RESEARCH ARTICLE

Drosophila $TAF_{II}150$: Similarity to Yeast Gene TSM-1 and Specific Binding to Core Promoter DNA

C. Peter Verrijzer, Kyoko Yokomori, Jin-Long Chen, Robert Tjian

In *Drosophila* and human cells, the TATA binding protein (TBP) of the transcription factor IID (TFIID) complex is tightly associated with multiple subunits termed TBP-associated factors (TAFs) that are essential for mediating regulation of RNA polymerase II transcription. The *Drosophila* $TAF_{II}150$ has now been molecularly cloned and biochemically characterized. The deduced primary amino acid sequence of $dTAF_{II}150$ reveals a striking similarity to the essential yeast gene, TSM-1. Furthermore, like $dTAF_{II}150$, the TSM-1 protein is found associated with the TBP in vivo, thus identifying the first yeast homolog of a TAF associated with TFIID. Both the product of TSM-1 and $dTAF_{II}150$ bind directly to TBP and $dTAF_{II}250$, demonstrating a functional similarity between human and yeast TAFs. Surprisingly, DNA binding studies indicate that purified recombinant $dTAF_{II}150$ binds specifically to DNA sequences overlapping the start site of transcription. The data demonstrate that at least one of the TAFs is a sequence-specific DNA binding protein and that $dTAF_{II}150$ together with TBP are responsible for TFIID interactions with an extended region of the core promoter.

The eukaryotic TFIID is an essential component of the RNA polymerase II machinery that is required for initiation of basal as well as regulated transcription of protein coding genes (*1–3*). The TBP subunit of TFIID was isolated and cloned first from yeast, and then from *Drosophila* and human cells (*2*). Although TBP was purified as a single polypeptide from yeast cells, subsequent studies with antibodies to *Drosophila* and human TBP revealed that TBP is almost exclusively present as part of large and stable complexes containing multiple associated subunits called TAFs. In these studies, TAFs were found to be essential for mediating activation by sequence-specific transcription factors (*4–6*). These and other studies established that TBP, in conjunction with distinct combinations of TAFs, serves as a universal factor contributing to the initiation of transcription by RNA polymerases I, II, and III (*1, 2, 4–10*).

Even though the basal transcription machinery is considered likely to be highly conserved among eukaryotes, a stable TFIID complex has not yet been isolated from yeast, and yeast genes homologous to RNA polymerase II TAFs have been not yet identified. However, a number of studies have resulted in the identification of yeast genes encoding factors that behave like TAFs. For example, the products of the yeast ADA genes were reported to act like potential coactivators or adaptors in that they are required to mediate activation but are not necessary for basal transcription (*11*). Thus far, the products of the ADA genes have not been shown to be subunits of TFIID. In another study, a complex containing multiple polypeptides [so-called suppressor RNA polymerase B (SRBs)] and TBP were found to be associated with the COOH-terminal domain (CTD) of the RNA polymerase II large subunit (*12*). However, none of these genes encode proteins similar to the TFIID subunits that have been characterized in human and *Drosophila* cells. Thus the question of whether yeast contain TAFs that help mediate transcriptional activation by RNA polymerase II remains unresolved.

The original finding that enhancement of transcription by promoter-selective factors requires TAFs suggested a definitive and critical function for at least some subunits of TFIID as potential coactivators that mediate transcriptional activation (*5, 8, 13*). Evidence for coactivator function has been obtained in two interacting pairs—(i) the human transcriptional activator Sp1 and its target $TAF_{II}110$ (*14, 15*), and (ii) the viral transactivator VP-16 and $dTAF_{II}40$ (*16*). However, in addition to serving as targets for interaction with activators, some of the TAFs in the TFIID complex are expected to perform other functions. For example, some TAFs may contact components of the basal transcription machinery. Indeed, basal transcription factor TFIIA interacts selectively with $dTAF_{II}250$ as well as with $dTAF_{II}110$ whereas $dTAF_{II}40$ binds TFIIB in addition to VP-16 (*16, 17*). Other TAFs may contact different components of the transcriptional apparatus.

One striking property of TFIID that distinguishes it from TBP is the ability of the TAF-TBP complex to bind the core elements of the promoter and protect an extended region outside of the TATA box that encompasses the start site of transcription as well as downstream sequences (*10, 18–23*). Furthermore, ultraviolet cross-linking experiments, with a relatively crude TFIID preparation, identified two proteins with approximate molecular sizes of 150 kD and 60 kD that apparently contact downstream sequences in the *Drosophila* heat shock protein hsp 70 promoter (*20*). Thus, it is logical to assume that one or more of the TAFs associated with TBP may also be responsible for contacting DNA. Indeed, several studies suggest that TFIID interacts not only with the TATA box but also with sequences overlapping the initiator element of the core promoter (*23, 24*). Therefore, it is likely that some of the TAFs could bind DNA in the context of the TFIID complex. It is, however, of critical importance to determine whether TAFs could also recognize and bind DNA in a sequence-specific manner. Such a hypothetical sequence-specific DNA binding by TAFs may help explain how the TFIID complex can direct initiation at promoters that lack the TATA and may reveal new insights concerning the function of TAFs.

We describe below the molecular cloning, expression, and biochemical characterization of *Drosophila* $dTAF_{II}150$. The primary amino acid sequence of this large subunit of TFIID reveals an unexpected and striking similarity to the recently cloned yeast gene TSM-1, which is essential for viability (*25*). In that the product of TSM-1 is associated with yeast TBP (yTBP) in vivo, it is therefore a good candidate for a bona fide yeast TAF. Using a variety of biochemical assays including coimmunoprecipitation and affinity chromatography to map specific protein-protein interactions in vitro, we were able to identify TAF-TAF and TAF-TBP contacts involving $dTAF_{II}150$ as well as the product of TSM-1. In addition, we have tested the sequence-specific DNA binding properties of purified recombinant $dTAF_{II}150$ with several assays including ultraviolet cross-linking, gel mobility shifts, and deoxyribonuclease (DNase) footprinting. Our results suggest that $dTAF_{II}150$ is an essential subunit of TFIID that makes specific protein-protein as well as protein-DNA contacts and identifies the first (to our knowledge) DNA binding TAF in the TFIID complex.

One of the major subunits of dTFIID that had remained uncharacterized was a 150-kD TAF (*5*). In order to isolate complementary DNAs (cDNAs) encoding $dTAF_{II}150$, we purified dTFIID from embryo nuclear extracts by affinity chromatog-

The authors are with the Howard Hughes Medical Institute and the Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720–3202, USA.

raphy with antibodies to TBP (5). The dTFIID complex purified from approximately 800 g of embryos (Fig. 1A) was subjected to separation by SDS-PAGE (polyacrylamide gel electrophoresis) and transferred to a polyvinylidene difluoride (PVDF) membrane. The region of the membrane containing $dTAF_{II}150$ was excised and digested with trypsin. The eluted peptides were subsequently separated by reversed-phase high- performance liquid chromatography (HPLC) and subjected to microsequence analysis. Amino acid sequences from nine peptides were determined and the longest were used to design oligonucleotide probes. Two independent probes were used to screen a *Drosophila* embryo cDNA library, from which 11 clones were obtained (26).

The two phage with the longest inserts each contained a 3′ poly(A) tail and a 3639–base pair (bp) open reading frame preceded by two in-frame stop codons. A protein of 1213 amino acids was deduced from the cDNA sequence (Fig. 2A). All nine peptide sequences obtained from the tryptic digest were found within this open reading frame (Fig. 2A, underlined sequences), thus providing evidence that this clone encodes the 150-kD protein that we sequenced. To further ascertain that this clone encodes $dTAF_{II}150$, we expressed a 60-kD COOH-terminal portion of the protein in *Escherichia coli* and raised polyclonal antibodies to this truncated product. These antibodies recognized (as tested with immunoblotting) the endogenous $dTAF_{II}150$ in the immunopurified TFIID complex (Fig. 1A). We also obtained expression of the complete cDNA of $TAF_{II}150$, using a baculovirus expression system. In SDS-PAGE, migration of the recombinant protein was indistinguishable from that of the endogenous protein, confirming that this cDNA most likely encodes full-length $dTAF_{II}150$ (Fig. 1B) (27).

To provide direct evidence that the cloned gene product is a bona fide TAF, we used antibodies to $dTAF_{II}150$ (anti-$dTAF_{II}150$) to immunoprecipitate dTFIID and confirmed by immunoblots the presence of other dTAFs including $dTAF_{II}60$, $dTAF_{II}80$, and $dTAF_{II}250$ in the complex (Fig. 1C). In addition, dTFIID immunopurified with monoclonal antibodies to dTBP and to $dTAF_{II}250$ contained $dTAF_{II}150$, as determined by immunoblot analysis with antibodies to the recombinant protein. These results, taken collectively, establish that $dTAF_{II}150$ is a bona fide TAF, and that we have isolated a cDNA that encodes this protein.

Sequence similarity of essential yeast gene TSM-1 to $dTAF_{II}150$ and association of the TSM-1 protein with TBP in vivo. The deduced amino acid sequence of $dTAF_{II}150$ predicted a protein of 142-kD with an estimated isoelectric point of 8.9 (Fig. 2A). The protein contained abundant bulky hydrophobic residues and several serine- and threonine-rich regions. The COOH-terminal portion of the protein was highly charged (Fig. 2B). The most striking finding, however, was a 50 percent similarity of amino acid sequence between $dTAF_{II}150$ and the expression product of a recently cloned essential *Saccharomyces cerevisiae* gene called TSM-1 (25) (Fig. 2A). The similarity in primary sequence extended throughout the entire length of the coding region, suggesting that TSM-1 is a yeast homolog of $dTAF_{II}150$. Interspersed between regions of high similarity are local patches of divergent sequences. For example, a region of about 150 amino acids in TSM-1 that is abundant in charged residues is absent in $dTAF_{II}150$ (Fig. 2, A and B). The high degree of similarity between TSM-1 and $dTAF_{II}150$ suggests that TSM-1 may encode a yeast TAF that takes part in RNA polymerase II transcription. We have mapped the location of the $dTAF_{II}150$ gene in *Drosophila* to chromosome III at position 67D5-6 (28). This region has been extensively analyzed and contains multiple essential genes. Because there are temperature-sensitive mutations in TSM-1 and because the *Drosophila* gene is in a well-characterized chromosomal location containing many mutations, it might be possible to study the role of this TAF in vivo as well as in vitro.

The extensive sequence similarity between TSM-1 and $dTAF_{II}150$ suggested that the product of TSM-1 might be a bona fide TAF and therefore be associated with yeast TBP, perhaps in a complex similar to those observed for *Drosophila* and human TFIID. To test this possibility, we immunoprecipitated yTBP and any associated factors from yeast nuclear extract with affinity-purified antibodies to yTBP (anti-yTBP). The resulting immunoprecipitates were analyzed by SDS-PAGE and subsequent immunoblotting with a polyclonal antibody to recombinant TSM-1 protein expressed in *E. coli*. As expected, preimmune serum did not detect any proteins in the immunoprecipitated complex, but antibodies to TSM-1 selectively cross-reacted with a polypeptide of approximately 165 kD (Fig. 3). In addition, a mock precipitate of TBP with control antiserum failed to immunoprecipitate any TSM-1. We then used anti–TSM-1 to test yTBP for its ability to be coprecipitated with TSM-1. Immunoblot analysis revealed that anti–TSM-1 but not

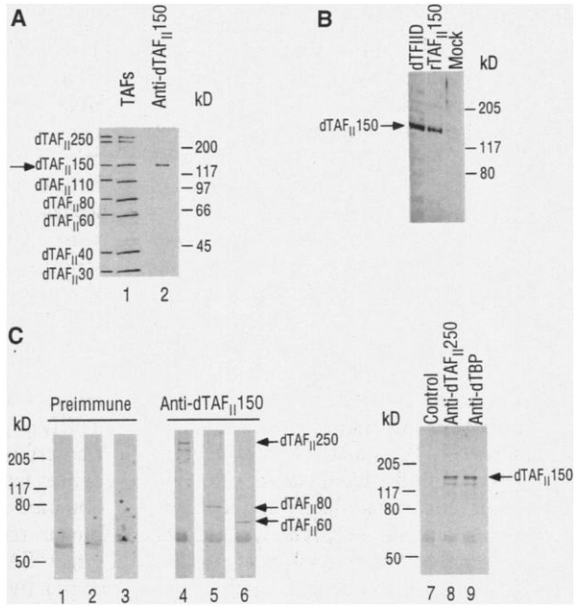

Fig. 1. (**A**) Antibodies to recombinant $dTAF_{II}150$ recognize the endogenous protein. Partially purified TFIID complex was immunoprecipitated with a monoclonal antibody to dTBP. TAFs were eluted with 1 M guanidine, resolved by SDS-PAGE, and visualized by silver staining (lane 1). The dTAFs are indicated. This gel represents about 2 percent of the material that was used to obtain the $dTAF_{II}150$ peptide sequence information. With the use of oligonucleotide fragments derived from peptide sequence information, the corresponding cDNA was cloned. Part of this cDNA was expressed in a bacterial T7 expression system, and polyclonal antiserum to encoded polypeptide was prepared. The antiserum recognized a band on an immunoblot corresponding to endogenous $dTAF_{II}150$ (lane 2). (**B**) Cloned $dTAF_{II}150$, overexpressed in Sf9 insect cells with the use of recombinant baculovirus, has the same apparent molecular mass as the endogenous $dTAF_{II}150$ from *Drosophila* embryos. Purified dTFIID, extracts from cells expressing recombinant $dTAF_{II}150$ ($rTAF_{II}150$), and extracts from cells infected with virus expressing an unrelated protein were resolved by SDS-PAGE and analyzed by immunoblotting with polyclonal anti-$dTAF_{II}150$. (**C**) $dTAF_{II}150$ is an integral part of the TFIID complex. The TFIID fraction was immunoprecipitated with either preimmune serum (lanes 1 to 3) or polyclonal antiserum to $dTAF_{II}150$ (lanes 4 to 6). The immunoprecipitates were resolved by SDS-PAGE and analyzed by immunoblotting with either anti-$dTAF_{II}250$ (lanes 1 and 4), anti-$dTAF_{II}80$ (lanes 2 and 5), or anti-$dTAF_{II}60$ (lanes 3 and 6). Bands corresponding to the different TAFs are indicated. The right panel shows an immunoblot analysis of the TFIID complex immunoprecipitated with antibodies to a non-TFIID protein (lane 7), $dTAF_{II}250$ (lane 8), or TBP (lane 9). The blot was probed with antiserum to $dTAF_{II}150$. The position of $dTAF_{II}150$ is indicated with an arrow.

control antibody could coprecipitate yTBP (*29*), a confirmation that TSM-1 protein behaves like a TAF and is associated with yTBP isolated from yeast nuclei. These results indicate that yeast has an RNA polymerase II TAF and establish that, as in the *Drosophila* and human systems, yeast TFIID may be a complex composed of TBP and associated TAFs.

Fig. 2. Amino acid sequence of $dTAF_{II}150$ and comparison with TSM-1. (**A**) Deduced amino acid sequence derived from the $dTAF_{II}150$ cDNA. Peptide sequences obtained from microsequencing of a tryptic digestion of the endogenous $dTAF_{II}150$ are underlined. Residues that are identical in the yeast gene TSM-1 are indicated by asterisks. Dashes indicate similar residues. Similarity rules are P=A=S, G=A=S, T=A=S, D=E=Q=N, K=R=H, V=I=L=M=F, F=Y. (**B**) Schematic representation of the $dTAF_{II}150$ protein sequence. The predicted amino acid sequence contains several regions that are relatively rich in particular residues. Indicated are leucine, isoleucine, valine-rich regions (28 to 30 percent), serine- and threonine-rich regions (24 to 33 percent), and the highly charged COOH-terminal region (19 percent acidic and 29 percent basic residues). The alignment of $dTAF_{II}150$ and TSM-1 is depicted schematically, with the deletions made in either protein to obtain the alignment indicated. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

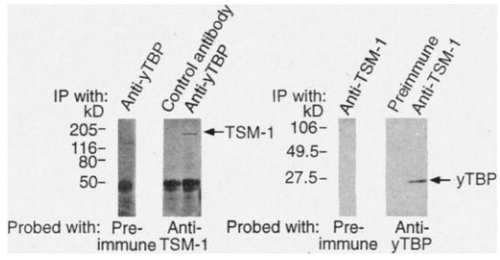

Fig. 3. The TSM-1 protein and yTBP are associated in yeast nuclear extracts. The yTBP was immunoprecipitated from a yeast nuclear extract with affinity-purified anti-yTBP or control antibody. The immunoprecipitates were resolved by SDS-PAGE and analyzed by immunoblotting with either anti–TSM-1 or preimmune serum. The band corresponding to TSM-1 is indicated. The right panel shows a western immunoblot analysis of protein complexes precipitated with either anti–TSM-1 or preimmune serum. Precipitated complexes were eluted with 1 M guanidine, separated by SDS-PAGE and the immunoblot was probed with a monoclonal antibody to yTBP (58C9). The position of yTBP is indicated.

Interaction of *Drosophila* TBP and $dTAF_{II}250$ with the COOH-terminal portion of $dTAF_{II}150$. Our previous studies of TAFs indicated that only some of the subunits bind directly to TBP while others are assembled into the TFIID complex via TAF-TAF interactions (*15, 30, 31*). We therefore performed a series of experiments to determine which subunits of dTFIID contact $dTAF_{II}150$. Specific protein-protein interactions were assayed by several different strategies (*32*). First, we tested the ability of $dTAF_{II}150$ to bind directly to dTBP and to a truncated version of the largest subunit of the TFIID complex, $dTAF_{II}250\Delta N$ (*15*) (Fig. 4A). The ^{35}S-labeled, in vitro translated $dTAF_{II}150$ bound efficiently (approximately 20 percent of input) to both immobilized hemagglutinin (HA) antigen epitope–tagged dTBP (HA-dTBP) and $dTAF_{II}250\Delta N$. In contrast, $dTAF_{II}150$ did not interact selectively with HA-$dTAF_{II}110$ (Fig. 4A) as well as some of the other TAFs tested. These initial findings suggest that $dTAF_{II}150$ interacts with at least two subunits of the TFIID complex, dTBP and $dTAF_{II}250$, which also contact each other (*15*).

These interactions were confirmed in an in vivo experiment where insect (Sf9) cells were coinfected with two recombinant baculoviruses, one expressing $dTAF_{II}150$ and the second expressing either dTBP or one of the other TAFs. Complexes were then immunopurified from crude cellular lysates and analyzed by SDS-PAGE and subsequent immunoblotting with antibodies to $dTAF_{II}150$ (Fig. 4B). In agreement with our experiments in vitro, coinfection of viruses expressing $dTAF_{II}150$ and either HA-dTBP or $dTAF_{II}250\Delta N$ resulted in the efficient formation and copurification of heteromeric complexes. In that there is a high degree of sequence conservation between *Drosophila* and human $TAF_{II}250$ (*15, 30, 33, 34*), we also tested the ability of the human protein to interact with $dTAF_{II}150$. As was expected, a full-length version of $hTAF_{II}250$ bound efficiently to $dTAF_{II}150$. In contrast, only very weak association was observed with $dTAF_{II}110$ and no significant interaction with $hTAF_I48$, a subunit of transcription factor SL1 involved in RNA polymerase I transcription (*35*). All proteins were well expressed and soluble.

To further establish the specificity of these interactions, we also analyzed the resulting complexes by silver staining of SDS gels (Fig. 4C). For these experiments, extracts from Sf9 cells expressing $dTAF_{II}150$ were mixed with HA-dTBP or HA-$dTAF_{II}250$ and the resulting complexes were subjected to immunopurification with anti-

body to HA. Silver-stained SDS gels of the purified complexes revealed selective and efficient copurification of dTAF$_{II}$150 with dTBP and hTAF$_{II}$250. Furthermore, a mixture of dTBP and dTAF$_{II}$150 could be purified by their interaction with immobilized hTAF$_{II}$250 (lane 8). Although we cannot conclude from this experiment that a triple complex was formed, the stoichiometry of the copurified species suggests the formation of a ternary complex. However, our data indicate that dTAF$_{II}$150 can make direct contacts with at least two other components of dTFIID, namely dTBP and dTAF$_{II}$250.

In order to determine in which region of dTAF$_{II}$150 these interactions take place, we tested an NH$_2$-terminal 786-residue portion (dTAF$_{II}$150ΔC) and a COOH-terminal 369-residue portion (dTAF$_{II}$150ΔN) of this protein for interaction with dTBP or dTAF$_{II}$250ΔN (Fig. 4D). ^{35}S-labeled, in vitro translated dTAF$_{II}$150ΔN bound dTBP and dTAF$_{II}$250ΔN with the same efficiency as the full-length protein. In contrast, no significant binding of the NH$_2$-terminal portion to either dTBP or dTAF$_{II}$250ΔN was observed. Therefore we conclude that the interaction interfaces for these proteins are located in the COOH-terminal portion of dTAF$_{II}$150.

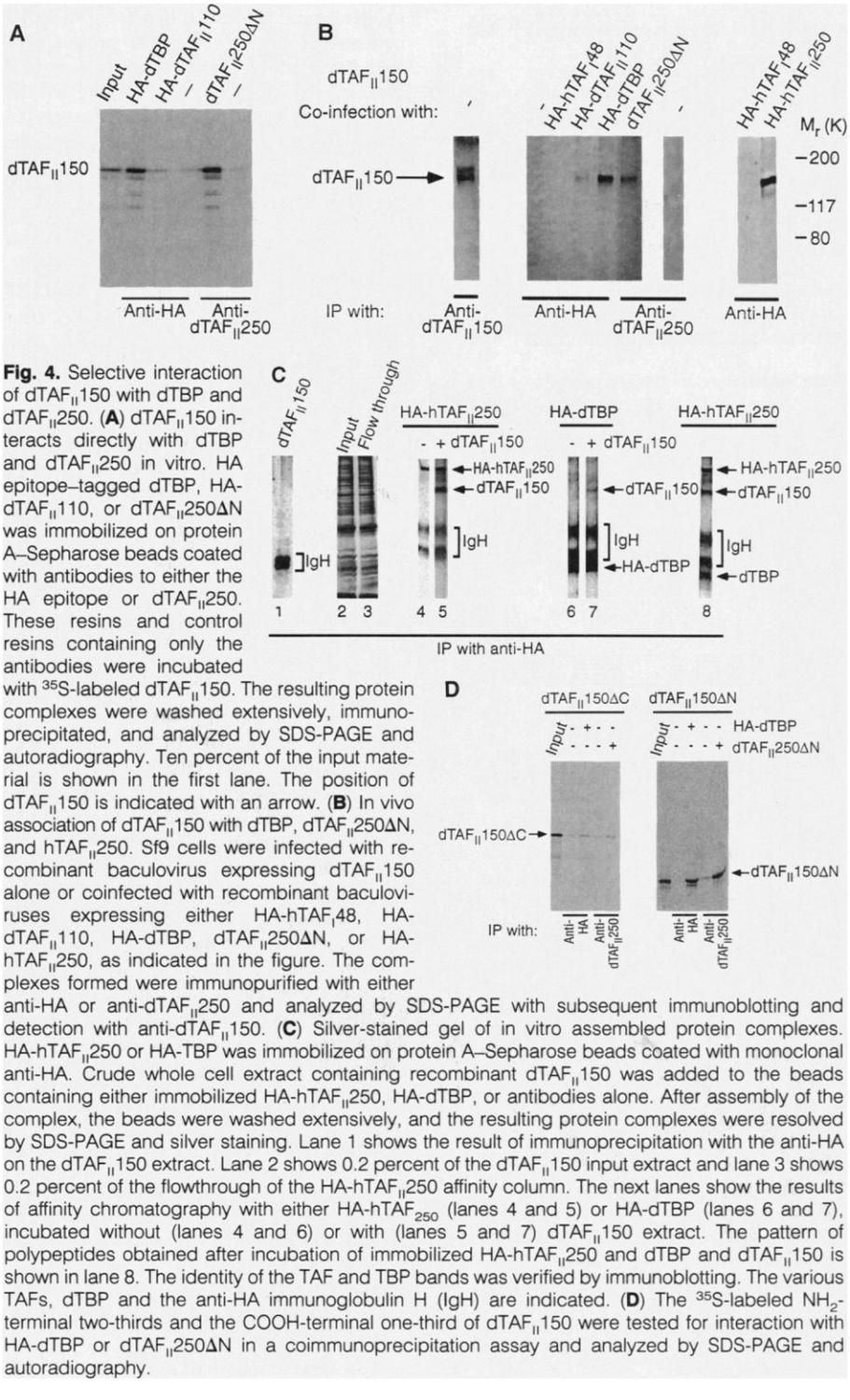

Fig. 4. Selective interaction of dTAF$_{II}$150 with dTBP and dTAF$_{II}$250. (**A**) dTAF$_{II}$150 interacts directly with dTBP and dTAF$_{II}$250 in vitro. HA epitope–tagged dTBP, HA-dTAF$_{II}$110, or dTAF$_{II}$250ΔN was immobilized on protein A–Sepharose beads coated with antibodies to either the HA epitope or dTAF$_{II}$250. These resins and control resins containing only the antibodies were incubated with ^{35}S-labeled dTAF$_{II}$150. The resulting protein complexes were washed extensively, immunoprecipitated, and analyzed by SDS-PAGE and autoradiography. Ten percent of the input material is shown in the first lane. The position of dTAF$_{II}$150 is indicated with an arrow. (**B**) In vivo association of dTAF$_{II}$150 with dTBP, dTAF$_{II}$250ΔN, and hTAF$_{II}$250. Sf9 cells were infected with recombinant baculovirus expressing dTAF$_{II}$150 alone or coinfected with recombinant baculoviruses expressing either HA-hTAF$_{I}$48, HA-dTAF$_{II}$110, HA-dTBP, dTAF$_{II}$250ΔN, or HA-hTAF$_{II}$250, as indicated in the figure. The complexes formed were immunopurified with either anti-HA or anti-dTAF$_{II}$250 and analyzed by SDS-PAGE with subsequent immunoblotting and detection with anti-dTAF$_{II}$150. (**C**) Silver-stained gel of in vitro assembled protein complexes. HA-hTAF$_{II}$250 or HA-TBP was immobilized on protein A–Sepharose beads coated with monoclonal anti-HA. Crude whole cell extract containing recombinant dTAF$_{II}$150 was added to the beads containing either immobilized HA-hTAF$_{II}$250, HA-dTBP, or antibodies alone. After assembly of the complex, the beads were washed extensively, and the resulting protein complexes were resolved by SDS-PAGE and silver staining. Lane 1 shows the result of immunoprecipitation with the anti-HA on the dTAF$_{II}$150 extract. Lane 2 shows 0.2 percent of the dTAF$_{II}$150 input extract and lane 3 shows 0.2 percent of the flowthrough of the HA-hTAF$_{II}$250 affinity column. The next lanes show the results of affinity chromatography with either HA-hTAF$_{250}$ (lanes 4 and 5) or HA-dTBP (lanes 6 and 7), incubated without (lanes 4 and 6) or with (lanes 5 and 7) dTAF$_{II}$150 extract. The pattern of polypeptides obtained after incubation of immobilized HA-hTAF$_{II}$250 and dTBP and dTAF$_{II}$150 is shown in lane 8. The identity of the TAF and TBP bands was verified by immunoblotting. The various TAFs, dTBP and the anti-HA immunoglobulin H (IgH) are indicated. (**D**) The ^{35}S-labeled NH$_2$-terminal two-thirds and the COOH-terminal one-third of dTAF$_{II}$150 were tested for interaction with HA-dTBP or dTAF$_{II}$250ΔN in a coimmunoprecipitation assay and analyzed by SDS-PAGE and autoradiography.

Functional comparison of dTAF$_{II}$150 and TSM-1. The high degree of conservation between dTAF$_{II}$150 and TSM-1 and the observation that in vivo both are part of a TBP-containing complex suggested that these proteins perform similar functions. In order to obtain further evidence that TSM-1 is a yeast TAF, we tested for its ability to bind directly to yTBP (36) and for its ability to associate with hTAF$_{II}$250. A ^{35}S-labeled in vitro translated COOH-terminal 920-residue portion of TSM-1 (TSM-1ΔN) or full-length dTAF$_{II}$150 were mixed with immobilized yTBP, HA-dTBP, or HA-hTAF$_{II}$250, and the resulting complexes were resolved by SDS-PAGE and autoradiography (Fig. 5). Like dTAF$_{II}$150, TSM-1ΔN bound efficiently to yTBP as well as to HA-dTBP. Although there are important functional differences between TBP species from yeast and higher eukaryotes (*37, 38*), this does not seem to be attributable to major differences in the interaction between TSM-1–TAF$_{II}$150 and TBP. Moreover, TSM-1ΔN also efficiently associates with HA-hTAF$_{II}$250, an indication that yeast most likely contains a TAF$_{II}$250 counterpart. The data suggest that the specificity for interaction with other TFIID subunits is conserved between TSM-1 and dTAF$_{II}$150.

The structural similarity and apparent functional homology between dTAF$_{II}$150 and TSM-1 described above suggested that mutants in TSM-1 might be rescued by expression of dTAF$_{II}$150 in yeast. To test this possibility, we expressed either wild-type TSM-1 or dTAF$_{II}$150 in a strain of *S. cerevisiae* carrying a temperature-sensitive mutation in TSM-1, JR1709 (39). The phenotypes of these transformants were subsequently examined at the permissive (25°C) and nonpermissive (37°C) temperatures. At 25°C all three transformants show normal growth. In contrast, at 37°C only transformants expressing the wild-type TSM-1 formed colonies of normal size whereas both the vector control and the strain expressing dTAF$_{II}$150 formed no colonies (Fig. 6). We verified by immunoblot analysis that expression of TSM-1 and dTAF$_{II}$150 was comparable. Our results indicate that, although dTAF$_{II}$150 and TSM-1 may have similar activities in vitro,

the *Drosophila* protein is not able to complement the defective yeast protein at the nonpermissive temperature. These results are reminiscent of experiments between *Drosophila* and *S. cerevisiae* TBP where the sequence similarity was high and where complementation was also not observed (*37, 38*). Thus, despite the extensive structural and functional similarity, there are critical species-specific differences between $dTAF_{II}150$ and TSM-1 which preclude functional interchangeability in vivo.

The $dTAF_{II}150$ sequence-specific DNA binding. It is generally accepted that TBP is the only component of TFIID that has sequence-specific DNA binding activity. However, compared to TBP, TFIID gives an extended footprint on certain promoters, revealing a broad range of interactions with core promoter elements encompassing the transcription start site (*10, 18–24*) (Fig. 7A). Using recombinant dTBP or immunopurified dTFIID we compared the DNase I footprint on the adenovirus major late promoter (AdML). As expected, dTBP gives a characteristic footprint of about 20 bp centered around the TATA box. In contrast, dTFIID gives a more than 70-bp footprint that extends downstream of the TBP binding site. Given the differences in DNA binding properties between TBP and TFIID, it seemed reasonable to test (*40*) whether $dTAF_{II}150$ or other TAFs might interact directly and specifically with core promoter elements.

We then used ultraviolet cross-linking to determine the potential specific or nonspecific DNA binding properties of dTAFs in the context of dTFIID. As expected, *Drosophila* TFIID isolated by antibody affinity chromatography with either antibody to $TAF_{II}250$ or antibody to $dTAF_{II}80$ contained $dTAF_{II}150$ (Fig. 7B). Furthermore, silver staining revealed the presence of dTBP and all the major dTAFs. These affinity-purified preparations of dTFIID were next allowed to bind a labeled AdML core promoter DNA fragment (position −44 to +38) substituted with bromodeoxyuridine (BrdU). The resulting protein-DNA complexes were subjected to ultraviolet cross-linking and nuclease digestion, and the isolated proteins were analyzed by SDS-PAGE and autoradiography. Under the assay conditions, we detected a labeled protein species of approximately 150 kD. Our results suggest that $dTAF_{II}150$ is selectively labeled and that this subunit makes contact with DNA when the TFIID complex is bound to the TATA box of the AdML promoter. In addition there is a somewhat weaker signal from a labeled protein species migrating with an apparent molecular mass of 70 kD which might correspond to $dTAF_{II}60$. We did not expect to detect dTBP in this assay since BrdU-substituted DNA should only cross-link proteins that make contacts in the major groove while TBP makes minor groove contacts (*2*).

The foregoing experiments do not address the critical question of whether $dTAF_{II}150$ alone can bind DNA. To test the DNA binding properties of $dTAF_{II}150$ in the absence of dTBP and the other dTAFs in the dTFIID complex, we used a baculovirus expression system to obtain $dTAF_{II}150$ and purified the protein from infected cell extracts by means of conventional column chromatography and immunopurification with anti-$dTAF_{II}150$. This highly purified preparation of $dTAF_{II}150$ was used in an ultraviolet cross-linking experiment (Fig. 7B, lane 4) and found that immunopurified, recombinant $dTAF_{II}150$ can be cross-linked to the AdML promoter. The mobility of the cross-linked species in purified $dTAF_{II}150$ was indistinguishable from the cross-linked 150-kD protein in the immunopurified TFIID; these data showed that recombinant $dTAF_{II}150$, in the absence of TBP and TFIID, could bind to sequences within the core promoter region.

As an independent test of $dTAF_{II}150$ DNA binding activity, we performed electrophoretic mobility-shift assays with purified recombinant $dTAF_{II}150$ alone or in complex with dTBP. Using an end-labeled DNA fragment bearing the TATA box and flanking sequences (position −53 to +45) from the AdML promoter, we found that purified $dTAF_{II}150$ produced a distinct shifted band (Fig. 7C). To ascertain that $dTAF_{II}150$ was responsible for this shifted species, antibodies to recombinant $dTAF_{II}150$ were used to supershift the $dTAF_{II}150$-DNA complex (Fig. 7C, lane 2). These experiments were performed in the presence of an excess amount of nonspecific DNA [poly(dG.dC)]. Furthermore, addition of unlabeled AdML promoter DNA, but not a nonrelated DNA fragment, severely inhibited the binding of $dTAF_{II}150$ to the labeled DNA. These results confirm that purified $dTAF_{II}150$ alone can specifically bind DNA sequences within the core promoter. We then tested $dTAF_{II}150$ for its ability to bind DNA when in a complex with dTBP and dTFIIB. As expected, when $dTAF_{II}150$ was added to a preparation of purified dTBP and dTFIIB, a slower migrating complex was observed (Fig. 7C, lane 8). We suspect that this slowest migrating species consisted of TBP, TFIIB, and $dTAF_{II}150$ since its mobility was distinct from that of $TAF_{II}150$ alone, or TBP plus TFIIB. In addition, the mobility of this complex was slower than that of TBP-150 complex. These results suggest that a complex between dTBP, dTBIIB, and $dTAF_{II}150$ can bind core promoter DNA efficiently.

Because we now know that $dTAF_{II}150$ binds to both TBP and $TAF_{II}250$ through direct protein-protein contacts, we tested the DNA binding properties of the dimeric and trimeric complex. This was necessary because it had been suggested by others that full-length $TAF_{II}250$ might inhibit binding

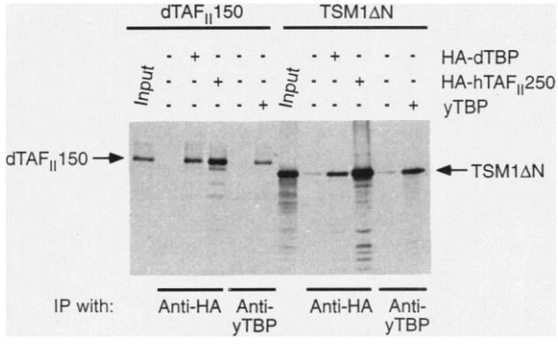

Fig. 5. TSM-1 associates with TBP and $TAF_{II}250$. HA-dTBP, HA-$hTAF_{II}250$ or yTBP was immobilized on protein A–Sepharose beads coated with antibodies to either the HA epitope or yTBP. These resins and control resins containing only the antibodies were incubated with either ^{35}S-labeled $dTAF_{II}150$ or a truncated version of TSM-1 consisting of the COOH-terminal 75 percent of the protein. After being extensively washed the resulting protein complexes were immunoprecipitated and analyzed by SDS-PAGE and then by autoradiography. Ten percent of the input material is shown. The positions of $dTAF_{II}150$ and TSM-1ΔN are indicated with an arrow.

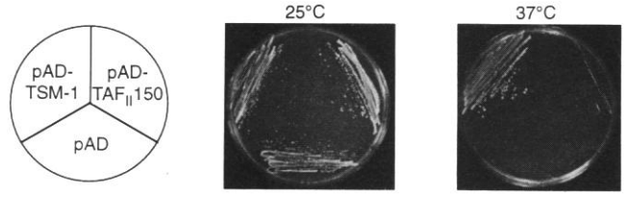

Fig. 6. $dTAF_{II}150$ does not complement a temperature-sensitive mutant of TSM-1. The *S. cerevisiae* strain JR1709 that contains a *ts* lethal mutation in TSM-1 was transformed with a vector (pAD) expressing either no recombinant protein, wild-type TSM-1 (pAD-TSM-1) or $dTAF_{II}150$ (pAD-$dTAF_{II}150$). Transformants were streaked out on plates and incubated at either 25°C or at 37°C.

of DNA by TBP (33). In order to assemble a complex that contained dTBP, $hTAF_{II}250$, and $dTAF_{II}150$, we used an HA epitope–tagged version of dTBP. First we attached purified recombinant HA-dTBP to the affinity resin, and then we added molar excess of either $dTAF_{II}150$ or $hTAF_{II}250$ and $dTAF_{II}150$ to the affinity resin. The excess recombinant subunits not bound to TBP were removed by washing and the resulting double or triple complexes were eluted with HA peptide. The affinity-purified preparations of HA-dTBP, HA-dTBP plus $dTAF_{II}150$, and HA-dTBP plus $dTAF_{II}150$ plus $hTAF_{II}250$ were then assayed for DNA binding by gel shift. As expected, HA-dTBP alone gave a modest amount of fast migrating shifted species (Fig. 7D). The addition of full-length $dTAF_{II}150$ to HA-dTBP shifted the complex to a prominent slower migrating form which presumably represented the double complex bound to DNA. Surprisingly, the addition of $TAF_{II}250$ to form the triple complex did not inhibit the binding of DNA. Instead, there was measurable enhancement of the shifted species, and the appearance of a supershifted species. When a control extract was passed over the affinity resin containing anti-HA, there was no detectable DNA binding activity in the HA peptide eluate. We confirmed the presence of stoichiometric amounts of $hTAF_{II}250$ by SDS-PAGE. These results suggest that preassembled complexes containing dTBP and $dTAF_{II}150$ or dTBP, $dTAF_{II}150$, and $hTAF_{II}250$ efficiently bound to DNA containing the TATA box and flanking sequences.

The sequence specificity of $dTAF_{II}150$ was obtained from DNase I footprint protection experiments. Purified recombinant $dTAF_{II}150$ was allowed to bind sequences within the AdML core promoter fragment (nucleotides −116 to +61) labeled at the 5′ end of either the coding or noncoding strands (Fig. 8). Under standard DNase footprinting conditions, we observed that $dTAF_{II}150$ recognizes and protects a region of about 30 to 40 bp from digestion by DNase I. This large protected region begins near the start site of transcription, nucleotide −1 on the transcribed strand and nucleotide −2 on the nontranscribed strand and extends to at least position +33 on the transcribed strand and position +38 on the nontranscribed strand. Like the native TFIID footprint, an enhanced cleavage was observed at position +9 in the nontranscribed strand and position +4 in the transcribed strand. Our experiments established that $dTAF_{II}150$ is indeed a sequence-specific DNA binding protein that can recognize and bind to elements overlapping the initiator and that extend at least 30 bp into the transcribed region. These findings also identified a component

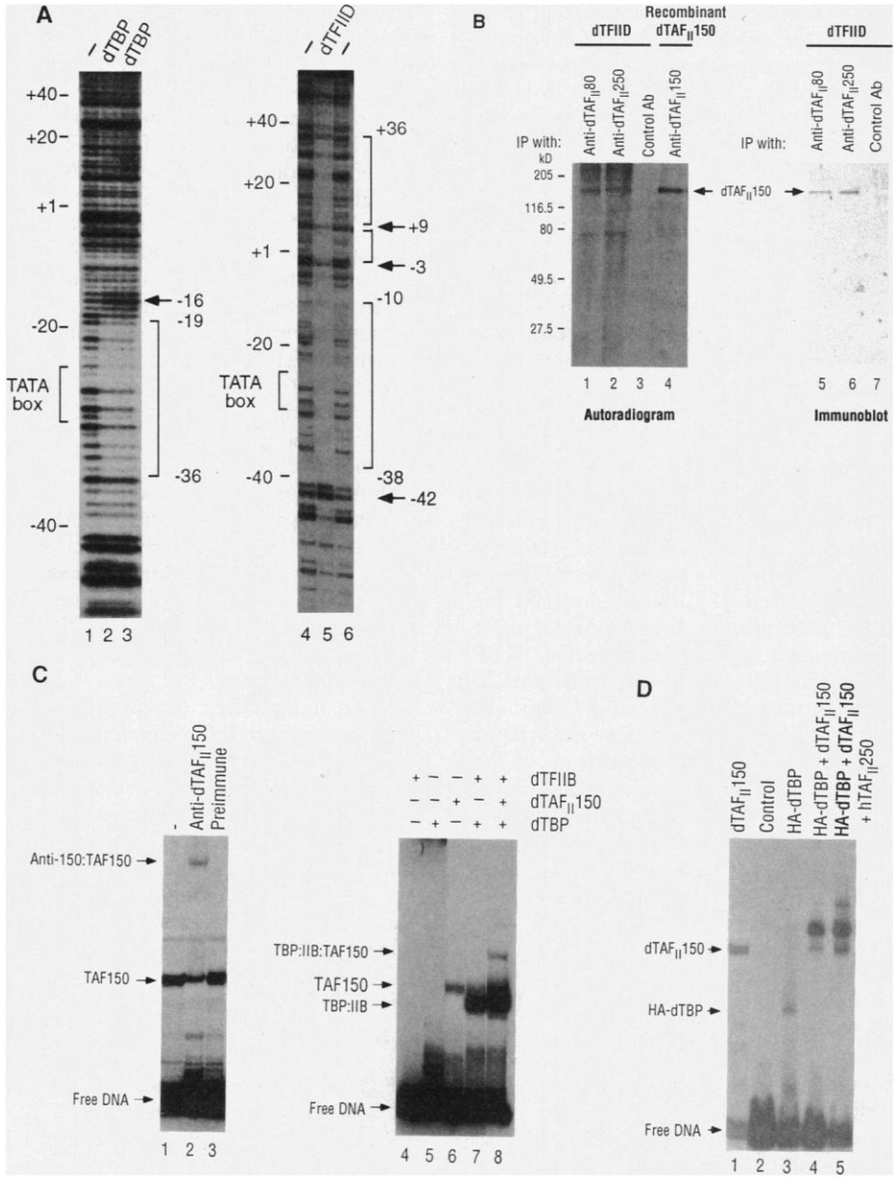

Fig. 7. The $dTAF_{II}150$ is a sequence-specific DNA binding protein. (**A**) DNase I footprinting analysis of dTBP and dTFIID on the AdML promoter. An AdML promoter fragment was labeled on the nontranscribed strand and was incubated with no protein (lanes 1, 4, and 6), with purified recombinant dTBP (lanes 2 and 3), or with dTFIID immunopurified with monoclonal anti-$dTAF_{II}250$ (lane 5) followed by partial DNase I digestion. The location of the fragments on the gel relative to the transcriptional start site are indicated. Protected region and enhanced cleavage sites are also indicated. (**B**) Ultraviolet cross-linking of $dTAF_{II}150$ to the AdML core promoter. Immunoaffinity-purified dTFIID with either anti-$dTAF_{II}80$ (lane 1), anti-$dTAF_{II}250$ (lane 2), or control (anti-HA, lane 3) monoclonal antibodies or immunopurified recombinant $dTAF_{II}150$ (lane 4) was allowed to bind a ^{32}P-labeled BrdU-substituted AdML promoter fragment. After ultraviolet cross-linking and nuclease treatment, proteins were analyzed by SDS-PAGE and then by autoradiography. The right panel shows an immunoblot analysis with anti-$dTAF_{II}150$ on the TFIID immunoprecipitates. (**C**) DNA binding of $dTAF_{II}150$ was assayed in an electrophoretic mobility-shift experiment with an end-labeled AdML promoter fragment (lane 1). Addition of anti-$dTAF_{II}150$ (lane 2) but not preimmune serum (lane 3) supershifted the retarded band. In the right panel various combinations of purified recombinant dTBP, dTFIIB, and $dTAF_{II}150$ were assayed in a gel shift experiment. Indicated are the proteins added and the resulting DNA-protein complexes. (**D**) Electrophoretic mobility-shift analysis of preformed TBP-TAF complexes. The same strategy as that described in Fig. 4C was used, HA-dTBP immobilized on protein G beads coated with anti-HA antibodies was used to build dimeric (dTBP + $dTAF_{II}150$) and trimeric (dTBP + $dTAF_{II}150$ + $hTAF_{II}250$) complexes. As a control, an extract from mock-infected Sf9 cells was passed over the anti-HA resin. Lane 1 shows the DNA binding of purified $dTAF_{II}150$ alone. After assembly and extensive washing, proteins were eluted with HA-peptide and tested for DNA binding activity using the AdML promoter as a probe.

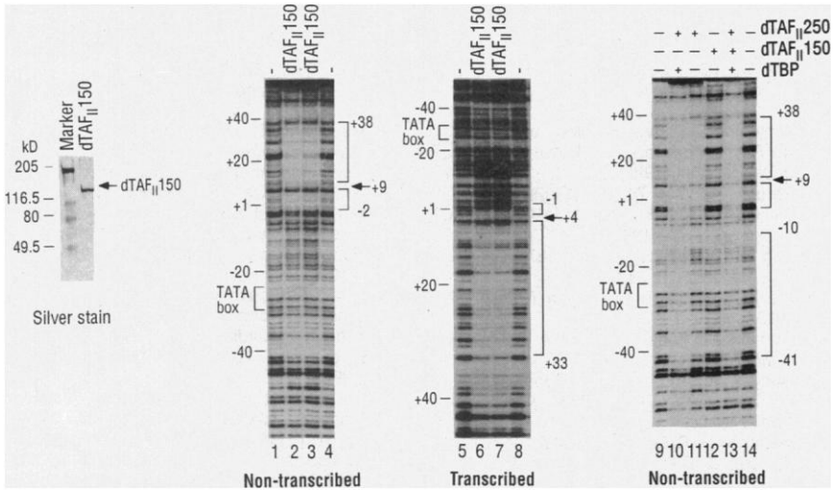

Fig. 8. DNase I footprinting analysis of dTAF$_{II}$150 on the AdML promoter. DNase I digestion patterns on the AdML promoter labeled on the nontranscribed strand (lanes 1 to 4) or the transcribed strand (lanes 5 to 8). The DNase I digestion was either in the absence of protein (lanes 1, 4, 5, and 8) or in the presence of recombinant, purified dTAF$_{II}$150 (lanes 2, 3, 6, and 7). A silver-stained gel of the dTAF$_{II}$150 preparation that is used is shown. Binding reactions contained between 10 and 40 ng of dTAF$_{II}$150. The DNase I footprinting activity of dTAF$_{II}$150 was also examined in the presence of dTBP (lane 10) or dTBP and hTAF$_{II}$250 (lane 13). Lane 11 shows dTAF$_{II}$150 alone, lane 12 shows hTAF$_{II}$250 alone, and in lanes 9 and 14 no protein was added.

of the "core" transcriptional apparatus (other than TBP) that directs sequence-specific DNA binding interactions with the core promoter.

Since TAF$_{II}$150 is not expected to act alone in vivo to bind DNA but is instead predominantly if not exclusively part of a larger complex containing TBP and other TAFs, we have also performed preliminary footprinting experiments with partial TFIID complexes. A complex containing dTBP, dTAF$_{II}$150, and hTAF$_{II}$250 binds and protects an extended region of DNA overlapping the TATA box, initiator, and downstream sequences up to position +38. Indeed, this extended region of protection is reminiscent of but not identical to the footprint pattern of the complete TFIID complex (Fig. 7A). For example, the characteristic enhanced cleavage at position +9 is observed with either dTAF$_{II}$150 alone or the TFIID complex. Thus, it appears that TBP and dTAF$_{II}$150 may be largely responsible for the classical "extended footprint" characteristic of the TFIID complex, which is quite distinct from the 20-bp protected region of purified TBP.

Identification of TSM-1 as a yeast RNA polymerase II TAF. Although genes homologous to RNA polymerase II TAFs had not been identified, several studies indicated that, like the *Drosophila* and human systems, yeast TBP and the other basal factors are not sufficient for activated transcription (*11, 41, 42*). Instead, additional factors and activities are required for regulation of transcription by activators. Whether the formation of these putative coactivators in yeast is analogous to TAFs in higher eukaryotes remains unclear. Although yTBP was thought to exist as an isolated entity in yeast, recent studies suggest that at least some TBP-TAF complexes can be found in yeast extracts. For example, TBP and associated subunits have been detected for RNA polymerase III transcription factor TFIIB (*43, 44*). Moreover, recent results indicate that about 70 percent of yTBP is stably associated as a complex with other proteins (*44*). Like the human and *Drosophila* systems, these large protein complexes could be immunopurified with the use of antibodies to yTBP.

Our finding that there is a significant structural and functional conservation between *Drosophila* dTAF$_{II}$150 and the essential yeast gene TSM-1 strongly suggests that yeast also makes use of a class of transcription factors that interact with TBP to mediate RNA polymerase II transcription. Further support for this hypothesis comes from the finding that complexes that are immunoaffinity-purified with anti-yTBP from yeast nuclei contain TSM-1. In addition, yTBP copurifies with TSM-1. Furthermore, the observation that TSM-1 not only efficiently associates with yTBP and dTBP but also with hTAF$_{II}$250 indicates that yeast may also contain a homolog of TAF$_{II}$250 as well as other subunits of *Drosophila* and human TFIID. This possibility is supported by our recent findings that other factors interacting selectively with dTAF$_{II}$150, like dTAF$_{II}$30B (*45*), also interact with TSM-1 (*46*). However, it is possible that the subunits in yeast TFIID will be fewer and possibly different compared to those in *Drosophila* or human TFIID. Despite the high degree of structure and function similarity, our experiments suggest that dTAF$_{II}$150 cannot replace TSM-1 in yeast complementation studies. A similar lack of functional interchangeability in vivo has also been observed for TBP; that is, hTBP and dTBP cannot complement TBP null mutations in yeast (*37, 38*). From these results we conclude that although the mechanism of RNA polymerase II transcriptional regulation in yeast and higher eukaryotes is more similar than was previously anticipated, it is certainly not identical. Furthermore, the critical function of TSM-1 for yeast viability supports an essential role of dTAF$_{II}$150 in gene regulation of all eukaryotes.

Our initial studies of TAFs focused on their potential role as coactivators that mediate by making direct contact with activators. Indeed, several examples of such interactions of TAFs and activators are now available (*14, 15, 16*). However, it has also become evident that at least some TAFs in the TFIID complex are likely to participate in other types of interactions to regulate transcription. For example, some TAFs are responsible for binding directly to other components of the basal transcriptional apparatus (*16, 17*). In addition to these protein-protein interactions, there have been various clues, suggesting that TAFs may also contribute to interactions with the DNA template at the promoter. It is these potential DNA contacts with subunits of TFIID that we report here.

Of the first seven TAFs that were cloned and characterized (250, 110, 80, 60, 40, 30α, and 30β) none exhibited any detectable sequence-specific DNA binding activity. Ironically, the last of the major TAFs to be isolated and characterized in *Drosophila* was dTAF$_{II}$150. Some reports have suggested that the 150-kD protein in dTFIID may be an artifact or contaminant (*47*). We sometimes also observe a nonspecific contaminant in the 150-kD range. However, this protein can easily be separated from the genuine dTAF$_{II}$150 on 7.5 percent polyacrylamide gels. We now know that dTAF$_{II}$150 is highly labile and sensitive to proteolytic degradation, which might explain why substoichiometric amounts of this protein are sometimes found in TFIID preparations. However, several lines of evidence reported in this article help establish that dTAF$_{II}$150 is an important and integral subunit of TFIID. First, if care is taken, dTFIID isolated with anti-dTBP contains stoichiometric amounts of dTAF$_{II}$150. Second, antibodies to recombinant dTAF$_{II}$150 immunoprecipitate the TFIID complex containing all the other dTAFs and dTBP. Third, dTAF$_{II}$150 turns out to be a ho-

molog of the essential yeast gene TSM-1, whose product coprecipitates with yTBP, establishing this subunit as a bona fide TAF. Finally, we document the sequence-specific DNA binding properties of $dTAF_{II}150$. Not only does $dTAF_{II}150$ bind to core promoter DNA sequences on its own, but in conjunction with TBP produces an extended footprint characteristic of TFIID binding to DNA. These results taken together establish that $dTAF_{II}150$ is a subunit of dTFIID that may turn out to be one of the more essential components of this transcription factor. For example, $dTAF_{II}150$ may be critical for stabilizing TFIID-DNA complexes, especially at promoters lacking a TATA box. This subunit of TFIID may also be in part responsible for recognizing initiator sequences, although additional experiments are needed to substantiate this point. What DNA sequence motifs are critical for $dTAF_{II}150$ DNA binding remains to be determined.

The discovery that $dTAF_{II}150$ can bind specifically to promoter sequences may also help explain the differences in transcriptional activity between different core promoters. For example, the AdML promoter is strong compared to the E4 promoter. Interestingly, when TFIID binds the E4 core promoter no obvious extended footprint is observed (*21*). Further study should reveal the potential role of TAFs in determining basal promoter strength. An interesting possibility is that TAF-DNA interactions induce distinct conformational forms of TFIID that might influence promoter-specific responses to upstream activators. Also DNA recognition and binding properties of $dTAF_{II}150$ could turn out to be important for mediating activation. For example, we have shown that the Gln-rich activation domains of Sp1 interact with $TAF_{II}110$ and that a TBP-$TAF_{II}250$-$TAF_{II}110$ complex is only weakly functional for transcription mediated by Sp1 (*15*). It will be interesting to determine whether $TAF_{II}150$ can help stabilize this partial complex via both protein-protein as well as protein-DNA interactions and therefore enhance the activity mediated by $TAF_{II}110$ during activation by Sp1. With the isolation of $dTAF_{II}150$ and its biochemical characterization as a sequence-specific DNA binding protein, we are now in a good position to attempt assembly of a functional TFIID complex.

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26. Nuclear extracts were prepared from 800 g of embryos (0 to 14 hour), and precipitated with polyvinyl alcohol (PVA). TFIID was either immunopurified directly or from a Q-Sepharose fraction as described (*5*). TAFs were eluted and precipitated with 100 percent trichloroacetic acid (TCA) containing deoxycholate (4 mg/ml), resolved by SDS-PAGE, and transferred to PVDF membrane and stained with ponceau S (Sigma). A band corresponding to $dTAF_{II}150$ was excised, digested with trypsin, eluted from the membrane, and, after fractionation by reversed-phase HPLC, subjected to microsequencing. The amino acid sequences of nine peptides were obtained (see Fig. 2A). $dTAF_{II}150$ was cloned by two different, partially degenerate oligonucletodides 5′-AACAACTTCTCI-AACTTCCAGCTITACTTCCTICAGAAG-3′, based on the peptide NNFSNFQLYFLQK, and 5′-AC(C/T)AT(C/T)GAGCA(G/A)CCIGA(C/T)TT(C/T)CA-(G/A)TGGCA(G/A)TA(C/T)CA/(G/A)-3′, based on the peptide IIEQPDFQWQYQ, these were used to screen a 3- to 9-hour *Drosophila* embryo λ gt-11 cDNA library. Approximately 8×10^5 recombinant phages were screened and 11 positive clones were isolated and analyzed. All contained related cDNAs, and the inserts of six recombinant phages were subcloned into Bluescript KS (Stratagene) and partially sequenced. After construction of deletions with various restriction enzymes, both strands of the longest cDNA were completely sequenced (Pharmacia ALF sequencer) with Bluescript specific primers, or with $dTAF_{II}150$-specific primers for conventional dideoxy sequencing.
27. A COOH-terminal polypeptide starting with Met^{786} (Fig. 2A) was expressed in *E. coli* with the T7 expression vector pRSETC (Invitrogen). The protein was gel-purified and used to produce a polyclonal antiserum in rabbits. The complete $dTAF_{II}150$ cDNA was cloned into the baculovirus expression vector pVL1392 (Pharmingen), and the resulting construct was cotransfected with BaculoGold viral DNA (Pharmingen) into Sf9 cells. The resulting recombinant virus was used for expression in Sf9 cells; 48 hours after infection extracts were prepared by sonicating the cells in 0.4 HEMG-NDAM [0.4 M KCl, 25 mM Hepes, pH 7.6, 0.1 mM EDTA, 12.5 mM $MgCl_2$, 10 percent glycerol, 0.1 percent NP-40, 2 mM dithiothreitol (DTT), 0.2 mM 4-(2-aminoethyl)-benzene sulfonyl fluoride, and 5 mM sodium metabisulfide]. The sonicate was centrifuged at 100,000*g* and the supernatant was divided into equal portions and stored at −80°C. Immunoprecipitations were performed with monoclonal antibodies to dTBP (25B4), $dTAF_{II}250$ (30H9), $dTAF_{II}80$ (3D10), $dTAF_{II}60$ (3B3) (*5, 15*).
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32. HA-dTBP, HA-$dTAF_{II}110$, and $dTAF_{II}250\Delta N$ were purified essentially as described (*14, 15, 31*). The $dTAF_{II}150$ in vitro transcription-translation vector pTB-150 was constructed by creating an Nco I site at the initiating methionine codon of the cDNA with a PCR-based (polymerase chain reaction) strategy and subcloning of the complete coding region into pTB-STOP (*5*). $dTAF_{II}150\Delta N$ and $dTAF_{II}150\Delta C$ are expressed by means of pBluescript II KS+; pTAF150ΔN expresses the COOH-terminal one-third of the protein starting from Met^{844}, and pTAF150ΔC expresses the NH_2-terminal two-thirds of the protein, from the first Met to Asn^{786}. The ^{35}S-labeled proteins were produced with the TNT coupled transcription-translation system (Promega). Construction of the baculovirus expression vectors for the other TAFs have been described or descriptions are in preparation (*14, 15, 30, 31*). For coimmunoprecipitation, protein A–Sepharose beads were first coated with monoclonal antibodies to $dTAF_{II}250$ (30H9) or to the HA epitope. Next, 1 to 2 μg of HA-tagged TAFs, HA-dTBP, or $dTAF_{II}250\Delta N$ was bound to about 15 μl of beads at 4°C in 0.1 HEMG-NDA. Unbound protein was washed away and the second protein (^{35}S-labeled $dTAF_{II}150$ or a crude Sf9 cellular extract) was added. After incubation for 2 hours at 4°C in 0.1 HEMG-NDA the beads were washed four times with a 100-fold excess volume of 0.25 HEMG-NDA, resuspended in SDS sample buffer, and analyzed by SDS-PAGE. For coinfection experiments, Sf9 cells were infected with $dTAF_{II}150$ expressing recombinant baculovirus together with baculoviruses expressing one of the other TAFs. At 48 hours after infection cell lysates were prepared by sonicating the cells in 0.1 HEMG-NDAM. The sonicate was centrifuged at 100,000*g*, and complexes were immunopurified from the supernatant with protein A–Sepharose beads coated with the indicated antibody, washed extensively with 0.25 HEMG-NDA and analyzed by SDS-PAGE and immunoblotting with anti-$dTAF_{II}150$.
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36. Experimental methods were as described (*31*). The yTBP purified from overexpressing bacteria, and the anti-yTBP have been described (*38*). The TSM-1 in vitro transcription-translation vector was constructed by subcloning of the 2850-bp Nde I–Nde I fragment from the TSM-1 gene into pTB-STOP. The encoded polypeptide started with Met at position 489.
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40. The DNase I footprinting and bandshift assays

were performed as described (*5, 48*) and a typical binding reaction contained 50 to 100 ng of poly (dG-dC) and about 1.5 fmol of labeled DNA. Bandshift reactions contained between 5 to 25 ng of $dTAF_{II}150$; footprinting reactions contained between 10 and 40 ng of $dTAF_{II}150$. Ultraviolet cross-linking was performed under standard DNA binding conditions (*5, 48*) in the presence of 100 ng of poly (dG-dC). After binding of the proteins to a BrdU-substituted, body-labeled AdML probe generated by PCR, reactions were irradiated with ultraviolet (304 nm) light for 15 minutes at room temperature and then treated with DNase I and micrococcal nuclease for another 15 minutes at 30°C. Samples were then analyzed by SDS-PAGE. The $dTAF_{II}150$ was purified by a combination of DEAE, SP-Sepharose, and Heparin-agarose column chromatography. Immunopurifications of $dTAF_{II}150$ and partially purified TFIID (*5*) were performed as described (*32*).

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