



HAL
open science

Multiple Interactions between hTAFII55 and Other TFIID Subunits

Anne-Claire Lavigne, Gabrielle Mengus, Michael May, Veronika Dubrovskaya, Lászlo Tora, Pierre Chambon, Irwin Davidson

► **To cite this version:**

Anne-Claire Lavigne, Gabrielle Mengus, Michael May, Veronika Dubrovskaya, Lászlo Tora, et al.. Multiple Interactions between hTAFII55 and Other TFIID Subunits. *Journal of Biological Chemistry*, 1996, 271 (33), pp.19774 - 19780. <10.1074/jbc.271.33.19774>. <hal-03108453>

HAL Id: hal-03108453

<https://hal.science/hal-03108453v1>

Submitted on 13 Jan 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/14499030>

Multiple Interactions between hTAF(II)55 and other TFIID subunits: Requirements for the formation of stable ternary complexes between hTAF(II)55 and the TATA-binding protein

Article in *Journal of Biological Chemistry* · September 1996

DOI: 10.1074/jbc.271.33.19774 · Source: PubMed

CITATIONS

47

READS

15

7 authors, including:



Anne-Claire Lavigne

French National Centre for Scientific Research

28 PUBLICATIONS 826 CITATIONS

SEE PROFILE



Gabrielle Mengus

Institut de Génétique et de Biologie Moléculaire et Cellulaire

64 PUBLICATIONS 1,533 CITATIONS

SEE PROFILE



László Tora

Institut de Génétique et de Biologie Moléculaire et Cellulaire

305 PUBLICATIONS 17,443 CITATIONS

SEE PROFILE



Pierre Chambon

Institut de Génétique et de Biologie Moléculaire et Cellulaire

1,159 PUBLICATIONS 167,880 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Gene mapping [View project](#)



Analysis of the composition and the function of the basal transcriptional machinery during development [View project](#)

Multiple Interactions between hTAF_{II}55 and Other TFIID Subunits

REQUIREMENTS FOR THE FORMATION OF STABLE TERNARY COMPLEXES BETWEEN hTAF_{II}55 AND THE TATA-BINDING PROTEIN*

(Received for publication, March 19, 1996, and in revised form, May 24, 1996)

Anne-Claire Lavigne†, Gabrielle Mengus, Michael May§, Veronika Dubrovskaya¶, Laszlo Tora, Pierre Chambon, and Irwin Davidson||

From the Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, Collège de France, B. P. 163, 67404 Illkirch Cédex, France

We have cloned and characterized the human TATA-binding protein (TBP)-associated factor hTAF_{II}55. hTAF_{II}55, which has no known *Drosophila* counterpart, is present in both of the previously described TFIID α and TFIID β subpopulations. We describe the interactions of hTAF_{II}55 with other subunits of the transcription factor TFIID. By cotransfection in COS cells, we show that hTAF_{II}55 interacts with hTAF_{II}250, hTAF_{II}100, hTAF_{II}28, hTAF_{II}20, and hTAF_{II}18, but not with hTAF_{II}30 or TBP. Analysis of the binding of hTAF_{II}55 and TBP to hTAF_{II}28 deletion mutants indicates that distinct regions of hTAF_{II}28 are required for these interactions. Although hTAF_{II}55 does not interact by itself with TBP, stable ternary complexes containing hTAF_{II}55 and TBP can be formed in the presence of hTAF_{II}250, hTAF_{II}100, or hTAF_{II}28. These results not only show that hTAF_{II}100 and hTAF_{II}28 interact with TBP, but also that they can nucleate the formation of partial TFIID complexes.

The transcription of protein-coding genes in eukaryotes involves the formation of a multiprotein complex containing the RNA polymerase II core enzyme and a series of auxiliary factors (TFIIA, TFIIB, TFIID, TFIIIE, TFIIF, and TFIIH) (reviewed in Refs. 1–4). Although these auxiliary factors can be assembled in an ordered fashion *in vitro* to form a functional preinitiation complex, in yeast and mammalian cells, many of these factors are associated with the RNA polymerase II core enzyme in a holoenzyme complex (5–7).

The transcription factor TFIID is itself a multiprotein complex comprising the TATA-binding protein (TBP)¹ and TBP-associated factors (TAF_{II}s) (Refs. 8–14; for reviews, see Refs. 15 and 16). In *Drosophila* embryos, TFIID has been reported to

exist as a homogenous complex comprising TBP and eight dTAF_{II}s (17), while we have shown that HeLa cell (h)TFIID exists in several chromatographically separable and functionally distinct forms (13, 18–20). Purification of hTFIID by chromatography and/or sequential immunoprecipitation with antibodies against TBP and hTAF_{II}30 identified two TFIID populations, TFIID α and TFIID β , which lack or contain hTAF_{II}30, respectively (21). Analysis of the TAF_{II} composition of the TFIID α and TFIID β complexes led us to propose the existence of core hTAF_{II}s (exemplified by hTAF_{II}250, hTAF_{II}135, hTAF_{II}100, and hTAF_{II}28), present in all TFIID complexes, and specific hTAF_{II}s (exemplified by hTAF_{II}30, hTAF_{II}20, and hTAF_{II}18), present in only a subset of TFIID complexes (21, 22).

The cDNAs encoding many *Drosophila* and human TAF_{II}s have been isolated (21–29). More recently, yeast homologues of the metazoan TAF_{II}s have been identified, and analysis of their sequence shows that the TAF_{II}s have been highly conserved during evolution (30, 31). Comparison of *Drosophila* and human TAF_{II}s indicates that, in general, the carboxyl-terminal regions are much better conserved than the amino-terminal regions (22, 29). Nevertheless, several human TAF_{II}s (hTAF_{II}30, hTAF_{II}18, and hTAF_{II}55) have no known *Drosophila* counterparts.

The isolation of cDNAs encoding the TAF_{II}s has facilitated the study of TAF-TAF and TAF-TBP interactions and the reconstitution of TFIID complexes using recombinant proteins. Comparison of TAF-TAF and TAF-TBP interactions observed with recombinant hTAF_{II}s or dTAF_{II}s indicates considerable similarities, but also some notable differences. For example, hTAF_{II}28 interacts with TBP both *in vitro* and in transfected COS cells (22), whereas no analogous interaction was observed with its *Drosophila* homologue, dTAF_{II}30 β (24). Similarly, hTAF_{II}100 interacts with TBP and can be stably incorporated into a partial TFIID complex with hTAF_{II}250 and TBP (29), whereas contradictory results concerning its *Drosophila* homologue, dTAF_{II}80, have been reported. Dynlacht *et al.* (32) observed that dTAF_{II}80 did not interact with dTBP and could only be incorporated into dTFIID following assembly of a dTAF_{II}250-dTAF_{II}110-dTAF_{II}60-dTBP complex, while Kokubo *et al.* (33) detected direct interactions between dTAF_{II}80 and TBP. These results highlight potential differences in the organization of dTFIID and hTFIID that may either be due to the different experimental protocols used or reflect real differences between dTFIID and hTFIID related to the presence of at least three additional TAF_{II}s in hTFIID.

Based on the observation that transactivation was supported by TFIID, but not TBP (11, 13, 34, 35), it was proposed that some TAF_{II}s may act as coactivators. This was first confirmed by Hoey *et al.* (23), who showed that direct interaction between

* This work was supported in part by grants from CNRS, INSERM, the Centre Hospitalier Universitaire Régional, the Ministère de la Recherche et de la Technologie, the Association pour la Recherche contre le Cancer, and the Collège de France. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) X97999.

† Supported by a fellowship from the Ligue Nationale contre le Cancer.

§ Supported by a grant from the Deutsche Forschungsgemeinschaft.

¶ Present address: Dept. of Chemistry, Dartmouth College, Hanover, NH 03755-3564.

|| To whom correspondence should be addressed. Tel.: 33-88-65-34-40/45; Fax: 33-88-65-32-01; E-mail: irwin@titus.u-strasbg.fr.

¹ The abbreviations used are: TBP, TATA-binding protein; TAF_{II}, TBP-associated factor; prefixes h and d, human and *Drosophila*, respectively; mAb, monoclonal antibody; PCR, polymerase chain reaction; GST, glutathione S-transferase.

the transactivator Sp1 and dTAF_{II}110 was required for transactivation *in vitro*. Subsequently, several studies confirmed our initial proposal (13, 18) that, depending on the nature of their activation domain, transactivators interact selectively and directly with different TAF_{II}s to activate transcription *in vitro* (21, 28, 36–38). However, TAF_{II}s such as dTAF_{II}150 and its putative human counterpart, CIF, may also play a role in promoter recognition (39–41). In addition, the ability of some TAF_{II}s to adopt a histone-like fold structure suggests that they also play a role in modeling DNA architecture around the promoter (42, 43).

We have characterized the roles played by hTAF_{II}30 and hTAF_{II}28 in the activation of transcription by members of the nuclear receptor superfamily (21, 44). This family comprises the receptors for steroid/thyroid hormones, retinoic acid, and vitamin D₃ (reviewed in Refs. 45–51), whose ability to activate transcription is regulated by the binding of their cognate ligands. We have shown that ligand-independent transactivation *in vitro* by the estrogen receptor involves direct interactions between the AF-2a domain, which is present in α -helices H1 and H2 of the estrogen receptor ligand-binding domain (21, 52) and which is also active in yeast, and hTAF_{II}30 (53). Moreover, we have also shown that coexpression of hTAF_{II}28 potentiates ligand-dependent transactivation by the RXR, the estrogen receptor, and the vitamin D₃ receptor in transfected COS cells (44). The coactivator activity of hTAF_{II}28 does not require direct hTAF_{II}28-receptor ligand-binding domain interactions, but requires hTAF_{II}28-TBP interaction. These results show that hTAF_{II}28 probably acts as a bridging factor between the transcriptional intermediary factors associated with the ligand-binding domains and the basal transcription factors via its interaction with TBP. These results highlight the important role that hTAF_{II}s may play in transcriptional regulation in mammalian cells.

In this study, we describe the properties of hTAF_{II}55. We show that hTAF_{II}55 is present in TFIID α , but is mainly associated with TFIID β . hTAF_{II}55 interacts with hTAF_{II}250, hTAF_{II}100, hTAF_{II}28, hTAF_{II}20, and hTAF_{II}18, but not with hTAF_{II}30 or TBP. Although it does not interact with TBP, hTAF_{II}55 can be incorporated into stable ternary complexes comprising TBP and hTAF_{II}250, hTAF_{II}100, or hTAF_{II}28 to form partial TFIID complexes. Furthermore, tagging hTAF_{II}55 with hTAF_{II}18 generates a fusion protein that can interact with TBP.

MATERIALS AND METHODS

Isolation of a cDNA Encoding hTAF_{II}55—HeLa cell nuclear extracts were prepared and purified on heparin-Ultrogel as described. TFIID was immunopurified using the anti-TBP mAb 2C1 and eluted with a peptide containing the 2C1 epitope. TFIID was concentrated, separated by SDS-polyacrylamide gel electrophoresis, and transferred to a polyvinylidene difluoride membrane as described previously. The hTAF_{II}55 subunit was excised and digested with trypsin. Four tryptic peptides were obtained: AVQSGHVNLIK, LTIELHPDGR, LLSTDAEAVSTR, and WEIAAEDETK. Based on these sequences, degenerate oligonucleotides were synthesized (5'-GC(C/T)GT(C/G)CA(A/G)(T/A)(G/C)(C/T)GG(A/C/G/T)CA(C/T)GT(C/G)AA(C/T)(C/T)T(C/G)AA-3', 5'-AC(A/C/T)AT(C/T)GA(A/G)CT(C/G/T)CA(C/T)CC(A/C/T)GA(C/T)AT-3', 5'-AC(A/C/T)GA(C/T)GC(C/T)GA(A/G)GC(C/T)GT(C/G)(T/A)(C/G)(C/T)(A/C)(A/C/T)(C/A)G-3', and 5'-GA(A/G)GA(A/G)AT(C/T)AT(C/T)GC(C/T)GA(A/G)GA(C/T)GA(A/G)AC(A/C/T)(C/A)G-3') and used to screen a HeLa cell random-primed cDNA library as described. Seven clones hybridizing to two or more probes were isolated and sequenced on both strands using an Applied Biosystems automated sequencer. The resulting data were analyzed using the Genetics Computer Group sequence analysis programs.

Construction of Expression Vectors—The complete open reading frame of hTAF_{II}55 was amplified by the polymerase chain reaction (PCR) from a cDNA clone using primers with *Bam*HI and *Xho*I restriction sites. The resulting fragment was cloned into the eukaryotic ex-

pression vector pXJ41. GST-hTAF_{II}55 was constructed by PCR amplification with primers containing *Bam*HI sites and cloning into the *Bam*HI site of pGEX2T. B10-tagged hTAF_{II}55 was constructed by PCR amplification with primers containing *Spe*I sites and cloning into the *Nhe*I site of pAT6. The hTAF_{II}55-hTAF_{II}18 fusion construct was constructed by ligating three fragments, a PCR fragment containing the entire hTAF_{II}55 coding sequence with *Bam*HI and *Xho*I restriction sites, a *Xho*I-*Pst*I restriction fragment containing the hTAF_{II}18 coding sequence, and *Bam*HI-*Pst*I-digested pXJ41. The sequences of all constructs were verified by automated DNA sequencing. The rationale for the construction of hTAF_{II}28-(1–179)-M1 stems from computer predictions using the Chou and Fasman algorithm in the Genetics Computer Group software package and using the PHD program (EMBL), which indicated that amino acids 161–179 (FVGEVVEEALDVCEKWGEM) of hTAF_{II}28 had the potential to form an amphipathic α -helix with a highly hydrophobic face (underlined) and a hydrophilic face where 6 out of the 7 amino acids (with the exception of K175) are acidic (shown in boldface). In hTAF_{II}28-(1–179)-M1, Glu-164, Glu-167, and Glu-168 were mutated to Pro, Pro, and Arg, respectively, both changing the charge and disrupting the putative α -helix (44). The other hTAF_{II} and TBP expression vectors are as described previously (22, 29, 44).

Immunization and Monoclonal Antibody Production—Monoclonal antibodies against hTAF_{II}55 were prepared essentially as described previously (22). Briefly, GST-hTAF_{II}55 was expressed in *Escherichia coli* and purified. Mice were injected intraperitoneally three times at 2-week intervals with 200 μ g of GST-hTAF_{II}55. Following fusion of spleen cells, the hybridoma culture supernatants were screened by immunofluorescence on COS cells transfected with pXJ41-hTAF_{II}55. Monoclonal antibodies against TBP (3G3), hTAF_{II}18 (16TA), hTAF_{II}20 (22TA), hTAF_{II}28 (15TA), hTAF_{II}30 (4G2 and 2F4), and the B10 tag were prepared as described previously (21, 22, 29, 54, 55).

Expression and Purification of Recombinant Proteins—GST-hTAF_{II}55 was overexpressed and purified from *E. coli* by standard procedures on glutathione-Sepharose. Native recombinant hTAF_{II}55 was prepared by immobilizing GST-hTAF_{II}55 on glutathione-Sepharose and adding thrombin ($1/400$ of the GST-hTAF_{II}55 mass). Digestion was performed by resuspending the glutathione-Sepharose-immobilized GST-hTAF_{II}55 in buffer A (50 mM Tris-HCl, pH 7.9, 20% glycerol, 0.1% Nonidet P-40, 0.5 mM EDTA, and 1.0 mM dithiothreitol) containing 0.1 M KCl and 2.5 mM CaCl₂ for 2 h at room temperature. The column was then allowed to elute, and the effluent was collected. The recombinant protein was >80% pure as determined by SDS-polyacrylamide gel electrophoresis and staining with Coomassie Brilliant Blue.

Transfection of COS Cells and Immunoprecipitations—COS cells were transfected by the calcium phosphate coprecipitation technique, and immunoprecipitations were performed as described previously (22, 29, 44). 48 h following transfection, the cells were harvested by three cycles of freeze-thawing in buffer A containing 0.5 M KCl. Expression of the transfected proteins was verified on Western blots. For immunoprecipitations, the cell extracts were incubated for 1 h at 4 °C with 1–2 μ g of the indicated monoclonal antibodies, after which time 50 μ l of protein G-Sepharose was added, and incubation was continued for another 2 h. The protein G-Sepharose was then washed four times for 10 min at room temperature with buffer A containing 1.0 M KCl and once with buffer A containing 0.1 M KCl. The resin was resuspended in loading buffer containing SDS, boiled for 5 min, and subjected to SDS-polyacrylamide gel electrophoresis. The bound proteins were detected on Western blots with the indicated antibodies using an ECL kit (Amersham Corp.).

RESULTS

Isolation of a cDNA Encoding hTAF_{II}55—HeLa cell nuclear extracts were fractionated on a heparin-Ultrogel column, and total TFIID was immunoprecipitated from the 0.6 M KCl fraction using the anti-TBP mAb 3G3. TFIID was separated by SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane, and the 55-kDa subunit was excised and digested with trypsin. The sequences of four tryptic peptides were obtained, and degenerate oligonucleotides based on these sequences were used to screen a HeLa cell cDNA library. Several overlapping clones hybridizing to more than one of the oligonucleotides were isolated and sequenced. The hTAF_{II}55 cDNA sequence deduced from these clones comprises an open reading frame encoding a protein of 349 amino acids preceded by a long 5'-untranslated region (at least 723

nucleotides) (Fig. 1A). Each of the four tryptic peptide sequences was found in the open reading frame (underlined in Fig. 1A). On Northern blots, a single mRNA species of ~2.4 kilobase pairs was detected in several human embryonic tissues (Fig. 1B) and in a variety of human cell lines (data not shown). Thus, the deduced nucleotide sequence in Fig. 1A corresponds to the almost complete cDNA. Interestingly, the sequence of hTAF_{II}55 shows no homology to any of the known *Drosophila* TAF_{II}s. This observation, together with those of previous studies, confirms that hTFIID comprises three hTAF_{II}s (hTAF_{II}18, hTAF_{II}30, and hTAF_{II}55), with no known *Drosophila* homologues.

The hTAF_{II}55 open reading frame was fused to the GST protein and expressed in *E. coli*. The purified fusion protein was injected into mice to generate mAb 19TA. mAb 19TA specifically recognizes hTAF_{II}55 in total TFIID immunopurified with mAb 3G3 (Fig. 2A, lane 1). When mAb 19TA was used to reimmunoprecipitate total hTFIID, both hTAF_{II}55 and TBP were immunoprecipitated (Fig. 2A, compare lanes 1–3 with lanes 4–6). This confirms that the isolated cDNA encodes a *bona fide* hTAF_{II}. However, a significant fraction of hTAF_{II}55 and TBP remained in the supernatant, indicating that not all of the hTAF_{II}55 present in TFIID reacted with mAb 19TA (Fig. 2A, lanes 2 and 5). As mAb 19TA efficiently precipitated recombinant hTAF_{II}55 (see below and data not shown), this suggests that the corresponding epitope is partially hidden in the TFIID complex.

We have previously shown that hTFIID can be separated by immunoprecipitation with an antibody directed against hTAF_{II}30 into two populations, hTFIID α and hTFIID β , either lacking or containing hTAF_{II}30, respectively (Fig. 2B) (21, 22). Total TFIID was reimmunoprecipitated with the hTAF_{II}30 antibody (mAb 2F4), and the presence of hTAF_{II}55 in the TFIID α and TFIID β fractions was determined by Western blotting with mAb 19TA. Both hTAF_{II}55 and hTAF_{II}30 were detected in the total TFIID fraction (Fig. 2B, lanes 1 and 4). Reimmunoprecipitation with the mAb against hTAF_{II}30 generated the TFIID α fraction, which was almost completely devoid of hTAF_{II}30, and the TFIID β fraction, which contained hTAF_{II}30 (Fig. 2B, lanes 2 and 3). In contrast, hTAF_{II}55 was detected in both the TFIID α and TFIID β fractions, although it is preferentially associated with TFIID β (Fig. 2B, lanes 2, 3, 5, and 6).

Interactions between hTAF_{II}55 and Other Subunits of the TFIID Complex—To systematically characterize the interactions of hTAF_{II}55 with the other TFIID subunits, native hTAF_{II}55 or derivatives of hTAF_{II}55 tagged with the B10 epitope of the estrogen receptor (22, 54) were coexpressed in COS cells with native or B10-tagged derivatives of other TFIID subunits. Using this technique, we have previously characterized TAF-TAF and TAF-TBP interactions (22, 29). We have shown that these interactions occur intracellularly as efficient coimmunoprecipitation was only observed when the target proteins were coexpressed, but not when extracts from independently transfected cells were mixed together (Ref. 22; for hTAF_{II}55, data not shown). It is also likely that the overexpressed proteins interact directly rather than indirectly via interactions with endogenous COS TAF_{II}s, which are much less abundant than the overexpressed proteins and are already stably associated in endogenous COS cell TFIID (see also below). Furthermore, we have previously noted TAF-TAF interactions in transfected COS cells that were not observed *in vitro*, e.g. hTAF_{II}28-hTAF_{II}20 (22). Following transfection, the cell extracts were immunoprecipitated with mAbs directed against TBP, the hTAF_{II}s, or the B10 tag, and the immunoprecipitated proteins were analyzed on Western blots. The analysis of interactions with hTAF_{II}55 is somewhat complicated by the fact

A	1 61 121 181 241 301 361 421 481 541 601 661 721 (1) 781 (20) 841 (40) 901 (60) 961 (80) 1021 (100) 1081 (120) 1141 (140) 1201 (160) 1261 (180) 1321 (200) 1381 (220) 1441 (240) 1501 (260) 1561 (280) 1621 (300) 1681 (320) 1741 (340) 1801 1861 1921 1981 2041 2101	GGAATCCCCCGGACGGAGGGCCGGCGAGGTGCGGGTCTGTTGATGCGAGTCCGCCCTC TCGGCAAGATTTCGGCGTGCCTACCCGCGCCCTTCATCAGTAATCGTGTAGTGGATCAC TCTGCCAAGCGGGCAGGAGGATTAAGGAAACACAGGAGAGGAGCGCTCCGCTCTCCCGCT TGCTCCTTCGGCCCTCCTCTCCCTCGCTCAAGCCCGGGAACCTGAACCTAFAAICG TCACATGGATTGTAAGTACCGGAGGCGAGAGAGAGTCCGTGAGCCCTGATTTTTGAGTGT CTTTGTTCCGGGAGAGTGTGAGTGTGAAAGATCTCTGCTGGCTGGCTTTCTGGCCGAAA CCGTTCGGGGGAGCGCCCTTTGCTTCTCTCCCTGCTGCTGCTGCTGCTGCTGCTGCTGCT GCCTTCGGGCGAGCGCCCTCAGAGAGTGGAGCTTGGCATTTGATTAAGAAC TTAAGTCTGTGAATAGCCCTTTGTTGTTAAGCAGTGGTATTCGGTACAGCATATTAGAG AAAAGACTTGGAGCTTAATATAAACTAAGGCAAAATAGACGCTTAGCTGATCTACA GAGAATCTTCTAATTAAGATTTCAATCATAGCAAACTGGTGTTTAAACTATTGCG AGTACTGGAACTTTTAGTGTAAACAGCATTTATTTGGAAGTGAATCACAGGAAATA AAAGAGTAAAAGCAAAGATGATGCTCCTCACGAAGTGGAGAGCCGATTTATCTACGCT (1) M S K S K D D A P H E L E S Q F I L R CTGCCTCCAGAAATATGCTCTACTGTGAGAGGCGAGTACAGTCTGGTCATCTCAACCTC (20) L P P E Y A S T V R R A C S G H V N L I 1 AAGACAGACTGACAATTGAGTTAGATCCGTGAGGCGTCAATGGAATCGTCAGAGTGGAC (40) K D R L T T I E L H P D G R H G I V R V D 2 CGTGTCCATTGGCCCTCAAATTAAGTACAGTCCCTGCTGTTATGAAAGCTTGAAGAAC (60) R V P L A S K L V D L P C V M B S L K K T ATTGATAAAAAAATTTTACAGAGCTGATATCTGTCAGGAGCTTGTATCCACAGTT (80) I D K K T F Y K T A D I C Q M L V S T V GATGTTGATCTCTATCTCCTCTGGAGAGCCAGTGTGATGAGCATGCTCAAGCAAGC (100) D G D L Y P P V B E P V A S T D P K A S AAGAAAGCATAGGACAAAGAAAAGTATTTATGCAACCCAGGAATTAATCTGCGCT (120) K K K D K D K E K K F I W N H G I T L P CTAAGAAATGTCAGGAGAGAGGTTCCGGAGAGCAGCAAGAAAGAAATATTAATGAACT (140) L K N V R K R R F R K T A K K K Y I E S CCAGATCTTGAAGAAGTGAAGCAATGCTGAGTACAGTGTGAGCTGTAGTGTACT (160) P D V E K E V K R L L S T D A R A V S T 3 CGTGGGAAATAATTCGGGAGATGAAACAAAGGAGGCGCAAAATCAAGCCCTGGATATC (180) R W E I T A E D E T K E A E N Q G L D I 4 TCTTCTCCAGGAATCTGCTGCTCACAGGCGAGGCCATGACTCATTGAACATGATGAGCTT (200) S S P G M S G H R Q G H D S L E H D E L CGGGAGATAATCAATGACCTCAGCAGCAGCAGTGAAGATGAAGATGAGACCAGCATCAA (220) R E I F N D L S S S S E D E D E T Q H Q GATGAAGAAGATATAAATCAATGACACCGGAGGAAGATCTGGAGACAGCTACAGGAC (240) D E E D I N I I D I G A D E L R Q L D AAGCTAATGAATCAGATGAACAGCACCAGGAAAATGAAGAACCAATCAGCTGGTTATG (260) K L N E S D E Q H Q E N E G T N Q L V M GGAATTCAGAAAGCAGATTGACAACTGAAGAGCAGCTCCAGAGAACCCAGGACAGGGCA (280) G I Q K Q I D N M K R Q G H D S L E Q E T Q D R A AAACGACAGAGGATCTCATCATGAAAGTGAAGAAATCTGCTCCTCAAGAACAGATTCAG (300) K R Q E D L I M K V E N L A L K N R F Q GCTTACTGGATGAGCTCAACAAAGGAGAGCCGAGAAAGAGGAGCACTCAGCTCTTTG (320) A V L D E L K Q G L E R D E K E Q L S L S CAAGAGGAGCTAGAACTCACTCCTAGAGAAAGTGAAGAAATGATATTTAATTCAGCTTT (340) Q E E L E S L L E K * CGACTGGTCAGCATTAGAAATTTCTGGCTTTATGTTACTGGGTATTAAGACCTTGCTC TTCCTAGCTTTTAAATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT TTTTGAGCAATTTAATTTGTTTCTGAGTGTCTCATCTATAAGAGCAGGATTTGATATA GGCAGAAATGATTTAGGAAAGTGCAGGATTAGCGAATGATGGTTCAACCTTAAT TATAGCTTATGCAAGACTTACTGTTTCTCCATTTCTAGAAGCTGCTGTCTGCTCTT TGTGATGACGTGAGATCAATAAGAAGAAC
---	--	---

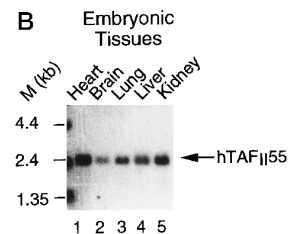


FIG. 1. A, the nucleotide and amino acid sequences of hTAF_{II}55. The nucleotide sequence of hTAF_{II}55 and the deduced amino acid sequence are shown. The peptides derived from sequencing of endogenous HeLa cell hTAF_{II}55 are underlined. The ATG and stop codons of the open reading frame are **boxed**. The *numbers* indicate the nucleotide coordinates, and those in *parentheses* indicate the amino acid coordinates. Nucleotides that differ between this sequence and that of Chiang and Roeder (26) are underlined. The two amino acid substitutions, one of which is a polymorphic variation reported by Chiang and Roeder (26), are shown in **boldface**. B, Northern blot analysis of hTAF_{II}55 expression. A commercial blot (CLONTECH) containing poly(A)⁺ mRNA from the human embryonic tissues shown above each lane was hybridized with a hTAF_{II}55 ³²P-labeled DNA probe generated by PCR amplification in the presence of [α -³²P]dCTP. Hybridization and washing were performed under standard conditions. The hTAF_{II}55 mRNA is indicated along with the positions of radiolabeled molecular size markers (*M*) in kilobase pairs (*kb*).

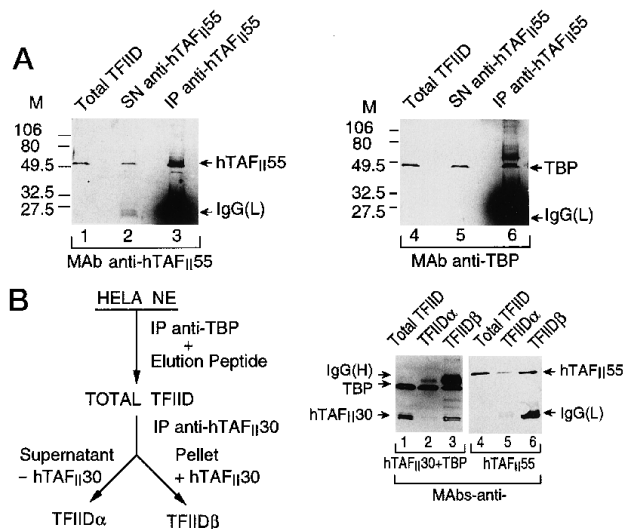


Fig. 2. A, precipitation of hTAF_{II}55 and TBP with mAb 19TA. Total TFIIID prepared by immunoprecipitation with the anti-TBP mAb 3G3 was reimmunoprecipitated with the anti-hTAF_{II}55 mAb 19TA. Aliquots of the supernatant (SN) and immunoprecipitate (IP) fractions were analyzed by Western blot analysis with the antibodies shown below each panel. TBP and hTAF_{II}55 are indicated. The positions of molecular mass standards (M) in kilodaltons and the light chain of the antibody used in the immunoprecipitation (IgG(L)) are indicated. B, distribution of hTAF_{II}55 in TFIIID α and TFIIID β . The experimental protocol is outlined in the left panel, and the results of Western blot analysis are shown in the right panel. NE, nuclear extract. The loaded fractions are indicated above each lane, and the antibodies used to reveal the blots are shown below each panel. The positions of TBP, hTAF_{II}55, and hTAF_{II}30 are shown. IgG(H) and IgG(L) show the heavy and light chains, respectively, of the antibodies used in the immunoprecipitations, which are selectively revealed depending on which conjugated secondary antibody is used.

that it comigrates with heavy chains of the mAbs used in the immunoprecipitations, and often the other coprecipitated proteins comigrate with the antibody light chains. Thus, for the sake of clarity, the Western blots that are presented were revealed only with mAbs either against hTAF_{II}55 or the coprecipitated proteins and with secondary antibodies against either the light or heavy chains. However, as shown in Fig. 3A, when aliquots of the immunoprecipitations were probed on two different blots, both hTAF_{II}55 and the coprecipitated proteins could be detected.

When native hTAF_{II}55 and B10-hTAF_{II}20 were coexpressed in COS cells, both proteins were immunoprecipitated by mAb B10 (Fig. 3A, see B10-hTAF_{II}20 in lanes 1 and 2 and hTAF_{II}55 in lanes 5 and 6), whereas hTAF_{II}55 was not immunoprecipitated by mAb B10 in the absence of coexpressed B10-hTAF_{II}20 (lanes 3 and 4). Similarly, in the converse experiment, neither hTAF_{II}18 nor hTAF_{II}28 was immunoprecipitated by mAb B10 in the absence of coexpressed B10-hTAF_{II}55 (Fig. 3B, lanes 1, 2, 5, and 6), whereas both proteins were immunoprecipitated when B10-hTAF_{II}55 was coexpressed (lanes 3, 4, 7, and 8). Analogous results were obtained when native hTAF_{II}28 or hTAF_{II}20 was coexpressed with hTAF_{II}55 and immunoprecipitated with mAb 19TA (see below and data not shown). In contrast, hTAF_{II}30 was not precipitated by mAb 19TA either in the presence or absence of coexpressed hTAF_{II}55 (Fig. 3C, lanes 2 and 5), and accordingly, hTAF_{II}55 was not precipitated by the anti-hTAF_{II}30 mAb 2F4 (lanes 6 and 7) in the presence of coexpressed hTAF_{II}30. In addition to the above interactions, it has also been shown that hTAF_{II}55 interacts with hTAF_{II}250 and hTAF_{II}100 (Refs. 26 and 29 and see below). Taken together, all of these observations indicate that hTAF_{II}55 interacts in transfected COS cells with hTAF_{II}18, hTAF_{II}20, hTAF_{II}28, hTAF_{II}100, and hTAF_{II}250, but not with hTAF_{II}30.

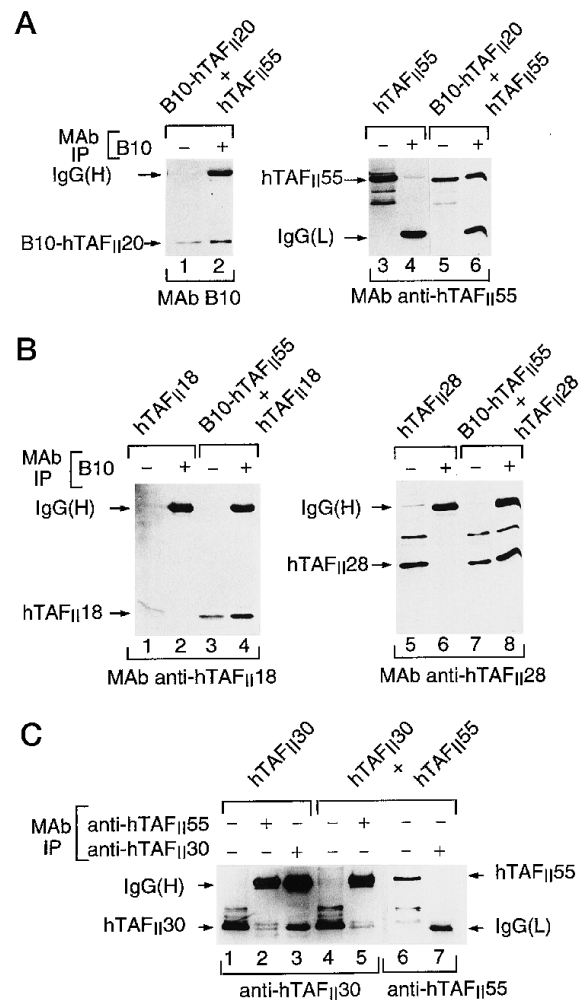


Fig. 3. Interaction between hTAF_{II}55 and other TFIIID subunits. Extracts from COS cells transfected with the vectors expressing the proteins indicated above each panel were immunoprecipitated (IP) with the mAbs shown above each lane. The minus sign above the lane indicates that an aliquot of the cell extract used for the immunoprecipitation has been loaded for reference. The Western blots were revealed with the antibodies shown below each panel. The positions of the immunoprecipitated proteins are indicated along with, where relevant, those of the IgG heavy (H) and IgG light (L) chains. A, shown is the interaction between hTAF_{II}55 and hTAF_{II}20. B, shown is the interaction between hTAF_{II}55, hTAF_{II}18, and hTAF_{II}28. C, hTAF_{II}55 does not interact with hTAF_{II}30. Note that the bands revealed in lanes 2 and 5 that migrate at positions similar to hTAF_{II}30 result from interaction of the secondary antibody with protein G (see also Ref. 21).

A possible interaction between hTAF_{II}55 and TBP was also investigated. Transfected TBP was precipitated by mAb 3G3, but not by mAb 19TA (Fig. 4A, lanes 8 and 9). When coexpressed with hTAF_{II}55, TBP could not be precipitated by mAb 19TA, and hTAF_{II}55 was not precipitated by mAb 3G3 (Fig. 4A, lanes 11 and 12). Similarly, TBP was not precipitated by mAb B10 either in the presence or absence of coexpressed B10-hTAF_{II}55 (Fig. 4B, lanes 2 and 5). These results show that hTAF_{II}55 does not interact with TBP in cotransfected COS cells.

Distinct Determinants of hTAF_{II}28 Are Required for Interactions with hTAF_{II}55 and TBP—We have previously shown that distinct determinants of hTAF_{II}28 are required for interactions with TBP and hTAF_{II}18 (22). A series of deletion mutants were used to map the region of hTAF_{II}28 required for interaction with hTAF_{II}55. In agreement with the results shown above, wild-type hTAF_{II}28(1–211) was precipitated by mAb 19TA only in the presence of coexpressed hTAF_{II}55 (Fig. 5A, lanes

FIG. 4. The organization of each panel is as described for Fig. 3, where the transfected vectors are indicated above each panel along with the mAbs used for the immunoprecipitations (IPs), and the mAbs used to reveal the Western blots are indicated below each panel. In A, B, and D, only enlargements of the relevant parts of the autoradiogram are shown. A and B, hTAF_{II}55 does not interact with TBP. Note that in A, TBP migrates below the lower of the two IgG heavy (H) chains and that hTAF_{II}55 migrates above the higher of the two IgG heavy chains (see lanes 4, 7, and 10). C, formation of stable ternary complexes between hTAF_{II}55 and TBP can be mediated by several hTAF_{II}s. D, a hTAF_{II}55-hTAF_{II}18 chimera interacts with TBP. IgG(L), IgG light chain.

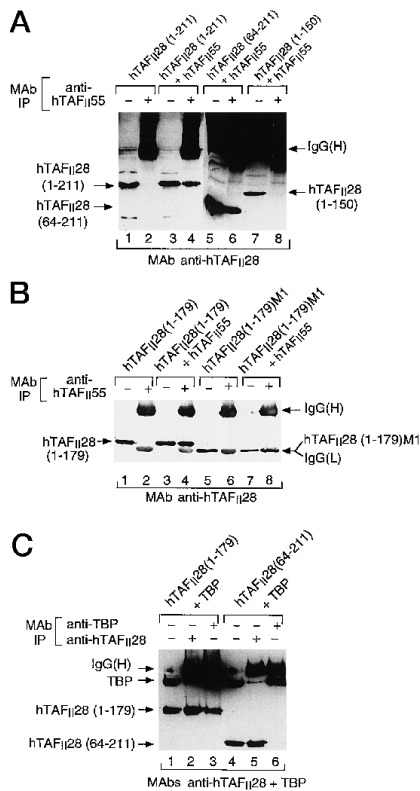
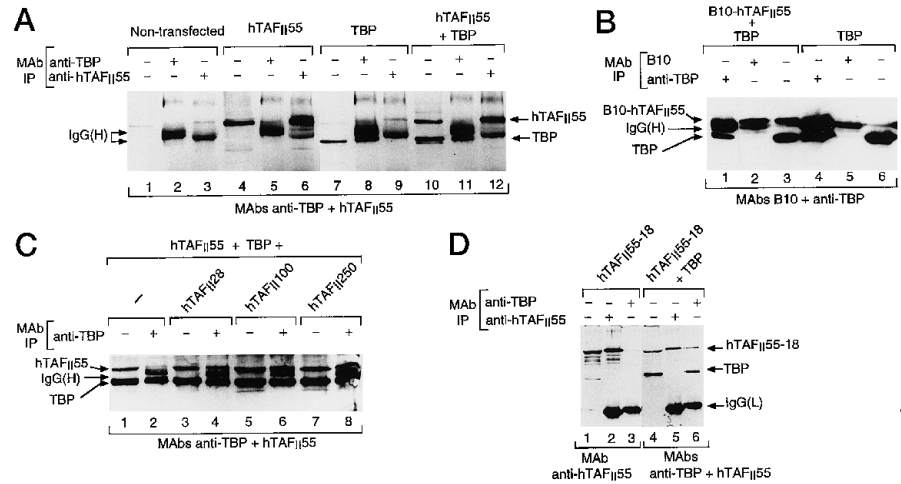


FIG. 5. A and B, interactions between hTAF_{II}55 and mutant derivatives of hTAF_{II}28. The organization is as described for Figs. 3 and 4. Note that the protein revealed in lane 6 of B is the IgG light (L) chain, not the hTAF_{II}28 mutant (compare lanes 6 and 8). C, interaction between hTAF_{II}28 and TBP. IgG(H), IgG heavy chain; IP, immunoprecipitate.

1-4). Similarly, mutants hTAF_{II}28-(64-211) and hTAF_{II}28-(1-179) were immunoprecipitated by mAb 19TA in the presence of hTAF_{II}55 (Fig. 5, A, lanes 5 and 6; and B, lanes 1-4). In contrast, deletion mutant hTAF_{II}28-(1-150) could not be precipitated by mAb 19TA in the presence of hTAF_{II}55 (Fig. 5A, lanes 7 and 8), suggesting that amino acids 150-179 of hTAF_{II}28 are required for interaction with hTAF_{II}55. We have previously reported that this region is also critical for interaction with TBP (44), and computer algorithms predict that amino acids 161-179 have the potential to form an amphipathic α -helix with a highly acidic face (see "Materials and Methods"). A triple amino acid substitution in this region (E165P/E167P/E168R, hTAF_{II}28-(1-179)-M1) that reduces in-

teraction with TBP (44) had no effect on interaction with hTAF_{II}55 (Fig. 5B, lanes 5-8). Moreover, mutant hTAF_{II}28-(64-211) did not interact with coexpressed TBP (Fig. 5C, lanes 4-6), but did interact with hTAF_{II}55 (Fig. 5A, lanes 5 and 6). Together, these results show that the hTAF_{II}28-TBP interaction requires determinants within both the amino- and carboxyl-terminal regions of hTAF_{II}28 and that these are distinct from those required for interactions with hTAF_{II}55.

Multiple hTAF_{II}s Mediate Stable Ternary Complex Formation between hTAF_{II}55 and TBP—As the above results suggested that hTAF_{II}28 could interact simultaneously with TBP and hTAF_{II}55, we coexpressed all three proteins in COS cells to test their ability to form a ternary complex. The abilities of hTAF_{II}250, hTAF_{II}100, hTAF_{II}20, and hTAF_{II}18, all of which interact with TBP, to form ternary complexes with hTAF_{II}55 and TBP were also tested. Following transfection, the cell extracts were immunoprecipitated with the anti-TBP antibody 3G3 and probed for the presence of hTAF_{II}55. Although no hTAF_{II}55 was immunoprecipitated with TBP in the absence of other coexpressed hTAF_{II}s (Fig. 4, A and B; and C, lanes 1 and 2), hTAF_{II}55 could be immunoprecipitated with TBP in the presence of coexpressed hTAF_{II}28, hTAF_{II}100, or hTAF_{II}250 (Fig. 4C, lanes 3-8). Analogous experiments could not be performed with hTAF_{II}20 and hTAF_{II}18 as coexpression of both hTAF_{II}55 and TBP reproducibly resulted in low expression of these proteins in the transfected cells (data not shown). These results indicate that stable ternary complexes can be formed by combining TBP and hTAF_{II}55 with hTAF_{II}250, hTAF_{II}100, or hTAF_{II}28. The observation that ternary complex formation requires the simultaneous expression of three TFIID subunits also indicates that endogenous COS cell TAF_{II}s cannot act as bridging factors.

We next "tagged" hTAF_{II}55 with hTAF_{II}18, which does not interact with TBP, and investigated whether the fusion protein would interact with TBP. The hTAF_{II}55-hTAF_{II}18 fusion protein was expressed in transfected COS cells both in the presence and absence of TBP. The fusion protein could be detected by mAbs directed against both hTAF_{II}18 and hTAF_{II}55 and was immunoprecipitated by mAb 19TA, but not by mAb 3G3 in the absence of coexpressed TBP (Fig. 4D, lanes 1-3; and data not shown). In contrast, in the presence of coexpressed TBP, the hTAF_{II}55-hTAF_{II}18 fusion protein was precipitated by mAb 3G3, showing that the fusion protein interacted with TBP (Fig. 4D, lane 6). Surprisingly, however, although the hTAF_{II}55-hTAF_{II}18-TBP complex was reproducibly precipitated by mAb 3G3, it could not be precipitated by mAb 19TA (Fig. 4D, lane 5). Thus, the formation of this complex seems to result in masking of the mAb 19TA epitope. These results show that hTAF_{II}18

can be used as a tag to direct the stable interaction of a heterologous protein with TBP in mammalian cells.

DISCUSSION

hTAF_{II}55 Interacts with Multiple Components of the TFIID Complex—In this study, we characterized novel interactions between hTAF_{II}55 and other components of the TFIID complex. We have previously described the molecular cloning of cDNAs encoding hTAF_{II}135, hTAF_{II}100, hTAF_{II}30, hTAF_{II}28, hTAF_{II}20, and hTAF_{II}18 (21, 22, 29).² Comparison of the sequences of these hTAF_{II}s with those of known dTAF_{II}s indicated that hTAF_{II}30 and hTAF_{II}18 have no known *Drosophila* homologues, although hTAF_{II}18 shares homology with the amino-terminal region of SPT3, a known yeast TAF_{II} (56). hTAF_{II}55 also has no known *Drosophila* homologue, confirming our previous suggestion that hTFIID comprises more subunits than dTFIID (13). These results suggest either that these dTAF_{II}s have not yet been isolated from the TFIID of *Drosophila* embryos or that they are not present in this dTFIID, but only in the dTFIID of differentiated adult tissues.

While this work was in progress, Chiang and Roeder (26) reported the molecular cloning of hTAF_{II}55. The hTAF_{II}55 cDNA sequence reported here is 32 nucleotides longer at the beginning of the 5'-untranslated region than that of Chiang and Roeder and contains several nucleotide changes. Our sequence contains a serine rather than an arginine at position 178, consistent with the proposed polymorphism (26). However, we reproducibly found a lysine rather than the reported asparagine at position 283. Although no other genes with homology to hTAF_{II}55 were found in the mammalian data bases, we did find a clone, isolated by random sequencing of a CpG island library (57), containing 270 nucleotides of the hTAF_{II}55 5'-untranslated region. This finding correlates with the observations that CpG islands often colocalize with the 5'-ends of human genes.

By sequential immunoprecipitation of HeLa cell extracts with antibodies against TBP and hTAF_{II}30, we have previously shown that hTAF_{II}18 and hTAF_{II}20 are specifically associated with the TFIID β subpopulation containing hTAF_{II}30. In contrast, hTAF_{II}250, hTAF_{II}100, and hTAF_{II}28 are present in both the TFIID α and TFIID β populations (21, 22). From silver nitrate staining of the hTAF_{II}s present in the TFIID α and TFIID β complexes, we previously suggested that hTAF_{II}55 (designated hTAF_{II}60 in Ref. 21) was also a core hTAF_{II}. However, as hTAF_{II}55 comigrates with the IgG heavy chain, which sometimes contaminates the TFIID preparations, it was difficult to unambiguously determine the identity of proteins in this region by silver nitrate staining. Here we confirm immunologically that hTAF_{II}55 is indeed present in both TFIID α and TFIID β , although it is mainly associated with TFIID β .

Chiang and Roeder (26) have reported that hTAF_{II}55 interacts *in vitro* with hTAF_{II}250. We show that, in COS cells, hTAF_{II}55 interacts with hTAF_{II}250, hTAF_{II}100, hTAF_{II}28, hTAF_{II}20, and hTAF_{II}18, but does not interact with hTAF_{II}30 or TBP. In this latter respect, hTAF_{II}55 differs from hTAF_{II}18, hTAF_{II}20, hTAF_{II}28, hTAF_{II}100, and hTAF_{II}250, all of which have been shown to interact with TBP (22, 29). Thus, hTAF_{II}55 can interact with several TFIID subunits in addition to hTAF_{II}250. It is unlikely that the interactions detected in the transfected COS cells can be explained by interaction of the overexpressed hTAF_{II}s with endogenous COS cell TAF_{II}s as not only do the transfected hTAF_{II}s accumulate to much greater levels than the endogenous TAF_{II}s, but the formation of ternary complexes between hTAF_{II}55 and TBP requires expres-

sion of other hTAF_{II}s, showing that endogenous COS cell TAF_{II}s cannot act as bridging factors. In contrast to what was observed in transfected COS cells, no interactions could be observed between GST-hTAF_{II}55 and purified recombinant hTAF_{II}18 and hTAF_{II}28 *in vitro*. This observation suggests either that the bacterially expressed proteins adopt an inactive conformation or that post-translational modifications of hTAF_{II}55, hTAF_{II}18, and/or hTAF_{II}28 are required to promote these TAF-TAF interactions. In this respect, it has been noted that hTAF_{II}55 contains potential sites for several protein kinases (26).

By using derivatives of hTAF_{II}28 containing deletion and point mutations, we have mapped determinants required for its interactions with hTAF_{II}55 and TBP. hTAF_{II}55 and TBP interact with hTAF_{II}28-(1-179), but not with hTAF_{II}28-(1-150), showing that interaction of hTAF_{II}28 with both of these proteins requires amino acids 150-179. However, the triple amino acid substitution in the putative amphipathic α -helix in hTAF_{II}28-(1-179)-M1 that affects interaction with TBP (44) does not affect interaction with hTAF_{II}55. Moreover, hTAF_{II}55 interacts with hTAF_{II}28-(64-211), but TBP does not interact with this mutant, indicating an additional requirement for amino acids 1-63 of hTAF_{II}28 for interaction with TBP. Together, these results indicate that distinct determinants of hTAF_{II}28 are required for binding to hTAF_{II}55 and TBP. As amino acids between positions 114 and 150 of hTAF_{II}28 are required for interaction with hTAF_{II}18 (22), it is possible that hTAF_{II}28 may interact simultaneously with hTAF_{II}55, hTAF_{II}18, and TBP. Our finding that interaction with TBP requires a determinant in the amino-terminal region of hTAF_{II}28, which is not conserved in dTAF_{II}30 β , may help to explain the observation that, despite the relatively high sequence homology in region 150-179, dTAF_{II}30 β does not interact with TBP (24).

hTAF_{II}55 Can Form Stable Ternary Complexes with TBP via Interactions with Multiple hTAF_{II}s—Initially, it was proposed that TAF_{II}250 was the only TAF_{II} to interact with TBP (11, 58, 59), but subsequent studies indicated that hTAF_{II}100, hTAF_{II}28, hTAF_{II}20, and hTAF_{II}18 (22, 29), dTAF_{II}150 and dTAF_{II}30 α (17, 24), and possibly other dTAF_{II}s (see Refs. 25 and 33) all interact with TBP. Our present results further demonstrate that not only do hTAF_{II}28 and hTAF_{II}100 bind to TBP, but that they can also mediate the formation of stable ternary complexes between TBP and hTAF_{II}55, which itself does not interact with TBP. The ability of several hTAF_{II}s to nucleate the formation of partial TFIID complexes raises the question as to how the hTFIID complexes are assembled intracellularly. It has previously been shown that interaction of dTAF_{II}250 with dTBP is an obligatory first step in the assembly of dTFIID *in vitro* (17). In addition, TAF_{II}250 competes for binding to TBP *in vitro* with hTAF_I110, hTAF_I63, or hTAF_I48 (60), and once either hTAF_{II}250 or hTAF_Is are bound, they earmark the TBP for formation of the TFIID or SL1 complexes. Our results suggest that hTAF_{II}250 may not be the only TAF_{II} to direct TBP into the formation of TFIID complexes and that, if present in the cell at the same time, other hTAF_{II}s may compete with hTAF_{II}250 for binding to TBP or may bind concomitantly with hTAF_{II}250 and nucleate the formation of TFIID complexes. A better understanding of how the formation of the different TBP-containing complexes is regulated will require the determination of the relative intracellular levels of each TAF and whether these levels are modulated by the cell cycle.

Acknowledgments—We thank L. Carré for excellent technical assistance; R. Frazer for critical reading of the manuscript; Adrien Staub for peptide sequencing of hTAF_{II}55; J. M. Garnier and T. Lerouge for the

² A.-C. Lavigne, G. Mengus, M. May, V. Dubrovskaya, L. Tora, P. Chambon, and I. Davidson, unpublished data.

HeLa cDNA library; S. Vicaire and P. Hamman for DNA sequencing; Y. Lutz and the monoclonal antibody facility; the staff of the cell culture and oligonucleotide facilities; P. Eberling and D. Stephane for peptide synthesis; and B. Boulay, J. M. Lafontaine, and R. Buchert for illustrations.

REFERENCES

- Conaway, R. C., and Conaway, J. W. (1993) *Annu. Rev. Biochem.* **62**, 161–190
- Buratowski, S., and Sharp, P. A. (1993) in *Transcriptional Regulation* (McKnight, S. L., and Yamamoto, K. R., eds) Vol. 1, pp. 227–246, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Buratowski, S. (1994) *Cell* **77**, 1–3
- Zawel, L., and Reinberg, D. (1995) *Annu. Rev. Biochem.* **64**, 533–561
- Wilson, C. J., Chao, D. M., Imbalzano, A. N., Schnitzler, G. R., Kingston, R. E., and Young, R. A. (1996) *Cell* **84**, 235–244, and references therein
- Kim, Y. J., Björklund, S., Li, Y., Sayre, H. M., and Kornberg, R. D. (1994) *Cell* **77**, 599–608, and references therein
- Ossipow, V., Tassan, J. P., Nigg, E. A., and Schibler, U. (1995) *Cell* **83**, 137–146, and references therein
- Dynlacht, B. D., Hoey, T., and Tjian, R. (1991) *Cell* **66**, 563–576
- Pugh, B. F., and Tjian, R. (1991) *Genes Dev.* **5**, 1935–1945
- Tanese, N., Pugh, B. F., and Tjian, R. (1991) *Genes Dev.* **5**, 2212–2224
- Zhou, Q., Lieberman, P. M., Boyer, T. G., and Berk, A. J. (1992) *Genes Dev.* **6**, 1964–1974
- Timmers, H. T. M., Meyers, R., and Sharp, P. A. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 8140–8144
- Brou, C., Chaudhary, S., Davidson, I., Lutz, Y., Wu, J., Egly, J. M., Tora, L., and Chambon, P. (1993) *EMBO J.* **12**, 489–499
- Chiang, C.-M., Ge, H., Wang, Z., Hoffmann, A., and Roeder, R. G. (1993) *EMBO J.* **12**, 2749–2762
- Hernandez, N. (1993) *Genes Dev.* **7**, 1291–1308
- Goodrich, J. A., and Tjian, R. (1994) *Curr. Opin. Cell Biol.* **6**, 403–409
- Chen, J. L., Attardi, L. D., Verrijzer, C. P., Yokomori, K., and Tjian, R. (1994) *Cell* **79**, 93–105, and references therein
- Brou, C., Ali, S., Wu, J., Scheer, E., Lang, C., Davidson, I., Chambon, P., and Tora, L. (1993) *Nucleic Acids Res.* **21**, 5–12
- Chaudhary, S., Brou, C., Valentin, M. E., Burton, N., Tora, L., Chambon, P., and Davidson, I. (1994) *Mol. Cell. Biol.* **14**, 5290–5299
- Chaudhary, S., Tora, L., and Davidson, I. (1995) *J. Biol. Chem.* **270**, 3631–3637
- Jacq, X., Brou, C., Lutz, Y., Davidson, I., Chambon, P., and Tora, L. (1994) *Cell* **79**, 107–117
- Mengus, G., May, M., Jacq, X., Staub, A., Tora, L., Chambon, P., and Davidson, I. (1995) *EMBO J.* **14**, 1520–1531
- Hoey, T., Weinzierl, R. O. J., Gill, G., Chen, J. L., Dynlacht, B. D., and Tjian, R. (1993) *Cell* **72**, 247–260
- Yokomori, K., Chen, J. L., Admon, A., Zhou, S., and Tjian, R. (1993) *Genes Dev.* **7**, 2587–2597
- Kokubo, T., Gong, D. W., Wootton, J. C., Horikoshi, M., Roeder, R., and Nakatani, Y. (1994) *Nature* **367**, 484–487
- Chiang, C.-M., and Roeder, R. G. (1995) *Science* **267**, 531–536
- Lu, H., and Levine, A. J. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 5154–5158
- Klemm, R. D., Goodrich, J. A., Zhou, S., and Tjian, R. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 5788–5792
- Dubrovskaya, V., Lavigne, A.-C., Davidson, I., Acker, J., Staub, A., and Tora, L. (1996) *EMBO J.*, in press
- Reese, J. C., Aponne, L., Walker, S. S., Griffen, L. A., and Green, M. R. (1994) *Nature* **371**, 523–527
- Poon, D., Bai, Y., Cambell, A. M., Björklund, S., Kim, Y. L., Zhou, S., Kornberg, R. D., and Weil, P. A. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 8224–8228
- Dynlacht, B. D., Weinzierl, R. O. J., Admon, A., and Tjian, R. (1993) *Nature* **363**, 176–179
- Kokubo, T., Gong, D. W., Yamashita, S., Takada, R., Roeder, R. G., Horikoshi, M., and Nakatani, Y. (1993) *Mol. Cell. Biol.* **13**, 7859–7863
- Pugh, B. F., and Tjian, R. (1990) *Cell* **61**, 1187–1197
- Hoey, T., Dynlacht, B. D., Peterson M. G., Pugh, B. F., and Tjian, R. (1990) *Cell* **61**, 1179–1186
- Goodrich, J. A., Hoey, T., Thut, C. J., Admon, A., and Tjian, R. (1993) *Cell* **75**, 519–530
- Thut, C. J., Chen, J. L., Klemm, R., and Tjian, R. (1995) *Science* **267**, 100–104
- Sauer, F., Hansen, S. K., and Tjian, R. (1996) *Science* **270**, 1783–1788
- Verrijzer, P., Yokomori, K., Chen, J. L., and Tjian, R. (1994) *Science* **264**, 933–941
- Verrijzer, P., Chen, J. L., Yokomori, K., and Tjian, R. (1995) *Cell* **81**, 1115–1125
- Kaufmann, J., Verrijzer, P., Shao, J., and Smale, S. T. (1996) *Genes Dev.* **10**, 876–886
- Xie, X., Kokubo, T., Cohen, S. L., Mirza, U. A., Hoffmann, A., Chait, B. T., Roeder, R. G., Nakatani, Y., and Burley, S. K. (1996) *Nature* **380**, 316–322
- Hoffmann, A., Chiang, C. M., Oelgeschläger, T., Xie, X., Burley, S. K., Nakatani, Y., and Roeder, R. G. (1996) *Nature* **380**, 356–359
- May, M., Mengus, G., Lavigne, A.-C., Chambon, P., and Davidson, I. (1996) *EMBO J.* **15**, 3093–3104
- Parker, M. G. (1993) *Curr. Opin. Cell Biol.* **5**, 499–504
- Chambon, P. (1994) *Semin. Cell Biol.* **5**, 115–125
- Tsai, M. J., and O'Malley, B. W. (1994) *Annu. Rev. Biochem.* **63**, 451–486
- Kastner, P., Mark, M., and Chambon, P. (1995) *Cell* **83**, 859–869
- Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1995) *Cell* **83**, 835–839
- Mangelsdorf, D. J., and Evans, R. M. (1995) *Cell* **83**, 841–850
- Beato, M., Herrlich, P., and Schutz, G. (1995) *Cell* **83**, 851–857
- Wurtz, J. M., Bouguet, W., Renaud, J. P., Vivat, V., Chambon, P., Moras, D., and Gronemeyer, H. (1996) *Nat. Struct. Biol.* **3**, 87–94
- Pierrat, B., Heery, D., Chambon, P., and Losson, R. (1994) *Gene (Amst.)* **143**, 193–200
- Ali, S., Lutz, Y., Bellocq, J.-P., Chenard-Neu, M.-P., Rouyer, N., and Metzger, D. (1993) *Hybridoma* **12**, 391–405
- White, J. H., Brou, C., Wu, J., Lutz, Y., Moncollin, V., and Chambon, P. (1992) *EMBO J.* **6**, 2229–2240
- Eisenmann, D. M., Arndt, K. M., Ricupero, S. L., Rooney, J. W., and Winston, F. (1992) *Genes Dev.* **6**, 1319–1331
- Cross, S. H., Charlton, J. A., Nan, X., and Bird, A. P. (1994) *Nat. Genet.* **6**, 236–244
- Hisatake, K., Hasegawa, S., Takada, R., Nakatani, Y., Horikoshi, M., and Roeder, R. G. (1993) *Nature* **362**, 179–181
- Weinzierl, R. O. J., Dynlacht, B. D., and Tjian, R. (1993) *Nature* **362**, 511–517
- Comai, L., Zomerdijs, J. B. C. M., Beckman, H., Zhou, S., Admon, A., and Tjian, R. (1994) *Science* **266**, 1966–1969

Nucleic Acids, Protein Synthesis, and
Molecular Genetics:

**Multiple Interactions between hTAF_{II}55
and Other TFIID Subunits:
REQUIREMENTS FOR THE
FORMATION OF STABLE TERNARY
COMPLEXES BETWEEN hTAF_{II}55 AND
THE TATA-BINDING PROTEIN**

Anne-Claire Lavigne, Gabrielle Mengus,
Michael May, Veronika Dubrovskaya, Laszlo
Tora, Pierre Chambon and Irwin Davidson
J. Biol. Chem. 1996, 271:19774-19780.
doi: 10.1074/jbc.271.33.19774

Access the most updated version of this article at <http://www.jbc.org/content/271/33/19774>

Find articles, minireviews, Reflections and Classics on similar topics on the [JBC Affinity Sites](#).

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 59 references, 19 of which can be accessed free at
<http://www.jbc.org/content/271/33/19774.full.html#ref-list-1>