with TFIIEβ binding to TFIIEα.

The stimulatory activity of the TFIIEβ subunit can be counter-acted by the inhibitory activity of its alpha partner subunit. The current data show that the stimulatory activity of TFIIEβ and the inhibitory activity of TFIIEα act independently, i.e. each works directly on XPB. This suggests that the TFIIE complex may be built so that it has the potential to either activate or inhibit. Photo-cross-linking data (46) suggest that ATP causes TFIIEα to re-arrange so as to better engage the downstream region of the transcription bubble (from +4 to +8) during promoter opening, while no accompanying change in the positioning of the beta subunit was detected. It is possible that this re-arrangement within TFIIE releases the beta subunit to stimulate the ATP-dependent XPB helicase to enhance the subsequent promoter escape steps. The data show that it is the C-terminus of TFIIEβ that accomplishes this. Because this region interacts with many components of the transcription complex (36,38), the data raise the possibility that it has a role in multiple re-modeling interactions subsequent to complex assembly (47–49).

Among RNA polymerases, only RNA polymerase II requires a helicase to transit to elongation mode following recruitment to the promoter (50–52), and TFIIH and TFIIE have evolved to meet this unique requirement. This mechanism enhances the potential diversity of regulation and factors may exist that target the TFIIEβ helicase stimulatory activity to control the entry of the promoter-bound RNA polymerase into elongation phase. This transition involves the opening of DNA around the start site (7,52) and the release of factors holding RNA polymerase to the promoter (48,49), leading to the addition of factors that couple transcription elongation and RNA processing (47). These steps cannot occur without the action of the ATP-dependent XPB helicase (13,30,31,36,38), which the current data suggest that it is controlled by TFIIEβ. Such considerations raise the possibility that any or all steps could be potential targets for regulation via the TFIIEβ-stimulated ATP-dependent helicase activity, although the recent demonstration that helicase action comes into play directly after promoter opening (Y. C. Lin and J. D. Gralla, accepted by Nature Structural and Molecular Biology) favors a primary role in providing the energy needed to re-model open pre-initiation complexes and drive RNA polymerase into elongation mode.

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