

Positive and Negative TAF_{II} Functions That Suggest a Dynamic TFIID Structure and Elicit Synergy with TRAPs in Activator-Induced Transcription

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Human transcription factor TFIID contains the TATA-binding protein (TBP) and several TBP-associated factors (TAF_{II}s). To elucidate the structural organization and function of TFIID, we expressed and characterized the product of a cloned cDNA encoding human TAF_{II}135 (hTAF_{II}135). Comparative far Western blots have shown that hTAF_{II}135 interacts strongly with hTAF_{II}20, moderately with hTAF_{II}150, and weakly with hTAF_{II}43 and hTAF_{II}250. Consistent with these observations and with sequence relationships of hTAF_{II}20 and hTAF_{II}135 to histones H2B and H2A, respectively, TFIID preparations that contain higher levels of hTAF_{II}135 also contain higher levels of hTAF_{II}20, and the interaction between hTAF_{II}20 and hTAF_{II}135 is critical for human TFIID assembly in vitro. From a functional standpoint, hTAF_{II}135 has been found to interact strongly and directly with hTFIIA and (within a complex that also contains hTBP and hTAF_{II}250) to specifically cooperate with TFIIA to relieve TAF_{II}250-mediated repression of TBP binding and function on core promoters. Finally, we report a functional synergism between TAF_{II}s and the TRAP/Mediator complex in activated transcription, manifested as hTAF_{II}-mediated inhibition of basal transcription and a consequent TRAP requirement for both a high absolute level of activated transcription and a high and more physiological activated/basal transcription ratio. These results suggest a dynamic TFIID structure in which the switch from a basal hTAF_{II}-enhanced repression state to an activator-mediated activated state on a promoter may be mediated in part through activator or coactivator interactions with hTAF_{II}135.

TFIID is a general transcription factor composed of a small TATA-binding polypeptide and a large number of TATA-binding protein (TBP)-associated factors (TAFs), all of which are highly conserved in evolution (reviewed in references 7 and 17). TFIID is involved, along with other general initiation factors (TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH), in both activator-independent (basal) and activator-dependent transcription. Furthermore, and in contrast to basal transcription, activator-dependent transcription in mammalian cell-free systems reconstituted with purified factors generally requires cofactor activities that include both USA (upstream factor stimulatory activity)-derived factors and TBP-associated factors (TAF_{II}s) within TFIID (for reviews, see references 28, 35, 37, and 38).

In general, the efficiency of preinitiation complex (PIC) assembly or function is controlled by the presence of transcription factors that are usually bound to specific distal sequences. Some models of how transcription regulatory factors influence PIC assembly invoke interactions with TFIID that, through qualitative and/or quantitative effects on TFIID binding, enhance the recruitment of downstream factors (reviewed in reference 7). Whereas TFIID from metazoans was found to mediate both basal and activator-dependent transcription in cell-free systems reconstituted with partially purified components, TBP elicited mainly basal transcription. This led to the

hypothesis that TAF_{II}s within TFIID interact directly with activators to promote PIC assembly. Conversely, and using reconstituted TFIID complexes, a seemingly good correlation was drawn between the activity of a specific activator and the ability of its activation domain to selectively bind a specific given TAF_{II} (for reviews, see references 7 and 43). In addition, in vitro studies have shown an important role for TAF_{II}s within TFIID in core promoter recognition and transcriptional strength, especially on TATA-less promoters that contain other core promoter elements such as the initiator (Inr) and/or downstream promoter elements (for a review, see reference 40). In this regard, early in vivo studies in *Saccharomyces cerevisiae* suggested that individual TAF_{II}s are dispensable for activated transcription of most genes (31, 44) and that core promoter elements, rather than upstream binding sites, confer TAF_{II}-dependence on some genes (39).

Consistent with these latter observations is the finding that the *S. cerevisiae* Mediator complex can support activated transcription in vitro with TBP alone (22, 25). In addition, it was also reported that TAF_{II}s are not required either for activated transcription by GAL4-VP16 in unfractionated HeLa nuclear extracts (34) or for activation by thyroid hormone receptor in association with the human TRAP/Mediator in a partially purified system (15). However, in a reexamination of the TAF_{II} requirement for activator function in *S. cerevisiae*, more extensive genetic analyses have suggested that at least some TAF_{II}s (notably the histone-related TAF_{II}s) are broadly required for transcription and that the TAF_{II} dependency, in some cases, may require upstream activators (reviewed in reference 17). Finally, and further complicating the interpretation

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of the *in vivo* assays, is the discovery that a subset of TAF_{II}s found in the TFIID complex are also integral components of histone acetyltransferase complexes in *S. cerevisiae* and humans (for review, see reference 6). Thus, TAF_{II}s have been shown to serve as conventional coactivators acting at the DNA level, as core promoter-selective factors, and within coactivators implicated in chromatin modifications, and at least one TAF_{II} has several catalytic activities that are potentially involved in transcription (reviewed in reference 17). It thus seems likely that different TAF_{II}s may function by distinct mechanisms that depend on the specific regulatory elements and chromosomal architecture of a given promoter.

An understanding of the various TFIID functions is based on a resolution of the overall architecture of TFIID that requires knowledge of both the primary sequences and structures of individual subunits and their interactions and topological organization within TFIID (1, 5). Studies of *S. cerevisiae*, *Drosophila melanogaster*, and human TAF_{II}s have provided valuable information on a number of protein-protein interactions and, of special interest, the potential for a histone octamer-like structure within TFIID that would comprise, in the human system, the H4-like (hTAF_{II}80), the H3-like (hTAF_{II}31), and the H2B-like (hTAF_{II}20) subunits (21).

Here we describe interactions of the H2A-related hTAF_{II}135 with the H2B-related hTAF_{II}20 through histone-like folds (16, 21) and the importance of this interaction for human TFIID assembly. We also report important new insights into TFIID function based on the demonstration of synergistic hTAF_{II}135-TFIIA interactions that relieve hTAF_{II}250-mediated inhibition of TBP binding and function, as well as a functional synergism between TAF_{II}s and the TRAP/Mediator complex.

MATERIALS AND METHODS

Isolation of cDNA clone for hTAF_{II}135. TFIID was affinity purified from either HeLa nuclear extract (NE) or a phosphocellulose (Whatman P11) fraction (0.85 KCl) of HeLa NE by use of an antibody against the N-terminal portion of hTAF_{II}100 or anti-TBP antibodies, respectively (42). The 135-kDa polypeptide was excised and digested with endoproteinase Lys-C (13). Earlier sequence analysis of the purified peptides yielded several sequences that were used to design degenerate oligonucleotides and to isolate the cDNA corresponding to the truncated hTAF_{II}135 sequence (amino acids 239 to 1083). Subsequent hTAF_{II}135 peptide sequence analysis revealed two additional peptides. The sequence of one of these two peptides matched the translation of a genomic DNA clone (accession number Z22478). The sequences of this genomic clone were used to obtain the missing 5' region of hTAF_{II}135 DNA coding region. The full-length coding DNA sequence (amino acids 1 to 1083) that was obtained is identical to the one reported by Mengus et al. (30).

Expression and purification of TFIID subunits, activators, and GST derivatives. The pVL derivatives for the expression of hemagglutinin (HA)-hTAF_{II}250, Flag-hTAF_{II}100, Flag-hTAF_{II}80, Flag-hTAF_{II}55, Flag-hTBP, Flag-hTAF_{II}31, and Flag-hTAF_{II}28 have been described (11, 18, 19, 42). Expression pVL plasmids for Flag-hTAF_{II}150, Flag-hTAF_{II}135, Flag-hTAF_{II}20, and HA-hTAF_{II}20 were constructed by PCR. In each case, an *Nde*I site at the N-terminal end and an appropriate restriction enzyme site at the C-terminal end following the natural stop codon were created. The large number of primers used in the PCRs has precluded description of their exact sequences, but the information is available upon request. The PCR-generated fragments were then inserted into adapter pFlag(S)-7 and pFlag(AS)-7 plasmids carrying the appropriate tag epitope (10) and subsequently subcloned into either pVL-1392 or pVL-1393 (41). Each construct was verified by sequencing.

For each TFIID subunit, an individual recombinant baculovirus was generated by cotransfecting corresponding cDNA and BacVector-3000-linearized baculovirus DNA (Novagen) into Sf9 cells. Each recombinant baculovirus was further amplified by repeated infection of Sf9 cells. For production of recombinant proteins, Sf9 cells were infected by the corresponding recombinant viruses and

harvested 48 h postinfection. For the coinfection experiments, the appropriate ratio between the viruses was determined by pilot assays before the large coinfection experiments were performed. Recombinant proteins were purified from infected cells. Nuclear extracts were prepared in buffer C (20 mM Tris [pH 7.9], 20% glycerol, 0.2 mM EDTA) containing 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 10 µg of leupeptin per ml, and 1 µg of pepstatin per ml, 400 mM KCl (BC400), and 0.1% NP-40 (13). Clarified extracts were subjected to the appropriate restriction method of purification, affinity purification on anti-Flag antibody (M2 agarose; Kodak) or anti-HA antibody (12CA5 monoclonal antibody) columns, and further purified by one or two steps of ion-exchange chromatography. The recombinant proteins were more than 90% pure as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie blue or silver staining.

Glutathione S-transferase (GST) constructs were created by inserting cDNA fragments corresponding to the different proteins and flanked, in frame, with the appropriate restriction sites into pGEX vector. GST fusion proteins were expressed in *Escherichia coli*, solubilized by sonication of cells in lysis buffer (18) and removal of insoluble debris by centrifugation, and purified on glutathione-Sepharose (Pharmacia); 1 µg of purified protein was used for each binding assay.

Bacterially expressed Flag-Gal4 fusion protein p65 was purified as described previously (18). Histidine-tagged full-length hTAF_{II}135 and hTAF_{II}135 deletion mutants were constructed and cloned in the 6HisT-pRSET vector.

Generation of antibodies against hTAF_{II}135. A cDNA corresponding to the C-terminal portion of hTAF_{II}135 was amplified by PCR with the appropriate restriction enzymes. The PCR-generated fragment was then inserted into bacterial expression construct pET11d (Novagen), which carries the six-histidine tag. The recombinant protein was then expressed in and purified from bacteria and subjected to preparative SDS-PAGE. Gel slices containing the corresponding protein were crushed and used for immunization of two rabbits.

Generation of hTAF_{II}135 cell line. A HeLa cell line constitutively expressing Flag-hTAF_{II}135 (f:135) was made using the pCIN4 expression vector (36).

Far Western blotting. The baculovirus-expressed and purified hTAF_{II}135 was labeled with ³²P at the heart muscle kinase site present in the Flag-tagged sequence (10) by incubation with heart muscle kinase and [γ -³²P]ATP for 30 min at 30°C. Labeled protein was then purified through a nick column (Pharmacia) and used for protein-blot interactions as described (4).

Gel filtration. Purified TFIID preparations from either f:135 or f:TBP cell lines were fractionated on Superose 6 (Smart System; Pharmacia) in buffer BC200 containing 0.05% NP-40. Fractionated proteins were resolved by SDS-PAGE and visualized by silver staining.

DNase I footprinting. Plasmid pML4, containing the major late promoter, was used for DNase I footprinting as described (10). Briefly, the *Eco*RI-*Hind*III DNA fragment from pML4 was isolated and end labeled with ³²P by T4 polynucleotide kinase. Cleavage of the DNA fragment with *Xba*I generated a specific labeled transcribed strand. DNase footprinting reactions and the processing of the labeled products were performed essentially as described previously (10).

In vitro RNA polymerase II transcription assays. Nuclear extracts were prepared as previously described (12). TFIID, TFIIF, and the Flag-thyroid hormone receptor alpha (TR α)-TRAP complex were purified from cell lines expressing Flag-TBP, Flag-hTAF_{II}135, Flag-ERCC3, and Flag-TR α , respectively, using affinity purification on anti-Flag antibody columns as previously described (10, 15, 18). Flag-TR α and Flag-retinoid X receptor alpha (RXR α) were purified from Sf9 cells as previously described (14). Native TFIIA was purified as previously described (18). For TFIIA (p55 and p12), TFIIB, TFIIE α , and TFIIE β recombinant Flag-tagged proteins were expressed in and purified from *E. coli* using an anti-Flag antibody column (M2 agarose; Kodak). Histidine-tagged TBP and TFIIF subunits (RAP30 and RAP74) were prepared as described previously (18). TFIIA and TFIIF were then reconstituted from individually purified components following denaturation and renaturation (45). RNA polymerase II was purified essentially as described previously (3).

Using the purified transcription factors described above, *in vitro* transcription assays were carried out in 25-µl reaction mixtures containing 20 ng of pML Δ 53 or pML200 templates and 50 ng of either pG₅E1b or pTRE₃pML Δ 53 templates. All transcription factors were added simultaneously to the reactions if not indicated otherwise in the figure legends. ³²P-labeled RNA was phenol-chloroform extracted, ethanol precipitated, analyzed directly by 4% polyacrylamide-urea gel electrophoresis, and visualized by autoradiography. Quantitation was done by phosphorimager.

Partial TFIID reconstitution. Following the method that we described earlier (18), human hTAF_{II}250 or hTAF_{II}20 containing a fused N-terminal HA epitope tag was immobilized on protein A-Sepharose containing covalently linked monoclonal antibodies directed against the HA epitope. After extensive washing (BC1000 with 0.1% NP-40), the beads were incubated sequentially (at 4°C for

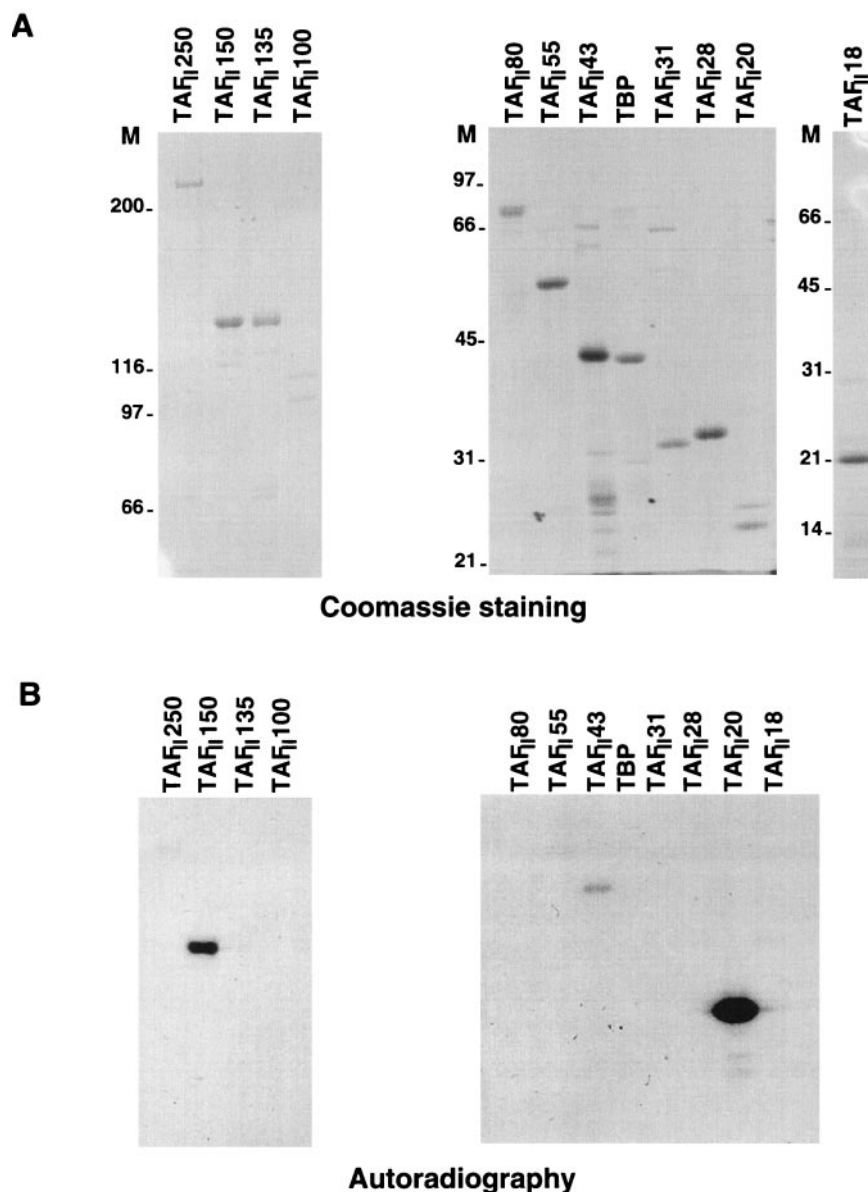


FIG. 1. Comparative far Western assays showing strong interactions between TAF_{II}135 and hTAF_{II}20. (A) Individually expressed (Sf9 cells via baculovirus vectors) and purified TFIID subunits were resolved on SDS-PAGE and visualized by Coomassie staining, M, protein markers, with molecular sizes indicated in kilodaltons on the left. (B) A set of gels equivalent to those in panel A were transferred onto nitrocellulose, denatured-renatured, and used for binding with ³²P-labeled and purified hTAF_{II}135. After extensive washing, bound material was analyzed by autoradiography.

4 h) with molar excesses of additional TFIID subunits. After each incubation, unbound materials were removed by several washes with 100 volumes of BC150 (with 0.1% NP-40). Finally, the resulting complex was eluted with HA peptide (1 mg/ml) in BC100 (with 0.1% NP-40).

In vitro protein-protein interaction assays. Sp1 activation domains A and B, TFIIA subunits p12 and p55, hTAF_{II}20, and hTBP were expressed in and purified from bacteria as GST fusion proteins. Histidine-tagged full-length hTAF_{II}135 and hTAF_{II}135 deletion mutant constructs were expressed in the TNT reticulocyte lysate system (Promega) and labeled with [³⁵S]methionine according to the manufacturer's instructions. Equivalent inputs of radioactive material of full-length hTAF_{II}135 and hTAF_{II}135 deletion mutants were used for binding studies with different GST derivatives. In each reaction, 1 μg of purified GST or GST derivative was immobilized on glutathione, and the appropriate input material was added to each reaction (in 300-μl total volume in BC300 plus 0.1% NP-40). After incubation at 4°C for 2 h, the beads were washed four times

with 300 μl of incubation buffer, and bound proteins were eluted with SDS loading buffer and analyzed by SDS-PAGE and autoradiography.

RESULTS

Strong and specific interaction of hTAF_{II}135 with hTAF_{II}20. The multisubunit nature of TFIID (TBP and TAF_{II}s) and the overall stability of human TFIID suggest a multiplicity of protein-protein interactions, with any single TAF_{II} expected to interact with a number of other subunits. To test for direct and comparative interactions of purified hTAF_{II}135 with affinity-purified TBP and other human TAF_{II}s (hTAF_{II}250, hTAF_{II}150, hTAF_{II}135, hTAF_{II}100, hTAF_{II}80, hTAF_{II}55, hTAF_{II}43,

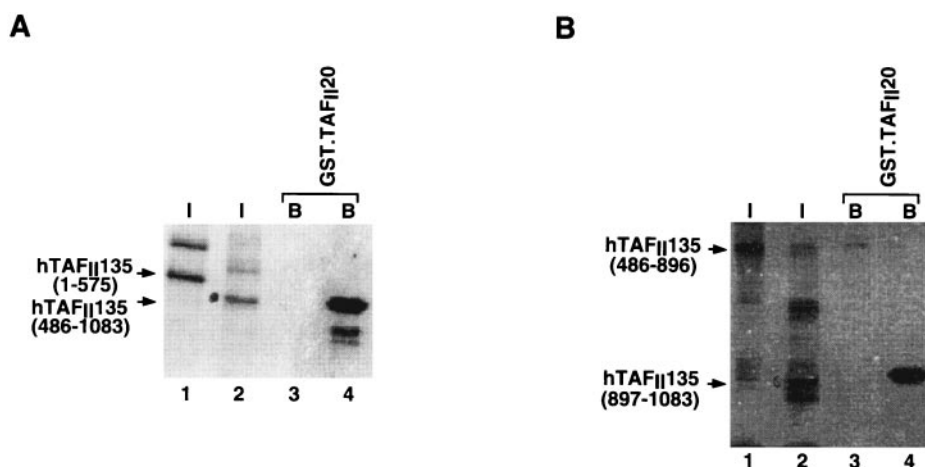


FIG. 2. The extreme C-terminal portion of hTAF_{II}135 interacts with hTAF_{II}20. The indicated deletion mutants of hTAF_{II}135 were [³⁵S]methionine-labeled and incubated with GST-hTAF_{II}20. Input samples (I) contained 10% of the amount used for binding (B). The arrow indicates the band corresponding to the appropriate mutant hTAF_{II}135 deletion.

hTAF_{II}31, hTAF_{II}28, hTAF_{II}20, and hTAF_{II}18), purified proteins (Fig. 1A) were probed with a Flag-tagged, ³²P-labeled hTAF_{II}135 in a far Western blot. This analysis (Fig. 1B) revealed a strong interaction of hTAF_{II}135 with hTAF_{II}20, which was unexpected on the basis of *Drosophila* studies (9), as well as a moderate interaction with hTAF_{II}150 and weak interactions with hTAF_{II}250 and hTAF_{II}43. Since the interaction of hTAF_{II}135 with hTAF_{II}20 was by far the strongest among all TFIID subunits, we speculated that it might have important consequences for de novo assembly of TFIID and, in particular, the recruitment of hTAF_{II}135. Furthermore, although one essential feature of the original octamer-like model was the presence of two hTAF_{II}20 molecules in TFIID, there was no apparent H2A-like partner for hTAF_{II}20 (which was assumed to heterodimerize).

To test the suggestion (above) of an hTAF_{II}20-interacting domain within hTAF_{II}135 as a potential candidate for an H2A-like partner for hTAF_{II}20, radiolabeled hTAF_{II}135 deletion mutants were analyzed for their ability to interact with hTAF_{II}20 (immobilized as a GST-hTAF_{II}20 fusion protein). hTAF_{II}20 interacted with the hTAF_{II}135 C-terminal fragment (486 to 1083), but not with the N-terminal fragment (1 to 575) (Fig. 2A). Further mapping revealed that an hTAF_{II}135 fragment comprised of residues 486 to 896 interacts weakly with hTAF_{II}20, whereas a fragment comprised of residues 897 to 1083 interacts strongly with hTAF_{II}20 (Fig. 2B). This shows that hTAF_{II}20 interaction domain in hTAF_{II}135 is located in the extreme C-terminal region. While this work was in progress, Gangloff et al. (16) showed that hTAF_{II}20 can interact with the C-terminal portion (residues 870 to 951) of hTAF_{II}135 in a yeast two-hybrid assay. Based on their mapping data between hTAF_{II}20 and hTAF_{II}135, as well as sequence alignments of H2A, hTAF_{II}135, hTAF_{II}105, and *Drosophila* TAF_{II}110 (dTAF_{II}110), they proposed a histone H2A-like domain for hTAF_{II}135 in a C-terminal region encompassing amino acids 876 to 944.

Functional in vivo relevance of interaction between TAF_{II}135 and TAF_{II}20. To assess the physiological relevance of the interaction of TAF_{II}135 and TAF_{II}20 and its potential involve-

ment in a histone octamer-like structure, we generated a cell line that stably expressed Flag-tagged TAF_{II}135 (f:135). TFIID complexes were purified from this cell line and from a cell line expressing a Flag-tagged TBP (f:TBP). A Western blot of nuclear extract from f:135 cells revealed a level of f:135 protein expression at least fivefold higher than that of endogenous TAF_{II}135 protein (Fig. 3A). Similar Western blots using antibodies against TBP and various TAFs (hTAF_{II}250, hTAF_{II}150, hTAF_{II}135, hTAF_{II}100, hTAF_{II}80, hTAF_{II}55, hTAF_{II}31, hTAF_{II}20, and hTAF_{II}15) revealed the presence of these components both in TFIID purified from the f:135 cell line and in TFIID prepared from the f:TBP cell line (Fig. 3B). Significantly, however, there was a clear enrichment of hTAF_{II}20 (and the hTAF_{II}15 isoform) in the TFIID preparation from the f:135 cell line compared to that from the f:TBP cell line when normalized to the content of TBP and other TAFs (Fig. 3B). These data are consistent with the observed in vitro interaction between hTAF_{II}20 and hTAF_{II}135. The data further show copurification of natural endogenous hTAF_{II}135 and exogenous Flag-hTAF_{II}135 (Fig. 3A) in the TFIID that was affinity purified (via f:135) on anti-Flag antibody columns and in the same ratio that they are expressed in the f:135 cell line (Fig. 3C). Furthermore, a Western blot with anti-Flag antibody revealed only one band, corresponding to the exogenous Flag-tagged TAF_{II}135, in the TFIID purified from the f:135 cell line and no reactive bands in TFIID purified from the f:TBP cell line (data not shown). Since there is no apparent self-association of hTAF_{II}135 (Fig. 1), this clearly indicates the presence of at least two molecules of hTAF_{II}135 within the TFIID complex.

The enrichment of hTAF_{II}20 in the TFIID preparation from the f:135 cell line could be due to an increased hTAF_{II}135 occupancy in the TFIID and/or to the simple association of hTAF_{II}20 with a fraction of overexpressed Flag-hTAF_{II}135 protein that is purified with, but not incorporated into, TFIID. To test this, we further fractionated purified TFIID complexes from f:135 and f:TBP cell lines on Superose 6 (Smart System). Silver staining of the Superose 6 fractions from the f:135 cell-derived TFIID revealed two hTAF_{II}135-containing peaks (Fig. 3D). The first peak eluted at a position corresponding to a size

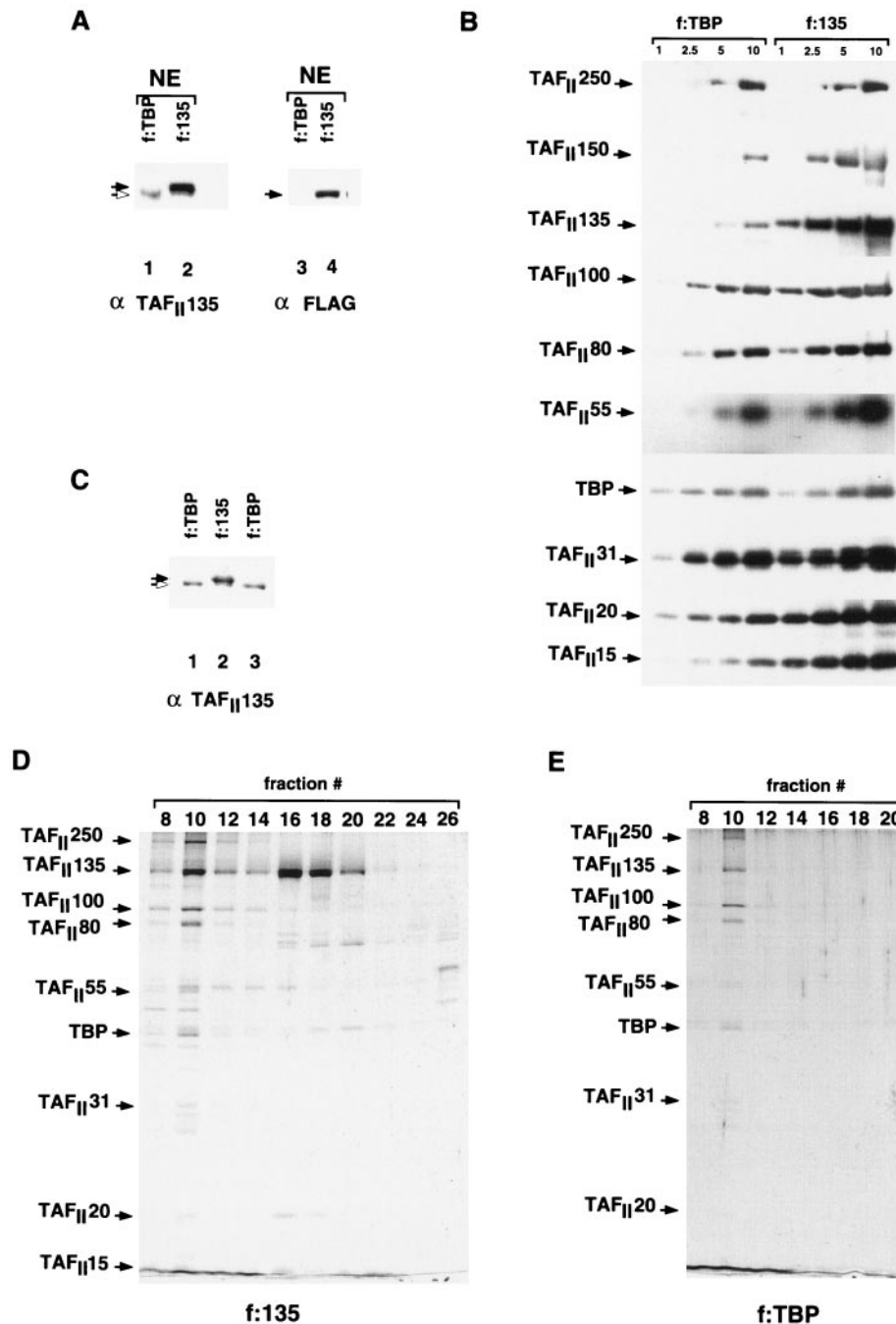


FIG. 3. At least two molecules of TAF_{II}135 coexist in TFIID. (A) Western blot analysis was performed using anti-hTAF_{II}135 (lanes 1 and 2) and anti-Flag antibodies (lanes 3 and 4) to probe SDS-PAGE-resolved proteins in nuclear extracts. Both the endogenous hTAF_{II}135 (open arrow) and Flag-TAF_{II}135 (solid arrow) are present in nuclear extract (NE) prepared from the Flag-TAF_{II}135 (f:135) cell line (lane 1), whereas only the endogenous hTAF_{II}135 band is present (lane 2) in NE from the Flag-TBP (f:TBP) cell line. (B) Comparison of TBP and TAF_{II} expression in the TFIID purified from f:TBP and f:135 cell lines. As indicated, increasing amounts (microliters) of purified TFIID from either cell line were analyzed by Western blotting with antibodies generated against each indicated TFIID subunit. (C) Western blot analysis was performed using anti-hTAF_{II}135 antibody. The endogenous hTAF_{II}135 (open arrow) and Flag-hTAF_{II}135 (solid arrow) proteins coexist in the purified TFIID prepared from the f:135 cell line (lane 2). In the TFIID purified from the f:TBP cell line, only the endogenous hTAF_{II}135 (open arrow) is detected (lanes 1 and 3). (D and E) Indicated fractions, obtained from gel filtration on Superose 6, of purified TFIID from f:TBP and f:135 cell lines, respectively, were resolved by SDS-PAGE and silver stained.

greater than 1 MDa (Fig. 3D, fraction 10) and coincided exactly with the single peak obtained upon fractionation of TFIID purified from the f:TBP cell line (Fig. 3E, fraction 10). It also contained TBP and a normal complement of TAFs and

thus corresponds to TFIID. Significantly, however, the TFIID preparation from the f:135 cell line showed an increased occupancy of both hTAF_{II}135 and hTAF_{II}20 with respect to TBP (and other TAFs such as TAF_{II}100 and TAF_{II}80) compared to

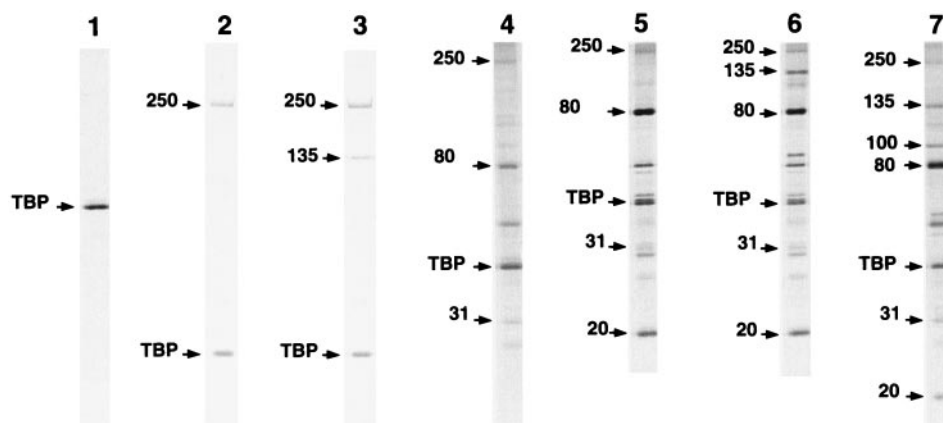


FIG. 4. Role of TAF_{II}135-hTAF_{II}20 interactions in human TFIID assembly. Partially reconstituted TFIID species were resolved by SDS-PAGE and visualized by silver staining. The complexes were assembled with purified subunits that were individually expressed in Sf9 cells via baculovirus vectors (see text). Lane 1 contained TBP alone. Complexes analyzed in other lanes contained hTBP-hTAF_{II}250 (lane 2), hTBP-TAF_{II}250-TAF_{II}135 (lane 3), hTBP-TAF_{II}250-TAF_{II}80-TAF_{II}31, to which added TAF_{II}135 failed to bind in a stoichiometric manner (lane 4), hTBP-TAF_{II}250-TAF_{II}80-TAF_{II}31-hTAF_{II}20 (lane 5), hTBP-TAF_{II}250-hTAF_{II}135-hTAF_{II}80-hTAF_{II}31-hTAF_{II}20 (lane 6), and hTBP-TAF_{II}250-hTAF_{II}135-hTAF_{II}100-hTAF_{II}80-hTAF_{II}31-hTAF_{II}20 (lane 7).

the TFIID from the f:TBP cell line (Fig. 3D, fraction 10, versus Fig. 3E, fraction 10). Apart from background proteins, the second peak from the Superose 6 fractionation contained mainly hTAF_{II}135 and hTAF_{II}20 and eluted at a position corresponding to a size of about 600 kDa; this suggests that hTAF_{II}135 and hTAF_{II}20 can form a stable oligomer (possibly a tetramer) in vivo.

Role of TAF_{II}20 and TAF_{II}135 interaction in human TFIID assembly. The interaction of hTAF_{II}135, through its histone fold, with the H2B-related hTAF_{II}20 constitutes a novel histone-like pair that parallels the well-characterized H3-related hTAF_{II}31 and H4-related hTAF_{II}80 pair. This is consistent with the possibility of a histone octamer-like substructure within TFIID and a central role for TAF-TAF interactions through histone-like folds in TFIID assembly. In this regard, we now show that the interaction between hTAF_{II}20 and hTAF_{II}135 is critical for TFIID assembly in vitro. By use of an earlier-described method for the assembly of human TFIID (18), several combinations of human TAFs and TBP were assembled on an immobilized HA epitope-tagged hTAF_{II}250 or hTAF_{II}20 and eluted with HA peptides. Although hTAF_{II}135 does assemble with the TAF_{II}250-TBP complex in a stoichiometric fashion (Fig. 4, lanes 2 and 3), the subsequent addition of other TAF_{II}s (hTAF_{II}150, hTAF_{II}100, and hTAF_{II}80) resulted in the dissociation of previously bound hTAF_{II}135 (data not shown). In addition, hTAF_{II}135 failed to stably associate with a preformed complex comprised of immobilized hTAF_{II}250, TBP, hTAF_{II}80, and hTAF_{II}31 (Fig. 4, lane 4), indicating that such an association requires an additional TAF(s). In this regard, addition to the TFIID assembly mixture of hTAF_{II}20, along with the histone H3- and H4-like TAF_{II}s (hTAF_{II}31 and hTAF_{II}80), greatly facilitated the incorporation of hTAF_{II}135 (Fig. 4, lane 6 versus lane 5) and, subsequently, hTAF_{II}100 (Fig. 4, lane 7). It is also possible that hTAF_{II}135 gets incorporated into de novo-assembled TFIID as a complex with hTAF_{II}20. These results show a requirement for hTAF_{II}20, along with other histone fold-containing TAFs (hTAF_{II}31 and hTAF_{II}80), for the stable incorporation of hTAF_{II}135 into assembling TFIID, which is

consistent with the in vivo data obtained with the f:135 cell line (Fig. 3). Our data clearly demonstrate a new pathway for the assembly of human TFIID that stresses the importance of hTAF_{II}135-hTAF_{II}20 interactions and is thus distinct from the pathway proposed for *Drosophila* TFIID. In the latter case, earlier assembly studies reported a simple association of dTAF_{II}110 (*Drosophila* homologue of hTAF_{II}135) with a TBP-dTAF_{II}250 complex, followed by sequential association of dTAF_{II}150 and the smaller *Drosophila* TAFs (9).

TAF_{II}135 interacts with TFIIA subunits. In light of previously described interactions of the *Drosophila* homologue of hTAF_{II}135, dTAF_{II}110, with general factor TFIIA and with activation domains of Sp1 (20, 46), we examined protein-protein interactions of hTAF_{II}135 with individual TFIIA subunits and with Sp1 activation domains A and B. The individual p35, p19, and p12 subunits of TFIIA, the p55 precursor of p35 and p19, and Sp1 domains A and B were expressed as GST fusion proteins and used for solution interaction studies with [³⁵S]methionine-radiolabeled full-length hTAF_{II}135. The results show that hTAF_{II}135 interacts with both the unprocessed TFIIA p55 and the derived p35 subunit, but not with the derived p19 subunit, and with the p12 subunit (Fig. 5A). Further studies with several deletion mutants of hTAF_{II}135 (Fig. 5B) revealed interactions of both TFIIA p55 (Fig. 5C) and TFIIA p12 (Fig. 5D) with the extreme C-terminal region (897 to 1083) of hTAF_{II}135.

Functional relevance of TAF_{II}135 interactions with TFIIA. To assess the functional significance of the observed hTAF_{II}135-TFIIA interactions, and because TFIIA can potentiate the binding of TBP to DNA (for reviews, see references 35 and 37), we asked whether hTAF_{II}135-TFIIA interactions could affect the binding activity of TBP within a minimal complex containing only TBP, hTAF_{II}250, and hTAF_{II}135. To this end we employed DNase footprinting to compare binding of TBP-hTAF_{II}250 and TBP-hTAF_{II}250-hTAF_{II}135 in both the absence and the presence of purified natural TFIIA. We also used TBP alone for binding experiments as a control. First, in the absence of TFIIA, limiting amounts of TBP alone bound weakly but specifically to the TATA box region of the adenovirus

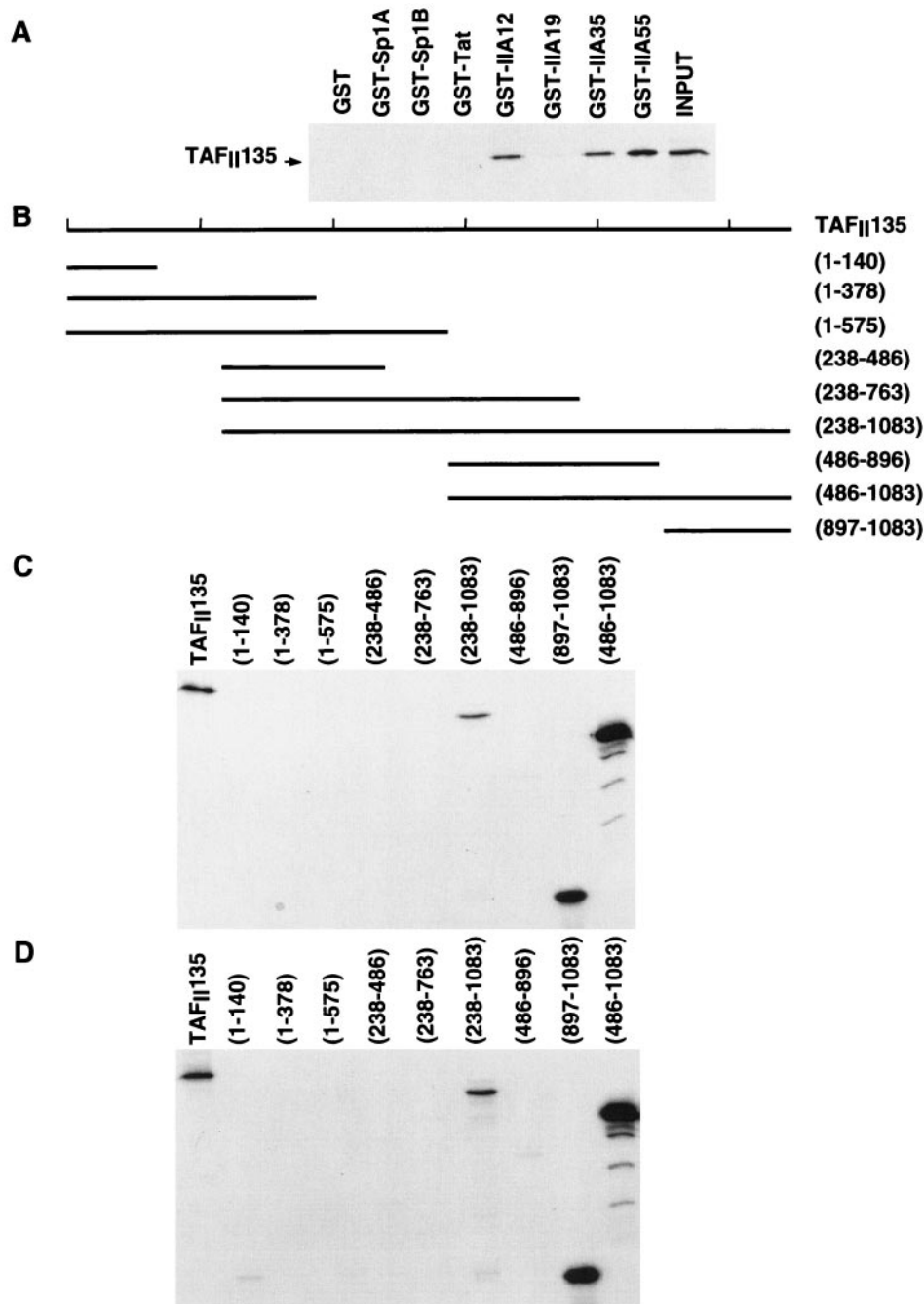


FIG. 5. Strong in vitro interactions between hTAF_{II}135 and TFIIA subunits. (A) Equivalent amounts of full-length [³⁵S]methionine-labeled hTAF_{II}135 were incubated with the indicated GST derivatives (1 μg of each). After extensive washing, bound proteins were resolved by SDS-PAGE and analyzed by autoradiography. Input samples contained 10% of the amount used for binding. The arrow indicates the band corresponding to full length hTAF_{II}135. (B) Deletion mutants of hTAF_{II}135 that were generated, [³⁵S]methionine labeled, and subsequently used for interaction studies (C and D). Equivalent amounts of [³⁵S]methionine-labeled full length hTAF_{II}135 and different deletion mutants were incubated with either GST-IIAp55 subunit (C) or GST-IIAp12 subunit (D). After washing, bound material was processed as in A.

major late promoter (Fig. 6A, lane 3 versus lane 2). As expected, the addition of TFIIA greatly potentiated TBP binding (Fig. 6A, lane 4 versus lane 3). Second, in the absence of TFIIA, the TBP-hTAF_{II}250 and TBP-hTAF_{II}250-hTAF_{II}135 complexes both failed to show significant binding to either the TATA box region or the downstream region of the adenovirus major late promoter (Fig. 6A, lane 5 versus lane 2, and Fig. 6B,

lanes 3 and 5 versus lane 2). This reflects the well-documented inability of TBP, when complexed to hTAF_{II}250 in the absence of a complete set of TAF_{II}s, to efficiently bind DNA (8, 23, 27, 33). Whereas addition of TFIIA to the TBP-TAF_{II}250 complex only marginally increased binding to DNA (Fig. 6A, lane 6 versus lane 5, and Fig. 6B, lane 4 versus lane 3), addition of TFIIA to the TBP-hTAF_{II}250-hTAF_{II}135 complex, which

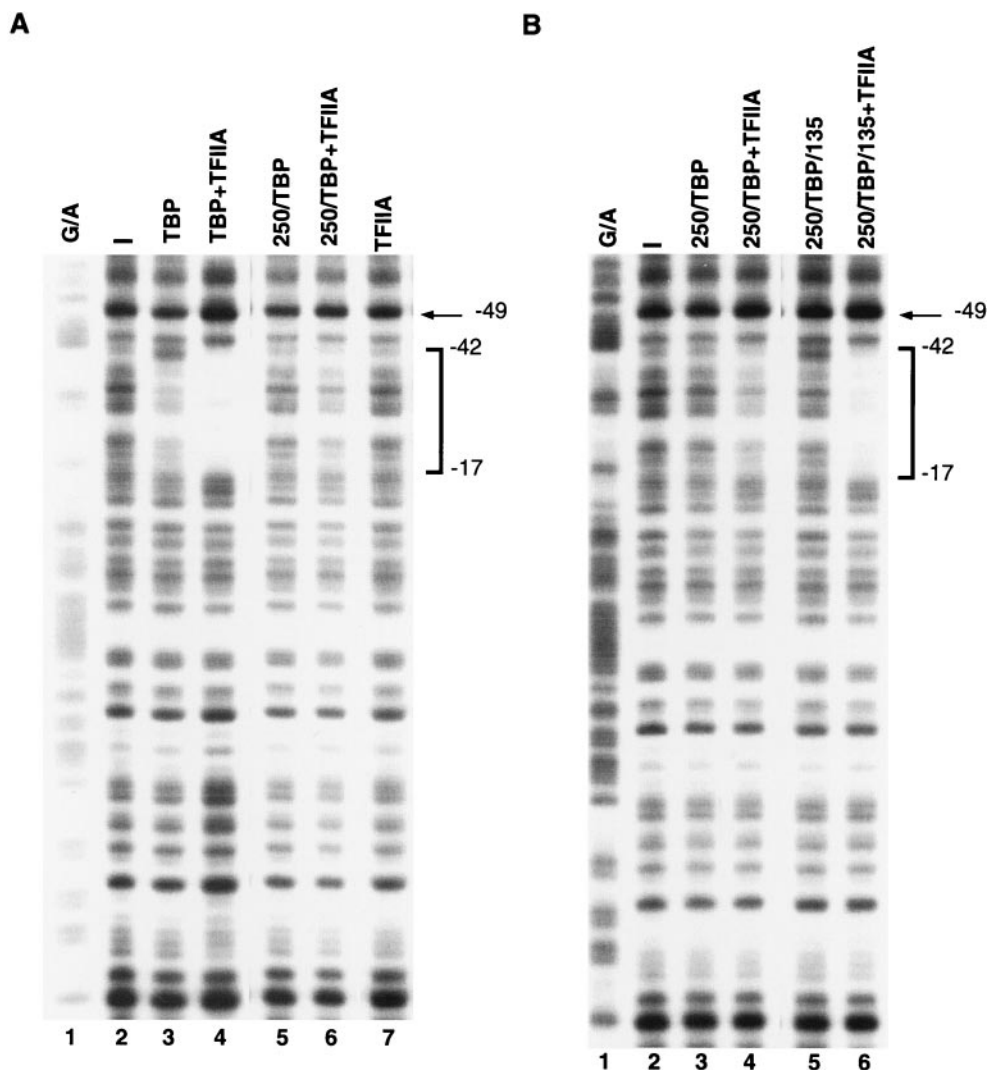


FIG. 6. hTAF_{II}135 and TFIIA synergize to specifically relieve the hTAF_{II}250-mediated inhibition of TBP binding. DNase I footprinting of hTBP, hTBP-hTAF_{II}250, and hTBP-TAF_{II}250-TAF_{II}135. The transcribed DNA template spanning positions -91 to +85 of the adenovirus major late promoter was prepared. The Maxam-Gilbert sequencing method was used to prepare the G/A footprinting marker (lanes A1 and B1). No protein (lanes A2 and B2), hTBP (2.5 ng for lanes A2 and A3), hTBP-hTAF_{II}250 (10 ng of TBP content for lanes A5, A6, B3, and B4), hTBP-TAF_{II}250-TAF_{II}135 (10 ng of TBP content for lanes B5 and B6). Purified TFIIA was added to reactions corresponding to lanes A4, A6, A7, B4, and B6.

alone showed no binding (Fig. 6B, lane 5), led to a strong binding of TBP to the TATA region (Fig. 6B, lane 6 versus lane 5). This clearly shows a synergy between TFIIA and hTAF_{II}135 that specifically relieves the inhibitory function of hTAF_{II}250 on TBP binding. These results are relevant to the natural TFIID, since the binding of natural TFIID on the adenovirus major late promoter is potentiated by TFIIA (data not shown). Furthermore, we have shown that a complex composed of TAF_{II}250, TAF_{II}80, and TBP failed to cooperate with TFIIA to relieve TAF_{II}250-mediated inhibition of TBP binding, thus demonstrating that the action of TAF_{II}135 and TFIIA is specific (data not shown).

Effects of TFIIA on basal and activated transcription in the presence of TFIID and partial TFIID complexes. To study the effects of TAF_{II}s on basal and activated transcription, we compared the effects of equimolar amounts (based on TBP

content) of f-TFIID and partial TFIID complexes. Partial in vitro-assembled TFIID species that contained hTAF_{II}135 in addition to TBP-TAF_{II}250 were designed to test the potential core promoter and coactivator functions of hTAF_{II}135. The transcription system consisted of recombinant and highly purified transcription factors from HeLa cells. The ability of this system to support both basal and activator-dependent transcription was tested simultaneously by using two templates whose correctly initiated G-less transcripts could be differentiated by their size. The activator-responsive template contained three thyroid hormone-responsive elements (TRE) upstream of the adenovirus major late promoter TATA box and natural initiator regions, and activation was mediated by TR, isolated in association with TRAP complex (14), and RXR. First, there was a higher basal activity with the TBP-TAF_{II}250-TAF_{II}135 complex compared to the TBP-TAF_{II}250 complex

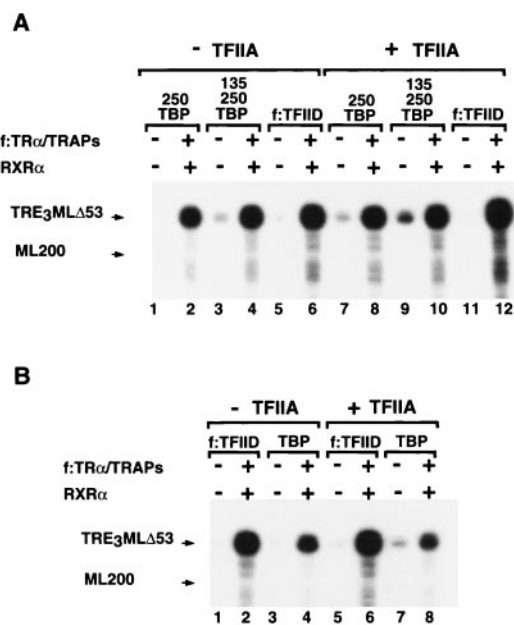


FIG. 7. Effect of TFIIA on basal and TR α -TRAP-activated transcription in the presence of TFIID and partial TFIID complexes. (A) The TFIID-dependent transcription system was supplemented with hTBP-TAF_{II}250 (lanes 1 to 2 and 7 to 8), hTBP-TAF_{II}250-TAF_{II}135 (lanes 3, 4, 9, and 10), and f-TFIID (lanes 5, 6, 11, and 12). Added amounts of TFIID and partial TFIID complexes contained the same amount of TBP (4 ng). Transcription was tested in the absence (lanes 1, 3, 5, 7, 9, and 11) or presence (lanes 2, 4, 6, 8, 10, and 12) of 80 ng of TR α -TRAP complex (12 ng of TR α). Recombinant TFIIA was added to lanes 7 to 12. The TR α -responsive template was TRE₃ML Δ 53 (50 ng), and the control template consisted of 20 ng of pML200, which contains the major late core promoter. Relative transcriptional activities (quantitated by phosphorimager analysis) from the TR-responsive template were 1 (lanes 1, 5, and 11), 2 (lane 3), 2.4 (lane 7), 4.6 (lane 9), 17 (lane 2), 24 (lane 4), 28 (lanes 8 and 10), 30 (lane 6), and 56 (lane 12). (B) Transcription was performed as in A except that f-TFIID was added to lanes 1, 2, 5, and 6 and TBP was added to lanes 3, 4, 7, and 8. Recombinant TFIIA was added to lanes 5 to 8. Relative transcriptional activities from the TR-responsive template were 1 (lane 3), 1.3 (lanes 1 and 5), 3 (lane 7), 11 (lane 8), 14 (lane 4), 42 (lane 2), and 56 (lane 6). The lower band, below the arrow indicating pML200, is a transcript generated from the activator-responsive template.

(Fig. 7A, lane 3 versus lane 1). This could be due, at least in part, to the above-described (Fig. 6) ameliorative effect of TAF_{II}135 on the TAF_{II}250-mediated repression of TBP binding to DNA, especially since these effects are more apparent in the presence of TFIIA (below). Second, basal transcription in the presence of the complete TFIID was totally repressed as a result of the strong repressive effect of the TAFs as a group on TBP function (Fig. 7A, lane 5 versus lanes 1 and 3). Thus, the complete complement of TAFs in TFIID appear to conditionally constrain the ability of TAF_{II}135 to reverse the TAF_{II}250-mediated repression of TBP function in basal transcription, but it is possible that this potential is reactivated in the presence of transcriptional activators. Third, and significantly, the absolute levels of TR-TRAP-activated transcription were slightly higher with TFIID than with the two partial complexes (Fig. 7A, lane 6 versus lanes 4 and 2). In addition, the fold activation (activated/basal transcription ratio) was much higher with TFIID

than with the partial TFIID complexes, due mainly to the potent inhibitory effect of the TAFs as a group, in the complete TFIID, on TBP function in basal transcription (for quantitation, see legend to Fig. 7).

Because of the observed stimulatory effect of TFIIA on binding of the partial TFIID complexes to the major later promoter, we next asked whether TFIIA could affect basal and/or activated transcription by the intact and partial TFIID complexes tested above. Whereas addition of TFIIA with TBP-hTAF_{II}250 and TBP-hTAF_{II}250-hTAF_{II}135 increased basal activity for these complexes (Fig. 7A, lanes 7 and 9 versus lanes 1 and 3, respectively), addition with TFIID had no discernible effect on the very low basal activity observed in its absence (Fig. 7A, lane 11 versus lane 5). Furthermore, TFIIA slightly potentiated the activated transcription observed with intact TFIID but had no apparent effect on the absolute levels of activated transcription observed with partial TFIID complexes (Fig. 7A, lanes 8, 10, and 12 versus 2, 4, and 6, respectively). These findings indicate a direct correlation between the effects of TFIIA on both the binding (Fig. 6) and the basal transcription of partial TFIID complexes (Fig. 7). Moreover, like the functional assays in the absence of TFIIA (above), they also point to roles both for hTAF_{II}135 and for other TAFs in modulating core promoter function. Thus, it is again apparent that the positive effect of hTAF_{II}135 observed in the partial complex is constrained by the full complement of TAFs within TFIID and possibly utilized in this context only in activated transcription. Although these studies with TAF_{II}135 were performed in the absence of its histone fold partner, TAF_{II}20, similar results were observed when a TBP-TAF_{II}250-TAF_{II}135-TAF_{II}20 complex was analyzed (data not shown). Thus, our analysis has allowed us to assign TAF_{II}20-independent functions to TAF_{II}135.

Since the experiments described above were performed with either natural TFIID or partial TFIID complexes, we next asked whether TBP alone could mediate activated transcription in the presence of the TR-TRAP complex in this highly purified transcription system. To this end we compared the ability of TBP and TFIID (at approximately equimolar TBP concentrations based on quantitative Western blot analysis) to mediate TR-TRAP complex-activated transcription. The TR-TRAP complex (in conjunction with RXR) activated transcription more than 30-fold in the presence of natural TFIID and 13-fold in the presence of TBP alone (Fig. 7B, lane 2 versus lane 1 and lane 4 versus lane 3, respectively). These findings, along with our previously published data (15), indicate that TAFs are not unconditionally required for a significant level of activated transcription at the level of free DNA template and that TRAPs might fulfill functional roles analogous or redundant to those performed by the TAFs in natural TFIID. Finally, and consistent with its effect on TBP binding (Fig. 6), TFIIA increased the basal activity obtained with TBP alone (Fig. 7B, lane 7 versus lane 3), but was without effect on the low basal activity with TFIID (Fig. 7B, lane 5 versus lane 1) or the absolute levels of TR-TRAP-mediated activity of either TBP (Fig. 7B, lane 8 versus lane 4) or TFIID (Fig. 7B, lane 6 versus lane 2). This results in a much higher fold stimulation by TR-TRAPs with TFIID than with TBP and is of significance because TFIID is the natural form of the TATA-binding factor

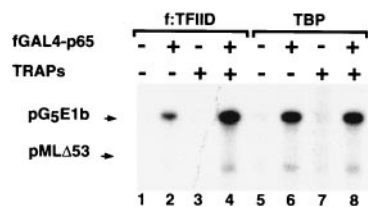


FIG. 8. Synergy of TAF_{II}s with the TRAP complex. The TFIID-dependent transcription system was supplemented with either f-TFIID (lanes 1 to 4) or hTBP (lanes 5 to 8). TRAP complex was added to lanes 3, 4, 7, and 8. Transcription was tested in the absence (lanes 1, 3, 5, and 7) or presence (lanes 2, 4, 6, and 8) of 20 ng of purified fGAL4-p65. Added amounts of f-TFIID and TBP alone contained the same amount of TBP (4 ng). The test template contains the adenovirus E1b TATA and initiator regions and consisted of 50 ng of pG₅E1b template. The control template consisted of 20 ng of pMLΔ53 and contains the major late core promoter sequence. Relative transcriptional activities from the fGAL4p65-responsive template were: 1 (lanes 5 and 7), 3 (lane 2), 10 (lanes 6 and 8), and 14 (lane 4). Basal levels of transcription with TFIID, either with or without TRAPs (lanes 3 and 1, respectively), were too low to be determined, in contrast to the low but measurable levels of basal transcription with TBP (lanes 5 and 7). The lower band, below the arrow indicating pMLΔ53, is a transcript generated from the activator-responsive template.

and since basal (activator-independent) transcription activities are not observed physiologically.

Functional synergism between the TRAP complex and TAF_{II}s. Since the above experiments concerned activated transcription in the presence of the activator TR and the interacting TRAP complex, we next asked whether the TRAP complex, which has been shown to mediate the function of many activators in concert with other positive cofactors such as PC4 (reviewed in reference 28), would exhibit synergistic or redundant functions with the TAFs in the presence of a different activator, fGAL4p65. fGAL4p65 consists of the DNA-binding domain (amino acids 1 to 94) of *S. cerevisiae* GAL4 fused to the potent C-terminal acidic activation domain of the NF-κB p65 subunit. First, to study the effects of TAF_{II}s on basal and activated transcription, we compared the effects of equimolar amounts (based on TBP content) of TBP and f-TFIID on both basal and fGAL4p65-activated transcription in the assay system described above. The test template contained five GAL4 binding sites upstream of the adenovirus E1b TATA and natural Inr regions. Basal transcription was assayed on a template containing only the adenovirus major late core promoter sequence and in a system reconstituted with general transcription factors and the general coactivator. In this assay, and in the absence of TRAPs, fGAL4-p65 strongly activated transcription in the presence of either natural TFIID or TBP (Fig. 8, lane 2 versus lane 1 and lane 6 versus lane 5, respectively; for quantitation, see figure legend). While absolute levels of activated transcription on the E1b promoter were threefold higher with TBP than with TFIID (Fig. 8, lane 6 versus lane 2), basal activity was significantly less with TFIID than with TBP alone (Fig. 8, lane 5 versus lane 1; see figure legend). This leads to a fold activation (activation/basal transcription ratio) that is actually higher for TFIID than for TBP alone. It is important to note that this high level of induction in the presence of TFIID is due to the potent inhibitory effect of TAFs as a group, within

the complete TFIID, on TBP basal activity and the partial reversal of these effects by the activator (18).

In this assay system, addition of the TRAP complex had no apparent effect on basal activity with either TFIID or TBP (Fig. 8, lane 3 versus lane 1 and lane 7 versus lane 5, respectively). Most significantly, however, the TRAP complex strongly (circa fivefold) enhanced the absolute level of activated transcription (mediated by GAL4p65) with TFIID (Fig. 8, lane 4 versus lane 2), while having no effect on the absolute level of activated transcription with TBP alone (Fig. 8, lane 8 versus lane 6). Moreover, in the presence of the TRAP complex, the absolute level of activated transcription was slightly higher with TFIID than with TBP (Fig. 7A, lane 4 versus lane 8). Thus, these results establish, for the first time, a synergism between the TRAP complex and the TAF_{II}s that are naturally present in TFIID and, at least in some cases, inhibitory to the function of TBP. It is important to note that while activation is readily observed in the absence of both TAFs and TRAPs, the TAFs lower basal transcription to a more physiological level and, in doing so also elicit a requirement for TRAPs both for a high absolute level of activated transcription and for a high induction ratio.

DISCUSSION

The TAF_{II} subunits of TFIID have been implicated both as targets for gene-specific activators (reviewed in references 7 and 43) and as modulators (negative and positive) of TFIID binding to diverse core promoter elements (for a review, see reference 38). Although not generally essential for basal transcription directed by TATA elements in core promoters, TAFs are essential for the function of other core promoter elements (initiator and downstream promoter element) either alone or in conjunction with the TATA elements (reviewed in references 17 and 38). As part of our ongoing effort to understand the assembly, structure, and function of human TFIID, we have focused, subsequent to cognate cDNA cloning and expression, on structure-function studies of hTAF_{II}135. We report both extended and novel functions of hTAF_{II}135 that include a critical role, dependent upon histone fold interactions, in TFIID assembly; a synergistic interaction with TFIIA that relieves the well-documented hTAF_{II}250-mediated inhibition of TBP binding and function (8, 23, 27, 33); and contributions to a functional synergy between TAFs and the human TRAP/Mediator complex in transcriptional activation. These findings support an increasing appreciation of TAFs as multifunctional components and TFIID as a dynamic complex subject to a variety of internal and external controls.

hTAF_{II}135-hTAF_{II}20 interactions, through the histone folds, are critical for human TFIID assembly. Taken together, the interaction and mapping data obtained with the baculovirus-expressed proteins and the biochemical characterization of the immunopurified TFIID from the Flag-tagged hTAF_{II}135 cell line show that hTAF_{II}135 and hTAF_{II}20 interact, through histone-like folds (16), to form a complex that coexists in TFIID. The multimeric nature of this complex, presumably a heterotetramer, within TFIID is suggested by the presence of at least two molecules each of hTAF_{II}20 (21) and hTAF_{II}135 (this report) in a single TFIID species. This hTAF_{II}135-hTAF_{II}20 complex may be involved, together with the H4-like hTAF_{II}80

and the H3-like hTAF_{II}31, in the formation of an octamer-like substructure, as previously proposed for the human TFIID (21). Other human TAFs, such as TAF_{II}28 (H3-like) and TAF_{II}18 (H4-like), have also been shown to contain histone folds (2) and to interact strongly with one another (29).

The presence within TFIID of multiple TAFs with histone folds points to the role of this fold in stable protein-protein interactions between TAFs. Furthermore, and more significantly, we have shown that the hTAF_{II}135-hTAF_{II}20 interaction is critical for human TFIID assembly, since this interaction helps stabilize the recruitment of hTAF_{II}135, along with the other histone-like TAF_{II}s (hTAF_{II}80, hTAF_{II}31 and hTAF_{II}20), to an hTAF_{II}250-TBP complex. This core complex is competent to recruit other TAFs, such as hTAF_{II}100, which may play a role in the stabilization of histone-like TAF complexes (42), and hTAF_{II}150. Our data on the assembly of the human TFIID indicate a novel pathway for the assembly of TFIID that is distinct from the one reported for the assembly of *Drosophila* TFIID (9) and point to important roles played by the histone-like motifs in this process. The conservation of this mechanism is suggested by the presence of hTAF_{II}20, hTAF_{II}31, PAF65 α (homologue of hTAF_{II}80), PAF65 β (homologue of hTAF_{II}100), and hTAF_{II}100 in human PCAF and GCN5 complexes and the presence of the *S. cerevisiae* homologues of hTAF_{II}20, hTAF_{II}31, hTAF_{II}80, and hTAF_{II}100 in the *S. cerevisiae* SAGA complex (6).

hTAF_{II}135 interacts with TFIIA to specifically relieve hTAF_{II}250-mediated inhibition of TBP binding and function. We have shown that hTAF_{II}135 interacts with two (the largest and the smallest) of the three subunits of TFIIA. These data confirm and extend an earlier observation of an interaction of dTAF_{II}110, the *Drosophila* homologue of hTAF_{II}135, with the large subunit of *Drosophila* TFIIA (46). A more detailed analysis with hTAF_{II}135 deletion mutants further shows that the human TFIIA subunit interactions are mediated through the C-terminal portion of hTAF_{II}135. Most importantly, from a functional standpoint, an analysis with highly purified TFIIA and in vitro-assembled TFIID subspecies has shown that the interaction of TFIIA with hTAF_{II}135 has a critical role in relieving hTAF_{II}250-mediated repression. Thus, these studies with partial TFIID species have revealed an internal mechanism involving hTAF_{II}135 that could be used in a structurally dynamic natural TFIID to facilitate the transition from TAF_{II}-mediated repression to activation. This leads to speculation that hTAF_{II}135 may be a direct or indirect (e.g., via TFIIA) target for factors that use it to relieve repression during activated transcription. Although TFIIA was shown to have a role in countering inhibitory interactions of the amino terminus of the *S. cerevisiae* TAF_{II}145 (the *S. cerevisiae* homologue of the human hTAF_{II}250) with TBP (24), our observations are the first to show a synergism between TFIIA and a specific TAF_{II} subunit in relieving hTAF_{II}250-mediated repression. They further demonstrate new core promoter functions for both TFIIA and hTAF_{II}135.

TAF_{II}135 core promoter function. In the context of TFIID, TAF_{II}s appear to have coactivator functions mainly on the basis of in vitro studies with metazoan factors (for reviews, see references 38 and 43), whereas core promoter-selective functions are evident from in vivo studies in *S. cerevisiae* (for a review, see reference 17) and from both in vivo and in vitro

studies in metazoans (for review, see reference 38). The transcriptional requirement of TAF_{II}s was assessed originally in purified (reconstituted) cell-free systems in which TBP alone efficiently supported basal but not activator-mediated transcription for several activators. Since TFIID supported both basal and activated transcription in vitro, one or more TAF_{II}s appeared to have a critical coactivator function under the conditions employed. These results, the demonstrations of in vitro interactions between activation domains and isolated TAF_{II}s, and studies with partial (reconstituted) TFIID complexes led to the proposal, consistent with earlier demonstrations of qualitative and quantitative effects of activators on TFIID binding, that TAF_{II}s are direct targets for activators (reviewed in references 7 and 43). The prototype for this paradigm was the activator Sp1 with its proposed "obligate" direct target, dTAF_{II}110, the *Drosophila* homologue of hTAF_{II}135 (9, 20).

Here we have shown that the partial TFIID complexes TBP-hTAF_{II}250 and TBP-hTAF_{II}250-hTAF_{II}135, like TBP alone (15), can mediate robust activated transcription by the TR-TRAP complex. However, the TBP-hTAF_{II}250-hTAF_{II}135 complex mediates higher basal transcription than the TBP-hTAF_{II}250 complex, and this effect of hTAF_{II}135 can be potentiated by TFIIA on the adenovirus major late promoter. Interestingly, similar data for basal transcription were also obtained with partial TFIID complexes using a different core promoter with Sp1-responsive elements (data not shown). Furthermore, the level (fold stimulation) of natural Sp1-activated transcription was comparable for TBP-hTAF_{II}250 and TBP-hTAF_{II}250-hTAF_{II}135 complexes and fivefold lower than that obtained with natural TFIID (data not shown). Taken together, our data underscore a role of hTAF_{II}135 in core promoter function rather than a coactivator function both for TR-TRAP and for Sp1, at least as assayed in these partial TFIID complexes. However, it is possible that hTAF_{II}135 also exhibits a coactivator function, alone or in concert with other TAFs, in natural TFIID. In this regard, it is important to emphasize that natural TFIID promotes relative (fold stimulation) and absolute levels of activation that are higher than those obtained either with TBP alone or with partial TFIID complexes. Natural TFIID achieves this both by inhibiting basal transcription and, in the presence of activator, by reversing this inhibitory effect along with an additional net increase in activation (18). As discussed earlier, the ability of hTAF_{II}135 (with TFIIA) to relieve hTAF_{II}250-mediated inhibition of TBP function in basal transcription, although constrained in natural TFIID relative to partial TFIID complexes, may nonetheless be utilized in TFIID-mediated transcription in response to an activator.

Synergy of TAF_{II}s with TRAP/Mediator complex. The Mediator complex has emerged as a major conduit for communication between gene-specific regulatory factors and the general transcription machinery in both *S. cerevisiae* (reviewed in references 26 and 32) and human (reviewed in reference 28). Given the observation that individual TAF_{II}s are not universally required for transcription activation in either *S. cerevisiae* or metazoans, as well as the function of some activators in the absence of TFIID-specific TAF_{II}s in certain cell-free systems from both metazoans and *S. cerevisiae*, a question of increasing importance is whether the TAF_{II}s may have either redundant or synergistic effects with other prominent coactivators, such as the Mediator.

Here, we have shown that TRAP/Mediator complex synergizes strongly with TAFs in the complete TFIID to potentiate TAF_{II}-mediated activated transcription. Thus, by using intact TFIID, partial TFIID complexes, and TBP, we have dissociated two major functions of TAFs in transcription regulation: intrinsic repressive effects on TBP binding and function that may be core promoter specific and activator-dependent coactivator functions that lead to the reversal of the repressive effects and a large concomitant increase in activation (manifested as synergy with the TRAP/Mediator complex). Because of the above-mentioned properties of TAFs, both the relative (fold stimulation) and absolute levels of activation in the presence of TFIID are usually much higher than those observed in the presence of TBP alone and thus recapitulate more closely the *in vivo* situation. It is important to note that while activation is observed with TBP, the basal levels are high and the levels of induction (activation/basal transcription ratio) are low; the effect of TAFs is both to lower basal transcription to a more physiological level and, in doing so, to elicit a TRAP requirement for simultaneously reversing the inhibition and effecting high absolute levels of activated transcription plus high levels of induction. Therefore, the presence of all TAFs in the natural TFIID increases the dynamic range of transcription regulation, and TAFs (as a group) can serve as both negative and positive cofactors. TAFs also may be subject to regulation (e.g., setting basal or activated levels) by other interacting cofactors, such as PC4 and the TRAP/Mediator.

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