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Human general transcription factor TFIIA: Characterization of a cDNA encoding the small subunit and requirement for basal and activated transcription

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ABSTRACT **The human general transcription factor TFIIA is one of several factors involved in specific transcription by RNA polymerase II, possibly by regulating the activity of the TATA-binding subunit (TBP) of TFIID. TFIIA purified from HeLa extracts consists of 35-, 19-, and 12-kDa subunits. Here we describe the isolation of a cDNA clone (hTFIIAγ) encoding the 12-kDa subunit. Using expression constructs derived from hTFIIAγ and TFIIAα/β (which encodes a 55-kDa precursor to the α and β subunits of natural TFIIA), we have constructed a synthetic TFIIA with a polypeptide composition similar to that of natural TFIIA. The recombinant complex supports the formation of a DNA–TBP–TFIIA complex and mediates both basal and Gal4-VP16-activated transcription by RNA polymerase II in TFIIA-depleted nuclear extracts. In contrast, TFIIA has no effect on tRNA and 5S RNA transcription by RNA polymerase III in this system. We also present evidence that both the p55 and p12 recombinant subunits interact with TBP and that the basic region of TBP is critical for the TFIIA-dependent function of TBP in nuclear extracts.**

Accurate transcription initiation on TATA-containing class II genes involves the ordered assembly of RNA polymerase (Pol) II and the general initiation factors TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (1). The first step involves recognition of the TATA element by the TATA-binding subunit (TBP) of TFIID (2, 3) and may be regulated by TFIIA, a factor which interacts with both TBP and a TBP-associated factor (TAF) in TFIID (3–9). TFIIA has been identified as a two-subunit (43 and 12 kDa) complex in yeast (7) and as a three-subunit complex in higher eukaryotes (8–12).

The mechanism(s) by which TFIIA influences transcription initiation by Pol II is not completely understood. TFIIA may alter the conformation of TBP, enhancing its ability to recognize and stably associate with various TATA elements (3, 12–14). Alternatively, TFIIA may compete for TBP binding with negative factors (NC1, Dr1/NC2, HMG1, and ADI; refs. 15–19) which prevent the formation of functional preinitiation complexes. Activator function requires TFIIA in some transcription systems (15, 16, 20) and, consistent with this, the activators Gal4-AH and Zta facilitate formation of activator–TFIID–TFIIA–promoter complexes (21–23). Finally, interactions of TFIIA with a $TAF_{II}110$ in *Drosophila* TFIID (9) and with the human coactivator PC4 (24, 25) are consistent with a specialized role of TFIIA in activation.

cDNAs isolated from *Drosophila* (dTFIIA-L) (9) and human (hTFIIAα/β) (10, 20) indicate that the p35 and p19 subunits of natural TFIIA are encoded, respectively, by N- and C-terminal regions of the large open reading frame within these clones (9, 10). In this paper we describe the isolation of hTFIIAγ, a cDNA clone encoding the p12 subunit of human TFIIA. We also describe properties of a completely recombinant TFIIA and demonstrate the functional importance, in a TFIIA-dependent transcription system, of a basic TBP domain that is required for the TFIIA interaction.†

MATERIALS AND METHODS

Isolation of hTFIIA/γ. Oligonucleotides P1 (5′-GTIYTIITICARTTTGAYAAM-3′) and P2 (5′-ATIARIGTCCAIACITYATCICAGAA-3′) were based on evolutionarily conserved protein sequences (see *Results*) and served as primers in PCRs using human mRNA (0.7 μg) as the template. The 119-bp product obtained was used to screen HeLa (Stratagene) and Namalwa cell cDNA libraries. Sequences of clones from the two libraries were identical, and one 615-bp Namalwa library clone, c12-1, was analyzed further.

Construction of Expression Plasmids. To produce the encoded protein in *Escherichia coli*, an *Nde* I–*Bam*HI histidine-tagged p12 open reading frame PCR product was subcloned into a prSET vector (Invitrogen) to give prSEThp12. Site-directed mutagenesis (Clontech) and restriction enzyme-mediated deletions were used to produce N- and C-terminal truncated hTFIIAα/β (7) constructs in prSET (pJD1-376, pJD1-325, pJD1-274, pJD1-59, pJD129-376, pJD129-325, and pJD129-274). An *Nde* I–*Bam*HI PCR product encompassing aa 275–376 of hTFIIAα/β was used to construct pJDGEX2t (L)275–376 from pGEX2t(L), a derivative of pGEX2t (Pharmacia). pGEX2t(L)hp12 and pGEX2t(L)hp55 were also constructed by inserting the respective *Nde* I–*Bam*HI fragments into pGEX2t(L). "FLAG"-tagged hTFIIAp12 and hTFIIAp55 expression plasmids were created by cloning *Nde* I–*Bam*HI fragments from prSEThp12 and pJD1-376 into an f:hTBP vector (26).

Purification and Reconstitution of Recombinant Proteins. Recombinant proteins were purified by NTA-resin affinity chromatography (7, 27) or as described for FLAG-tagged human TBP (26). The glutathione *S*-transferase (GST)-hTFIIAα/β-(275–376) fusion protein (GST-p19*) was purified on glutathione-Sepharose and digested with thrombin, and p19* was separated from GST (24). Three-subunit recombinant TFIIA was produced by co-renaturation of recombinant p35* and p12 proteins to prevent precipitation of p12, followed by the addition of soluble p19* (see *Results*).

Bandshift and Transcription Assays. Bandshift assays using an adenovirus major late promoter (AdMLP) oligonucleotide (−45 to −15) were performed (5, 9, 10) using ≈100 ng of each indicated polypeptide. For transcription assays, HeLa nuclear extracts were depleted of TFIIA either immunologically (10) or by an affinity matrix. In the latter case, nuclear extracts (0.75 ml) were rotated for 1 hr at 4°C with 0.25 ml of packed NTA

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Abbreviations: TBP, TATA-binding subunit; TAF, TBP-associated factor; Pol, RNA polymerase; GST, glutathione *S*-transferase; AdMLP, adenovirus major late promoter; TA, TBP–TFIIA–promoter.

†The sequence reported in this paper has been deposited in GenBank (accession no. U21424).

resin, centrifuged for 5 min, and dialyzed against BC100 (10). Neither extract contained detectable TFIIA-dependent bandshift activity. Pol II-dependent transcription assays (10) used 200 ng of an AdMLP core template with five upstream Gal4 sites (5GpMLG-less) or 100 ng of a related human immunodeficiency virus/AdMLP template (pG5HMC2AT) (15). Pol III-dependent transcription assays used 500 ng of tRNA or 5S RNA promoters (28). Nuclear extracts were heat treated according to Nakajima *et al.* (2). The highly purified transcription system has been described by Okhuma *et al.* (29). Natural TFIIA refers to an NTA-resin-purified fraction (10).

GST Interaction Assays. Glutathione-Sepharose-purified GST, GST-p12, and GST-p55 fusion proteins (100 ng/50 μl of resin) were incubated with ≈100 ng of target protein in the presence of 0.1% bovine serum albumin for 1 hr at 4°C. After the beads were washed, bound proteins were eluted for SDS/PAGE and immunoblot analysis. Bound FLAG-tagged TBP or p55-derived polypeptides were detected with antiserum raised against p55 (10) or the FLAG epitope (IBI).

UV Crosslinking. An oligonucleotide encompassing nt −51 to +1 of the coding strand of the AdMLP was annealed to a complementary oligonucleotide spanning nt −16 to −35 of the noncoding strand. The annealed oligonucleotide (0.5 pmol) was extended with Klenow DNA polymerase (GIBCO-BRL) to incorporate 5-[*N*-(*p*-azidobenzoyl)-3-aminoallyl]-dUTP (N_3R-dUTP) at positions −39 and −40 (8 and 9 bases upstream of the TATA box). Photocrosslinking assays (30) employed gel shift conditions (9) without carrier DNA. After incubation at 37°C, the tubes were subjected to UV irradiation at 312 nm for 7 min at a distance of 5 cm. Micrococcal nuclease and DNase I were added for 15 min at 37°C and the trichloroacetic acid-precipitated reaction mixture was analyzed by SDS/PAGE and autoradiography.

RESULTS

Isolation of a Human TFIIA Small-Subunit (p12) cDNA. An alignment of TFIIA small-subunit amino acid sequences from *Drosophila* (31), yeast (32), and rice (Genbank accession no. D15390) identified homologous regions (aa 36–42 and 67–75) that allowed the design of PCR primers for RNA-based PCR analysis. After analysis of isolates obtained by direct screening with the PCR product, one clone (c12-1) of 615 nt encoding a 12.5-kDa protein of 109 aa was designated hTFIIAγ (Fig. 1, top line). The high degree of sequence conservation between the human protein and the *Drosophila* (80%), yeast (54%), and rice (75%) proteins demonstrates that this clone encodes the human TFIIA small subunit.

Approximation of the Junction Site in hTFIIAα/β (p55). The mobilities of two recombinant proteins containing p55 residues 1–325 and 1–274 were compared with those of

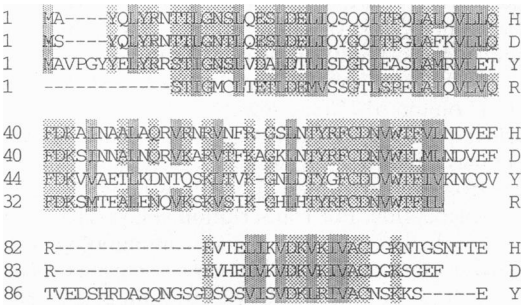

FIG. 1. Sequence analysis of human TFIIAγ. The deduced sequence of hTFIIAγ is shown on the top line (H). The sequence homology to *Drosophila* (D), yeast (Y), and rice (R) small subunits is shown by the light stippling of residues conserved in three of four sequences. Conserved branched-chain hydrophobic amino acids are shown in darker stippling. Amino acid numbers are indicated at left.

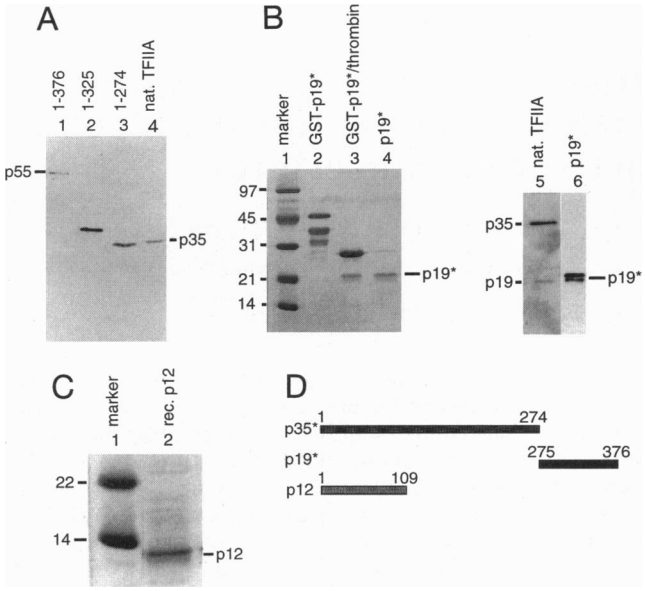

FIG. 2. Approximation of the processing site of hTFIIAα/β (p55) and the construction of a three-subunit recombinant TFIIA. (*A*) Comparison of the sizes of hTFIIAα/β (p55) and derived C-terminal deletion constructs relative to the p35 subunit of natural TFIIA. Lane 1, aa 1–376 of hTFIIAα/β; lane 2, aa 1–325; lane 3, aa 1–274; lane 4, natural TFIIA. Positions of the full-length recombinant p55 and the large subunit (p35) of natural TFIIA are shown. (*B*) (*Left*) Coomassie-stained SDS/polyacrylamide gel of various stages in the preparation of recombinant p19*. Lane 1, molecular weight markers [molecular masses (kDa) are at left]; lane 2, purified GST-p19* fusion proteins; lane 3, thrombin-digested GST-p19* fusion proteins; lane 4, purified recombinant p19*. (*Right*) Immunoblot analysis demonstrates that p19* (lane 6) migrates very similarly to the p19 subunit of natural TFIIA (lane 5). Polypeptides in *A* and *B* (lanes 5 and 6) were detected with anti-p55 antiserum (10). (*C*) Coomassie-stained SDS/polyacrylamide gel showing that hTFIIAγ migrates as a 12-kDa species. Lane 1, molecular weight markers; lane 2, *E. coli*-expressed recombinant hTFIIAγ (p12). (*D*) Schematic of the constructions used to express three-subunit recombinant TFIIA in *E. coli*. Numbers above the upper two lines indicate amino acid positions in the hTFIIAα/β clone; the bottom line represents the p12 subunit.

full-length p55 and the p35 subunit of natural TFIIA. As shown in Fig. 2*A* (lane 2), removal of the C-terminal ≈50 amino acids, which contain a cluster of basic amino acids (aa 340–355), resulted in a drastic change in apparent size (15 kDa). This observation explains the difference between the deduced molecular mass of hTFIIAα/β (42 kDa) and the apparent size by SDS/PAGE (55 kDa) (10, 20). The polypeptide composed of aa 1–274 of hTFIIAα/β (p35*) migrated slightly faster than the natural p35 TFIIA subunit (lanes 3 and 4), suggesting that the natural junction is very near our estimated 274/275 breakpoint.

A recombinant p19-like protein (p19*) synthesized as a fusion protein containing GST and aa 275–376 from hTFIIAα/β was purified on glutathione-Sepharose (Fig. 2*B*, lane 2), separated from GST by cleavage with thrombin (lane 3), and purified (lane 4). Immunoblot analysis (lanes 5 and 6) indicated a recombinant p19* doublet, the lower band having a mobility similar to that of natural p19. The substoichiometric production of p19* relative to GST, as well as the appearance of a doublet, may reflect premature translational termination in the highly acidic region (aa 280–330) of p19*. Recombinant p12 was overproduced from prSEThp12 (Fig. 2*C*, lane 2). The three constructs are schematicized in Fig. 2*D*.

Recombinant TFIIA Functions in a TBP-Dependent Electrophoretic Bandshift Assay. In the presence of recombinant human p55, recombinant TFIIA small subunits from yeast, *Drosophila*, and human supported formation of a TBP–

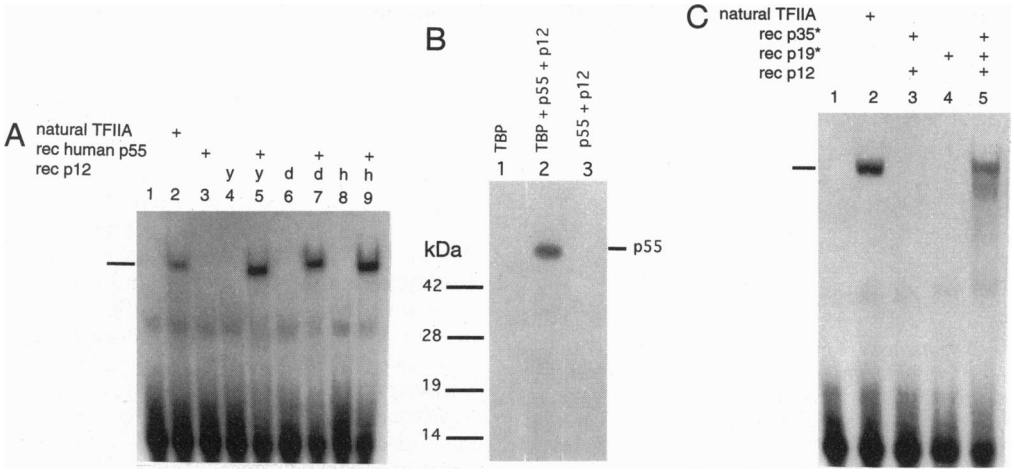

FIG. 3. Recombinant TFIIA functions in a TBP-dependent bandshift assay. (*A*) Both p55 and p12 recombinant TFIIA subunits are required to form a TA complex. Lane 1, recombinant yeast TBP alone; lane 2, natural TFIIA; lane 3, recombinant p55; lanes 4, 6, and 8, recombinant yeast (y), *Drosophila* (d), and human (h) p12 subunits, respectively; lanes 5, 7, and 9, same as lanes 4, 6, and 8 but with the addition of recombinant human p55. (*B*) UV crosslinking analysis demonstrates that p55 is in the TA complex and contacts the DNA upstream of the TATA box. Lane 1, recombinant yeast TBP alone; lane 2, yeast TBP, p55, and p12; lane 3, p55 and p12. Protein size markers are at left and the position of the p55–oligonucleotide radiolabeled complex is shown at right (p55). (*C*) Three-subunit recombinant TFIIA supports bandshift activity. Lane 1, recombinant yeast TBP alone; lane 2, natural TFIIA; lane 3, recombinant p35* and p12; lane 4, recombinant p19*; lane 5, recombinant p35*, p19*, and p12. All bandshift reaction mixtures in *A* and *C* contained 50 ng of yeast TBP. Lines at left indicate the position of the TA complex.

TFIIA–promoter (TA) complex (Fig. 3*A*, lanes 5, 7, and 9) similar in mobility to that seen with natural TFIIA (lane 2). To determine whether intact recombinant p55 was functional, TA complexes formed with an N_3R-dUMP-containing AdMLP oligonucleotide (−51 to +1) were subjected to UV crosslinking (Fig. 3*B*). A 55-kDa crosslinked species was observed only in the presence of all the components (TBP, p55, and p12) required for bandshift activity (lane 2 vs. lanes 1 and 3), whereas no crosslinking of p12 or TBP was observed. In addition, the three-subunit recombinant TFIIA also formed a TA complex with the correct mobility (Fig. 3*C*, lane 5). Thus intact p55 and the derived N- and C-terminal fragments (p35* and p19*) are functional proteins.

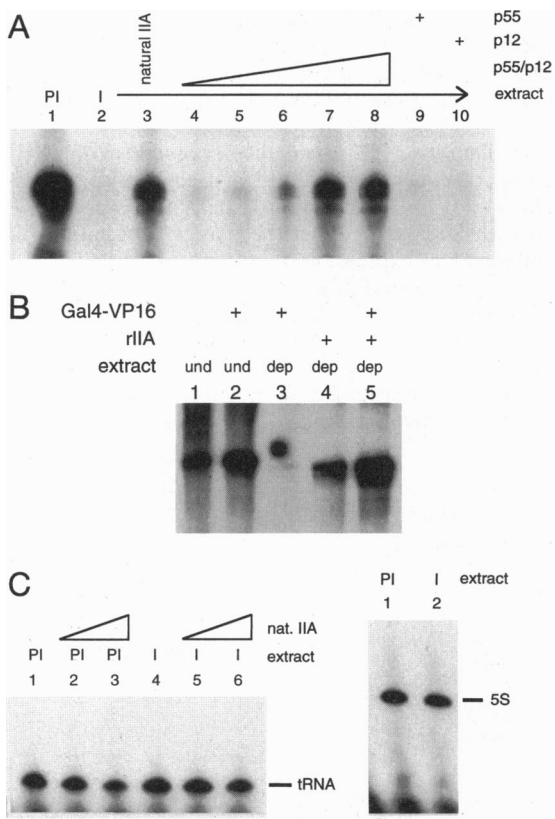

FIG. 4. TFIIA is essential for activator-independent and activator-dependent transcription by Pol II but is not required for Pol III-mediated transcription. (*A*) The loss of basal transcription from the AdMLP core in TFIIA-depleted HeLa nuclear extract can be reversed by the addition of the recombinant p55 and p12 TFIIA subunits. Lane 1, basal transcription in extract treated with preimmune (PI) serum; lanes 2–10, basal transcription in extract depleted with immune (I) serum and supplemented with the following factors: lane 2, no addition; lane 3, natural TFIIA; lanes 4–8, increasing amounts (10 ng, 100 ng, 0.5 μg, 1 μg, and 2 μg) of both p55 and p12; lane 9, p55 alone (1 μg); lane 10, p12 alone (1 μg). (*B*) Gal4-VP16-dependent transcription from an AdMLP containing five Gal4 sites requires TFIIA. HeLa nuclear extracts were either not depleted (und; lanes 1 and 2) or depleted (dep; lanes 3–5) of TFIIA by NTA-resin chromatography. In addition, reaction mixtures contained 20 ng of Gal4-VP16 (lanes 2, 3, and 5) and/or recombinant three-subunit TFIIA (≈0.5 μg of each subunit) (lanes 4 and 5). (*C*) Pol III-dependent transcription of tRNA and 5S genes does not depend on TFIIA. (*Left*) tRNA transcripts synthesized in preimmune (PI; lanes 1–3)- or immune (I; lanes 4–6)-depleted extract. In addition, reaction mixtures contained 1 μl (lanes 2 and 5) or 3 μl (lanes 3 and 6) of purified natural TFIIA. (*Right*) Transcription of the 5S gene in preimmune (PI; lane 1) or immune (I; lane 2) serum-depleted extract.

Transcriptional Properties of TFIIA. TFIIA-depleted nuclear extracts were used to determine the functionality of recombinant human TFIIA (Fig. 4*A*). Treatment of extracts with immune serum (10) virtually abolished basal transcription from the AdMLP core (compare lanes 1 and 2). However, increasing amounts of co-renatured p55 and p12 (lanes 4–8), but not p55 (lane 9) or p12 (lane 10) alone, restored basal transcription to levels seen upon readdition of natural TFIIA (lane 3). To investigate the involvement of TFIIA in activated transcription, and the competence of the recombinant three-subunit TFIIA, the effect of Gal4-VP16 was tested in TFIIA-depleted or undepleted extracts (Fig. 4*B*). Gal4-VP16 activated transcription ≈4-fold in TFIIA-containing extracts (lanes 1 and 2) but did not support transcription in TFIIA-depleted extracts (lane 3), which also lacked basal activity. However, readdition of recombinant three-subunit TFIIA to depleted extracts elevated basal transcription to control levels (compare lanes 4 and 1) and allowed a further activation of

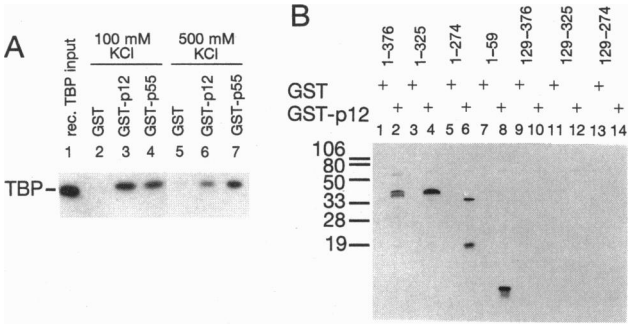

FIG. 5. Analysis of interactions between recombinant TFIIA subunits and TBP. (*A*) GST interaction assays show that TBP interacts independently with both p12 and p55 recombinant TFIIA subunits. GST (lanes 2 and 5), GST-p12 (lanes 3 and 6), and GST-p55 (lanes 4 and 7) were evaluated for their ability to retain the input TBP (lane 1, 20% input TBP). The FLAG-tagged TBP was detected by Western blotting using antibody directed against the FLAG tag. (*B*) The N-terminal region of p55 interacts with p12. GST (odd-numbered lanes) and GST-p12 (even-numbered lanes) were tested for their ability to retain N- and C-terminal deletion constructs of hTFIIAα/β (listed at the top). Retention of polypeptides was determined by Western analysis using antibody directed against p55. The additional smaller bands seen in lanes 2 (aa 1–376) and 6 (aa 1–274) reflect degradation in those particular preparations.

≈7-fold by Gal4-VP16 (lanes 4 and 5). Thus, in unfractionated extracts high levels of both basal and activated transcription require the presence of TFIIA.

To address the possibility that TFIIA is required for transcription by Pol III, tRNA and 5S RNA templates were assayed in control and TFIIA-depleted extracts. Similar levels of tRNA synthesis were observed for both extracts (Fig. 4*C Left*, lanes 1 and 4), and the addition of further amounts of natural TFIIA had no effect (lanes 2, 3, 5, and 6). Similarly, 5S rRNA gene transcription was unaffected by the removal of TFIIA (Fig. 4*C Right*, lanes 1 and 2).

Interactions Between p55, p12, and TBP. GST pulldown assays were performed to evaluate interactions between TBP and individual subunits of TFIIA. As shown in Fig. 5*A*, TBP was retained by immobilized GST-p12 and GST-p55 (lanes 3 and 4), but not by GST alone (lanes 2 and 5), indicating that these proteins interact independently with TBP. Fig. 5*B* compares the ability of GST (odd-numbered lanes) and GST-p12 (even-numbered lanes) to interact with polypeptides encoded by various N- and C-terminal p55 deletion constructs. These results show that a region of homology (aa 1–59) among human, *Drosophila*, and yeast large subunits is required for the interaction with p12.

Functional Analysis of a Mutant TBP Unable to Interact with TFIIA. TFIIA interacts with the basic region of TBP (13, 33), and a TBP double mutant in this domain (K138L, K145L) was used to ascertain the functional relevance of this interaction. As indicated in Fig. 6*A*, both wild-type and mutant TBPs formed complexes with an AdML promoter oligonucleotide (lanes 3 and 5), although the mobility of the complexes differed. However, only wild-type TBP supported the formation of a TA complex (lanes 4 and 6). In a highly purified TFIIA-independent transcription system (data not shown), wild-type (Fig. 6*B*, lanes 2–4) and mutant (lanes 5–7) TBPs were functionally indistinguishable. This suggested that the mutant TBP does not exhibit generalized defects in folding and, like wild-type TBP, can interact with other general transcription factors. In contrast, in unfractionated TFIIA-dependent nuclear extracts treated with mild heat to selectively inactivate endogenous TBP (2), exogenous wild-type TBP strongly enhanced transcription (lanes 2–4) above the residual level, whereas the mutant TBP showed little effect (lanes 5–7). These results demonstrate the functional importance of the basic region of TBP and, since the mutations eliminate TFIIA–TBP interactions, that of the TFIIA–TBP interaction as well.

DISCUSSION

This paper describes the isolation of a cDNA (hTFIIAγ) encoding the small subunit (p12) of human TFIIA, as well as functional properties of this factor. These results confirm and extend our previous study (31) of the small subunit of *Drosophila* TFIIA, and are in agreement with related studies of human (34, 35) and *Drosophila* (36) small subunits that were reported as this work was being completed. hTFIIAγ shares substantial sequence similarity with the TFIIA small subunits of three other species (Fig. 1), particularly in regions containing aa 5–23, 32–42, 67–75, and 87–98. This homology suggests a conserved function in evolution, an idea supported by the interchangeability of human, *Drosophila*, and yeast p12 subunits in reconstituting (with human p55) the TA complex (Fig. 3).

The availability of TFIIAγ makes it possible, with TFIIAα/β (which encodes a putative 55-kDa precursor to the 35- and 19-kDa natural TFIIA subunits), to design recombinant TFIIA subunits which closely reflect the structure of natural TFIIA. The independent expression of regions 1–274 and 275–376 of hTFIIAα/β generated proteins (p35* and p19*) with mobilities very near those of the natural p35 and p19 subunits, respectively. These results, which suggest that the actual junction between p35 and p19 is near aa 274 and 275, are of interest since this is the border between internal nonconserved (aa 50–230) and highly acidic (aa 280–320) regions. One intriguing

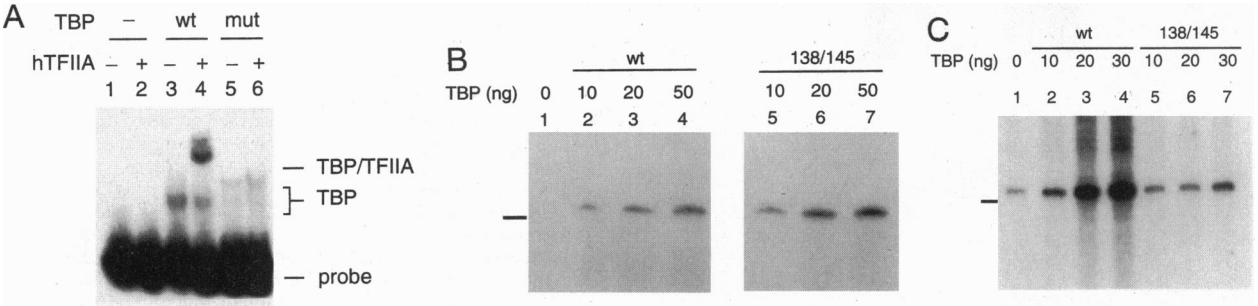

FIG. 6. Transcriptional properties of mutant (K138L, K145L) and wild-type TBPs. (*A*) Mutant TBP binds DNA but is unable to support TA complex formation. Reaction mixtures contained either no TBP (lanes 1 and 2), wild-type (wt) TBP (lanes 3 and 4), or mutant (mut) TBP (lanes 5 and 6). For lanes 2, 4, and 6 recombinant (p55 and p12) TFIIA was also present. (*B*) Mutant and wild-type TBPs function identically in a TFIIA-independent reconstituted transcription system. Lane 1, no TBP added; lanes 2–4, addition of increasing amounts of wild-type TBP; lanes 5–7, addition of increasing amounts of mutant TBP. The observed transcript is driven from the pG5HMC2AT (15) promoter. (*C*) Mutant TBP is functionally impaired in heat-treated nuclear extracts. Lane 1, transcription in heat-treated HeLa nuclear extracts; lanes 2–4, addition of increasing amounts of wild-type TBP; lanes 5–7, addition of increasing amounts of mutant TBP.

possibility is that the formation of the p35 and p19 subunits of natural TFIIA may be the result of specific ribosomal termination and reinitiation in this region.

These hTFIIAγ and hTFIIAα/β constructs allowed the generation of either two-subunit (p12 and uncleaved p55) or three-subunit (p12, p19*, and p35*) recombinant TFIIA species, both of which can support the formation of a TA complex. In addition, UV crosslinking demonstrated that the separation of p55 into N- and C-terminal domains was neither required for nor the consequence of promoter complex formation, consistent with the conservation of hTFIIAα and hTFIIAβ subunit homologies within a single large subunit in natural yeast TFIIA (9, 10, 20). Our ability to crosslink TFIIA at positions 8 and 9 nt upstream of the TATA box substantiates previous UV crosslinking (37) and DNase I footprinting (3, 6, 8) results. The inability to crosslink the p12 subunit may indicate that this highly hydrophobic subunit is sequestered in protein–protein interactions, consistent with the observed TBP and p55 interactions (Fig. 5).

Beginning with earlier studies in this laboratory (15), a number of observations have suggested that TFIIA may affect transcription activation by sequence-specific activators. This idea is based on reports of TFIIA involvement in activation by Gal4-AH (21) and by the Epstein–Barr virus activator Zta (22, 23) and of interactions of TFIIA with the coactivator PC4 (24) and a *Drosophila* Pol II-specific TAF (9). Fig. 4 shows effects of TFIIA on basal transcription (mediated by TFIID) and on Gal4-VP16-dependent activated transcription from the AdMLP. One simple explanation for the loss of activation by Gal4-VP16 is the absence of a TFIIA function that is required (with the natural TATA-binding factor TFIID) even for basal transcription. Alternatively, in making specific interactions with activators, coactivators, and TBP or TAF subunits of TFIID, TFIIA may perform activation-specific functions which are separate from the TFIIA function in basal transcription.

In addition, and in contrast to an earlier report (38), we have observed that the ability of Pol III to transcribe tRNA and 5S genes in nuclear extracts is independent of TFIIA content, at least under the assay conditions used. These results are consistent with the observation that immunoprecipitation of *Drosophila* TFIIA precipitates dTFIID (9), but apparently not SL1 or TFIIIB TBP-containing complexes, and supports the hypothesis that TFIIA is a Pol II-specific factor.

Specific lysine to leucine mutations in the basic region of TBP either abolish or sharply reduce its ability to interact with TFIIA (13, 33), but the present study shows that such a mutant TBP can still support a normal level of transcription by Pol II in a TFIIA-independent transcription system composed of highly purified and recombinant factors. In contrast, transcription assays performed in unfractionated TBP-deficient HeLa nuclear extracts show that ectopic wild-type TBP is able to mediate efficient core promoter transcription by Pol II, whereas the mutant TBP elicits only very low levels of transcription. These results could reflect the inability of TFIIA to displace negatively acting TBP-binding factors (Introduction) or an inability to form stable TBP–TFIIA complexes on TATA elements. These observations demonstrate the functional significance of the basic region of TBP and are consistent with a model in which TFIIA interactions in this region are critical for mediating TBP/TFIID function in a more physiologic environment of nuclear factors.

It is hoped that further examination of protein interactions and functional properties with the recombinant components described here will lead to a better understanding of the mechanisms by which TFIIA influences both basal and activated transcription.

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