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Cloning and expression of *Drosophila* $TAF_{II}60$ and human $TAF_{II}70$ reveal conserved interactions with other subunits of TFIID

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Regulation of transcription initiation by RNA polymerase II requires TFIID, a multisubunit complex composed of the TATA binding protein (TBP) and at least seven tightly associated factors (TAFs). Some TAFs act as direct targets or coactivators for promoter-specific activators while others serve as interfaces for TAF–TAF interactions. Here, we report the molecular cloning, expression and characterization of *Drosophila* $dTAF_{II}60$ and its human homolog, $hTAF_{II}70$. Recombinant $TAF_{II}60/70$ binds weakly to TBP and tightly to the largest subunit of TFIID, $TAF_{II}250$. In the presence of $TAF_{II}60/70$, TBP and $TAF_{II}250$, a stable ternary complex is formed. Both the human and *Drosophila* proteins directly interact with another TFIID subunit, $dTAF_{II}40$. Our findings reveal that *Drosophila* $TAF_{II}60$ and human $TAF_{II}70$ share a high degree of structural similarity and that their interactions with other subunits of TFIID are conserved.

Key words: evolutionary conservation/protein–protein interactions/RNA polymerase II transcription/TATA binding protein associated factors/TFIID

Introduction

Since the discovery that TFIID is not the same as the TATA binding protein, TBP, but instead consists of a multisubunit complex containing TBP and TBP-associated factors (TAFs), much interest has been focused on this essential transcription factor (Dynlacht *et al.*, 1991; Pugh and Tjian, 1991; Tanese *et al.*, 1991). The first clue that TAFs perform an important function was the finding that recombinant TBP could restore basal transcription *in vitro* (Hoey *et al.*, 1990; Peterson *et al.*, 1990) while promoter-specific activation by transcription factors such as Sp1, CTF and NTF1 requires the TFIID complex in both *Drosophila* and human reconstituted RNA polymerase (RNA pol) II systems (Dynlacht *et al.*, 1991; Tanese *et al.*, 1991; Zhou *et al.*, 1992). Interestingly, distinct sets of TAF–TBP complexes have also been found to be essential components of the transcriptional apparatus for RNA pol I (SL1) (Comai *et al.*, 1992) and RNA pol III (TFIIIB) (Kassavetis *et al.*, 1992; Lobo *et al.*, 1992; Taggart *et al.*, 1992; White and Jackson, 1992). These studies established the universal role of TBP in transcription and underscore the coactivator functions of TAFs (reviewed in Gill, 1992). In order to understand the mechanism of transcriptional regulation in eukaryotes we must therefore first define the biochemical properties of TAFs and dissect their involvement in the assembly of transcription initiation complexes.

For TFIID, progress in characterizing individual TAFs has been quite rapid. The first of the TFIID subunits to be purified and cloned and to have its functional properties studied was *Drosophila* $TAF_{II}110$ (Hoey *et al.*, 1993). This 110 kDa transcription factor binds to the largest subunit of TFIID, $TAF_{II}250$, and serves as one of the targets for interaction with the Gln-rich domains of the promoter-selective transcription factor Sp1 (Hoey *et al.*, 1993; Weinzierl *et al.*, 1993). The second subunit of TFIID to be characterized was $TAF_{II}250$, which binds directly to TBP and appears to serve as the core component that links other TAFs to TBP. Molecular cloning of human (h) $TAF_{II}250$ revealed an unexpected result: the $hTAF_{II}250$ gene is identical to a previously isolated gene called *CCG-1* which had been implicated in cell cycle progression from G_1 to S (Sekiguchi *et al.*, 1991; Hisatake *et al.*, 1993; Ruppert *et al.*, 1993). The *Drosophila* homolog, $dTAF_{II}250$, is highly similar in structure to the human protein with extensive stretches of homology throughout the protein coding region (Kokubo *et al.*, 1993; Weinzierl *et al.*, 1993). The most recent TAF to be isolated and characterized was $dTAF_{II}80$ which contains β-transducin repeats and assembles into the TFIID complex via specific TAF–TAF interactions (Dynlacht *et al.*, 1993). Because TFIID contains some eight distinct subunits, several TAFs remain to be isolated and characterized. One major issue that we hope to address is the relationship between TAFs and TBP, as well as specific contacts between TAFs which must occur in the assembled complex. In addition, studies of *Drosophila* and human TFIID suggest that there may be similarities in the composition and pattern of TAF subunits and thus there is likely to be both structural and functional conservation (Dynlacht *et al.*, 1991; Tanese *et al.*, 1991).

Antibodies directed against $TAF_{II}60/70$ were used to establish the identity and activity of this subunit. We also expressed recombinant versions of $TAF_{II}60/70$ and have begun to analyze specific interactions with other subunits of the TFIID complex. Finally, we tested the ability of *Drosophila* $dTAF_{II}60$ to bind a subunit of the human TFIID complex, and vice versa. Our results suggest that TAFs are structurally and functionally conserved between *Drosophila* and man, and that they assemble into the TFIID complex by contacting analogous subunits.

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Results and discussion

Cloning of Drosophila dTAF$_{II}$60 and its human homolog

In a systematic effort to isolate cDNAs encoding the various TAFs, we generated polyclonal antibodies against the subunits of *Drosophila* TFIID and then used these antisera to screen a λgt11 cDNA library (Zinn *et al.*, 1988) derived from *Drosophila* embryos (Dynlacht *et al.*, 1993; Hoey *et al.*, 1993; Weinzierl *et al.*, 1993). From this initial screen we obtained several clones encoding products that cross-reacted specifically with polyclonal and monoclonal antibodies directed against dTAF$_{II}$60. The largest of these cDNA clones, λD6, contained an insert of ~2.0 kb, and DNA sequence analysis revealed an open reading frame encoding a protein of 580 amino acids (Figure 1). Northern blot analysis indicated an mRNA species of ~2.1 kb (S.Ruppert, data not shown), and the deduced protein derived from this sequence has a calculated molecular weight of 64.1 kDa and an estimated isoelectric point of 9.4. Several peptide sequences generated by tryptic digests of purified endogenous dTAF$_{II}$60 protein were found within this open reading frame, thus confirming the identity of λD6.

Next, we used radiolabelled DNA probes derived from λD6 to screen a HeLa cell cDNA library to identify the human homolog. Several cDNAs were characterized and three of the longest clones were sequenced. DNA sequence analysis of these cDNAs revealed at least three distinct species (hTAF$_{II}$70α, β and γ) which appear to be the result of alternative splicing (Figure 1B). We chose a cDNA clone encoding hTAF$_{II}$70α for further characterization. The deduced amino acid sequence predicts a protein of 677 amino acids corresponding to a calculated molecular weight of 72.7 kDa and predicted isoelectric point of 9.1. Furthermore, two peptide sequences derived from tryptic digests of endogenous hTAF$_{II}$70 are found within this reading frame (see Figure 1A) thus confirming the identity of the clone. For convenience we shall henceforth refer to the *Drosophila* protein as dTAF$_{II}$60 and the human protein as hTAF$_{II}$70.

Southern blot analysis of genomic DNA confirmed that both the *Drosophila* and human genes encoding this TAF are single-copy (S.Ruppert, data not shown). Polytene chromosome *in situ* hybridization of the *Drosophila* clone localized the dTAF$_{II}$60 gene to position 76B9-10 on the left arm of chromosome 3. Interestingly, this map location lies at or very near the position of a previously characterized *Drosophila* locus bearing the genes of *Ash-1* (J.Tamkun and J.Kennison, personal communication). Mutations in *Ash-1* cause a wide variety of homeotic transformations affecting imaginal disc development (Shearn *et al.*, 1987). It will be of interest to determine whether *Ash-1* actually encodes dTAF$_{II}$60 and whether expression of this TAF in mutant flies can rescue the homeotic phenotype.

Sequence conservation between dTAF$_{II}$60 and hTAF$_{II}$70

A direct comparison of amino acid sequences between dTAF$_{II}$60 and hTAF$_{II}$70 indicates two regions of extensive (84%) similarity (Figure 1A), located toward the N-terminal two-thirds of these proteins. In contrast, the C-terminal one-third shares no significant sequence homology but contains sequences particularly rich in serine (dTAF$_{II}$60) or proline, serine and threonine (hTAF$_{II}$70). This localized high degree of conservation is reminiscent of the structure of TBP which also contains an evolutionarily highly conserved domain linked to diverged N-terminal sequences in various species [e.g. Peterson *et al.* (1990)]. We propose that many of the TAF–TAF interaction surfaces and/or functional domains evolved at an early stage of eukaryotic evolution whereas other portions of TAF sequences may have been free to diversify e.g. species-specific features. These results, taken together with the reported homology between human and *Drosophila* TAF$_{II}$250 (Kokubo *et al.*, 1993; Ruppert *et al.*, 1993; Takada *et al.*, 1992; Weinzierl *et al.*, 1993), confirm our previous hypothesis that many of the TAFs originally identified in the *Drosophila* complex have a counterpart in human TFIID even though the apparent molecular weights of individual proteins may differ (Dynlacht *et al.*, 1991; Tanese *et al.*, 1991; see also Figure 2A).

Interaction of dTAF$_{II}$60 and hTAF$_{II}$70 with other TFIID subunits

As a first step towards characterizing the biochemical properties of dTAF$_{II}$60 and hTAF$_{II}$70 we produced the *Drosophila* and human proteins either in a baculovirus expression system or by *in vitro* translation. In the case of hTAF$_{II}$70, we have also expressed a hemagglutinin (HA) epitope tagged version. As expected, the size of the virally expressed dTAF$_{II}$60 is indistinguishable from that of endogenous dTAF$_{II}$60, as determined by SDS–PAGE and Western blotting (Figure 2B, see also Figure 3B). In the case of hTAF$_{II}$70, we expressed a recombinant HA epitope tagged version which migrates slightly more slowly in SDS–PAGE than the endogenous TAF (Figure 2B) due to the presence of 16 additional amino acids (representing the tag) at the N-terminal end.

The first question we asked was whether the recombinant proteins can participate in the formation of a native TFIID complex. Since we had generated an HA epitope tagged version of hTAF$_{II}$70, we could easily distinguish exogenous recombinant protein from the endogenous species. We therefore expressed hTAF$_{II}$70 in HeLa cells by transient transfection under the control of the CMV promoter (Gorman *et al.*, 1989) and tested whether TFIID purified from these transfected cells contain the tagged hTAF$_{II}$70. Figure 2C indicates that the TFIID complex purified from the nuclei of transfected HeLa cells after anti-TBP affinity chromatography indeed contains recombinant hTAF$_{II}$70, as detected by a monoclonal antibody specific to the HA epitope. In contrast, TFIID purified from untransfected control HeLa cells failed to react with the anti-HA antibody. A similar result was obtained when we used a monoclonal antibody directed against hTAF$_{II}$100 (E.Wang, unpublished) to immunoprecipitate endogenous TFIID from transfected cells (N.Tanese, data not shown). These results establish that recombinant hTAF$_{II}$70 can assemble efficiently into the TFIID complex in the presence of the other subunits.

Next, we asked which components of TFIID interact directly with dTAF$_{II}$60 or hTAF$_{II}$70 *in vitro*. First, we tested the ability of the *Drosophila* protein to interact with dTBP. Coimmunoprecipitation of recombinant dTAF$_{II}$60 produced in baculovirus-infected cells with dTBP using anti-TBP antibodies revealed a very weak but reproducible interaction between these two subunits (Figure 3A). A

A

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                 PEP 1
dTAF 60      MLYGSSISAESMKVIAESIGVGSLSDDAAKELAEDVSIKLKRIVQDAAKFMNHAKRQKLSVRDIDMSLKVRNV
                *   ^ ^^^****** *** *^^ ^ ^^^    *^^^*** ^^* * *** ***    *****^  *** ^****^**
hTAF 70   MAEEKKLKLSNTVLPSESMKVVAESMGIAQIQEETCQLLTDEVSYRIKEIAQDALKFMHMGKRQKLTTSDIDYALKLKNV

PEP 2                  PEP 3
dTAF 60   EPQYGFVAKDFIPLRFASGGGRELHFTEDKEIDLGEITSTNSVKIPLDLTLRSHWEVVEGVQPTVPENPPPLSKDSQLLD
          ** *** * ^****^*********** * *^***^***^*   *   ^^****^ **^** ^*** ** ^****** **^ *  ^
hTAF 70   EPLYGFHAQEFIPFRFASGGGRELYFYEEKEVDLSDIINTPLPRVPLDVCLKAHWLSIEGCQPAIPENPPPAPKEQQKAE
             PEP8

dTAF 60   SVNPVIKMDQGLNKDAAGKPTTGKIHKLKNVETIHVKQLAT------------HELSVEQQLYYKEITEACVGSDEPRRG
          ^  ^^      * ^ * ^ *                ^                 ********************** *^^*^
hTAF 70   ATEPLKSAKPGQEEDGPLKGKGQGATTADGKGKEKKAPPLLEGAPLRLKPRSIHELSVEQQLYYKEITEACVGSCEAKRA

dTAF 60   EALQSLGSDPGLHEMLPRMCTFIAEGVKVNVVQNNLALLIYLMRMVRALLDNPSLFLEKYLHELIPSVMTCIVSKQLCMR
          *****^^^*** ^*****^ ***^***^*********************^***^***^*^****^******^*********^**^*
hTAF 70   EALQSIATDPGLYQMLPRFSTFISEGVRVNVVQNNLALLIYLMRMVKALMDNPTLYLEKYVHELIPAVMTCIVSRQLCLR

                                                PEP 4
dTAF 60   PELDNHWALRDFASRLMAQICKNFNTLTNNLQTRVTRIFSKALQNDKTHLSSLYGSIAGLSELGGEVIKVFIIPRLKFIS
          *^^************^***^****** * * ***^**^**^ *^*^  ^^** ^^ ********^*** ^*** ^*^****   ^
hTAF 70   PDVDNHWALRDFAARLVAQICKHFSTTTNNIQSRITKTFTKSWVDEKTPWTTRYGSIAGLAELGHDVIKTLILPRLQQEG

                                    PEP 5       PEP 6                     PEP7
dTAF 60   ERIEPHLLGTSISNTDKTAAGHIRAMLQKCCPPILRQMRSAPDTAEDYKNDFGFLGPSLCQAVVKVRNAPASSIVTLSSN
          ***    * *  ^** *^ ^* *^ ^^* * *^*^*  ^*^^** ^ *^ ^** *** **  *** *  ^*
hTAF 70   ERIRSVLDGPVLSNIDRIGADHVQSLLLKHCAPVLAKLRPPPDNQDAYRAEFGSLGPLLCSQVVKARAQAALQAQQVNRT

dTAF 60   TINTAPITSAAQTATTIGRVSMPTTQRQGSPGVSSLPQIRAIQANQPAQKFVIVTQNSPQQGQAKVVRRGSSPHSVVLSA
          *^     ^    ^       ^         ^ ^^^    *          ^^                 ^ ^              *   **
hTAF 70   TLTITQPRPTLTLSQAPQPGPRTPGLLKVPGSIALPVQTLVSARAAAPPQPSPPPTKFIVMSSSSSAPSTQQVLSLSTSA
                                 PEP9

dTAF 60   ASNAASASNSNSSSSGSLLAAAQRSSENVCVIAGSEAPAVDGITVQSFRAS
          ^^ ^^^^^ ^ ^^^  *^ ^     *      ^^^ ^^^  ^^      ^
hTAF 70   PGSGSTTTSPVTTTVPSVQPIVKLVSTATTAPPSTAPSGPGSVQKYIVVSLPPTGEGKGGPTSHPSPVPPPASSPSPLSG

hTAF 70   SALCGGKQEAGDSPPPAPGTPKANGSQPNSGSPQPAP
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B hTAF$_{II}$70 — Predicted MW

Variant	Upstream stop	Initiation	Coding length	Stop	Predicted MW
γ	TGA	ATG	667 aa; Δ(VNRTTLTITQ)	TGA	71.5 kD
α	TAA	ATG	677 aa	TGA	72.7 kD
β	TGA	ATG	726 aa	TGA	77.9 kD

Fig. 1. Predicted amino acid sequences of *Drosophila* dTAF$_{II}$60 and human hTAF$_{II}$70. (**A**) Alignment of *Drosophila* TAF$_{II}$60 and human TAF$_{II}$70 primary sequences reveals a high degree of conservation in the N-terminal portions of the two proteins. Identical or homologous amino acid residues are highlighted with asterisks (*) and carets (^), respectively. The following similarity rules were used: G = A = S, P = A = S, S = T = A, D = E = Q = N, K = R = H, V = I = L = M = F, F = Y = W. The two sequence portions containing extensive stretches of similarity are also highlighted by boxes. Peptide sequences derived from protein microsequencing of tryptic digestion products of purified endogenous dTAF$_{II}$60 (PEP1–7) and hTAF$_{II}$70 (PEP8, 9) are indicated. (**B**) Alternatively spliced transcripts encode multiple versions of hTAF$_{II}$70. 17 cDNA clones were characterized and the splicing pattern of the three cDNA clones is shown. Exons encoding identical sequences are identified by identical shading patterns. hTAF$_{II}$70α includes an additional stretch of 10 amino acids in the C-terminal half of the protein which is absent in hTAF$_{II}$70γ. hTAF$_{II}$70β might utilize a different initiation codon thus altering the N-terminus. The initiation codons are preceded by in-frame stop codons. None of these splicing variants show any variability within the evolutionarily conserved N-terminal two-thirds of hTAF$_{II}$70 (see A).

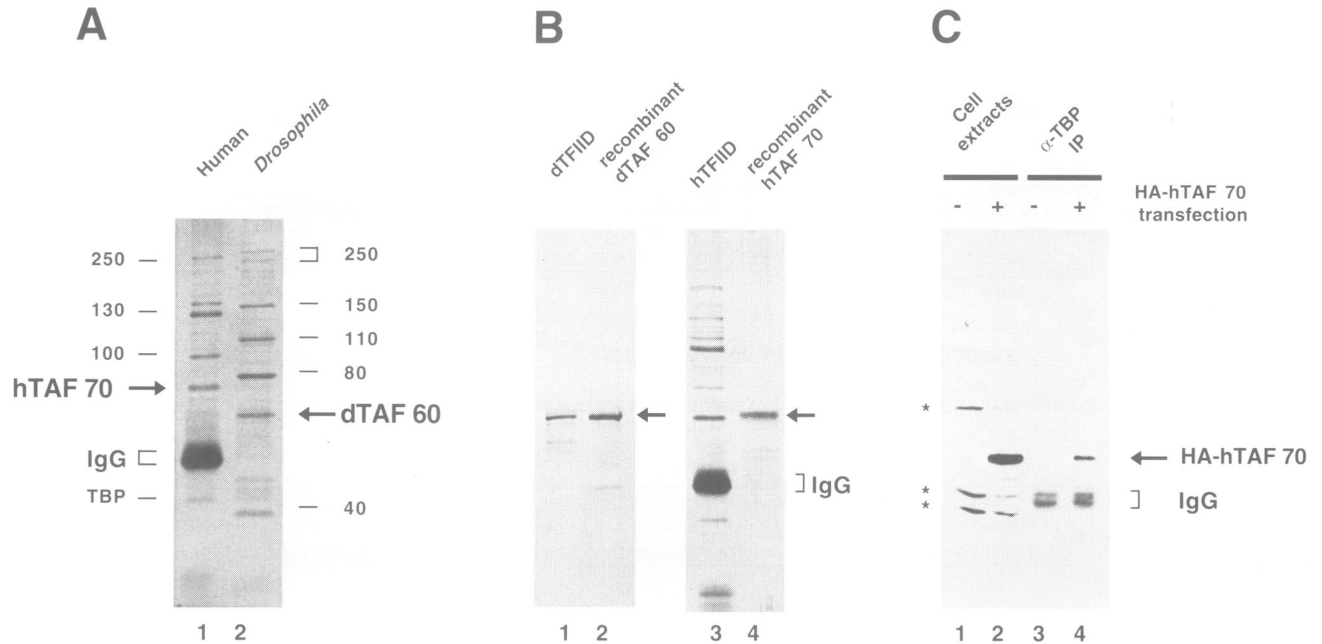

Fig. 2. $dTAF_{II}60$ and $hTAF_{II}70$ are components of the TFIID complex. (**A**) TFIID complexes from *Drosophila* and HeLa nuclear extracts. Polyclonal antisera directed against either *Drosophila* (Dynlacht *et al.*, 1991) or human TBP (Tanese *et al.*, 1991) were used to immunopurify TFIID. The immunopurified material was subsequently analyzed by SDS–PAGE and silver staining. $dTAF_{II}60$ and $hTAF_{II}70$ are indicated by arrows. (**B**) Recombinant $dTAF_{II}60$ and $hTAF_{II}70$ comigrate with the endogenous proteins. Whole cell extracts prepared from baculovirus-infected Sf9 cells overexpressing $dTAF_{II}60$ (lane 2) or the HA-tagged version of $hTAF_{II}70$ (lane 4) were run next to immunopurified *Drosophila* (lane 1) or human TAFs (lane 3) and visualized by Western blotting (lane 2) or silver staining (lane 4). (**C**) A recombinant, tagged version of $hTAF_{II}70$ becomes stably incorporated into the TFIID complex *in vivo*. $hTAF_{II}70$ was tagged with the HA epitope and expressed in HeLa cells under CMV promoter control (Gorman *et al.*, 1989). TFIID was immunopurified with a polyclonal antiserum directed against hTBP (Tanese *et al.*, 1991) from nuclear extracts derived from transfected and non-transfected cells. After SDS–PAGE and Western blotting the extracts were probed with an anti-HA monoclonal antibody, 12CA5. The presence of tagged $hTAF_{II}70$ in the immunoprecipitate from transfected cells (lane 4) demonstrates the presence of the recombinant protein in the assembled TFIID complex. In the crude nuclear extracts (lanes 1 and 2) some proteins cross-reacting non-specifically with the monoclonal antibody are indicated by asterisks.

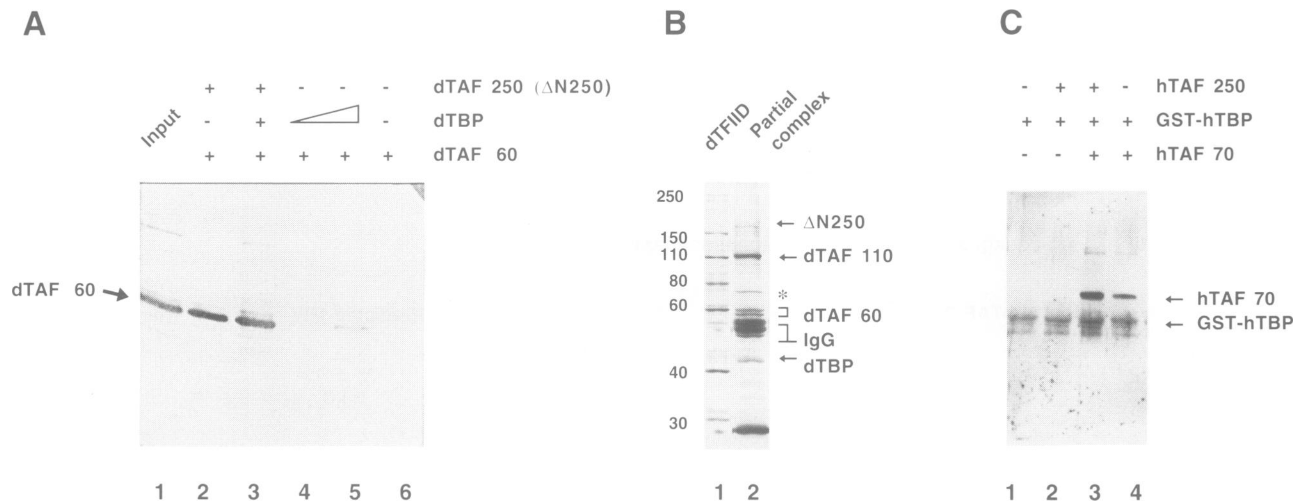

Fig. 3. TAF–TBP and TAF–TAF interactions revealed by *in vitro* assembly of $dTAF_{II}60$ and $hTAF_{II}70$. (**A**) $dTAF_{II}60$ forms a ternary complex with $TAF_{II}250$ and dTBP. Recombinant $dTAF_{II}60$, dTBP and the C-terminal portion of $dTAF_{II}250$, ΔN250 (Weinzierl *et al.*, 1993), were incubated in various combinations and immunoprecipitated with either an anti-$TAF_{II}250$ mAb, 30H9 (lanes 2, 3 and 6) (Weinzierl *et al.*, 1993) or an anti-dTBP mAb, 25B4 (R.O.J.Weinzierl, unpublished; lanes 4 and 5) as shown in the figure. After elution of the immunoprecipitated proteins with 1 M guanidine–HCl-containing buffer the eluate was analyzed by Western blotting and probed with an anti-$dTAF_{II}60$ mAb, 3B3. The position of $dTAF_{II}60$ is indicated by an arrow. The preparation and partial fractionation of extracts containing recombinant ΔN250, dTBP and $dTAF_{II}60$ was carried out as previously described (Dynlacht *et al.*, 1993). (**B**) Assembly of $dTAF_{II}60$ into a partial TFIID complex *in vitro*. ΔN250 immobilized on protein G beads with an anti-$dTAF_{II}250$ mAb, 30H9, was incubated in the presence of $dTAF_{II}110$, dTBP and $dTAF_{II}60$-containing fractionated baculovirus extracts. Unbound proteins were washed away as previously described (Dynlacht *et al.*, 1993). The assembled partial TFIID complex was then analyzed by SDS–PAGE and silver staining. The positions of ΔN250, $dTAF_{II}110$, dTBP and $dTAF_{II}60$ are shown. The band marked with an asterisk represents a proteolytic breakdown product of $dTAF_{II}110$. (**C**) $hTAF_{II}70$ shows TAF–TAF interactions similar to those of $dTAF_{II}60$. hTBP was immobilized on glutathione beads as a GST–hTBP fusion protein and subsequently incubated in the presence of baculovirus extracts containing recombinant $hTAF_{II}70$ or $hTAF_{II}250$ in the combinations shown. Bound material was visualized by Western blotting with an anti-$dTAF_{II}60$ mAb, 3B3, which recognizes an epitope conserved between human $hTAF_{II}70$ and *Drosophila* $dTAF_{II}60$ (N.Tanese, unpublished observation). The positions of the GST–hTBP fusion protein (which cross-reacts weakly with the antibody) and $hTAF_{II}70$ are marked with arrows.

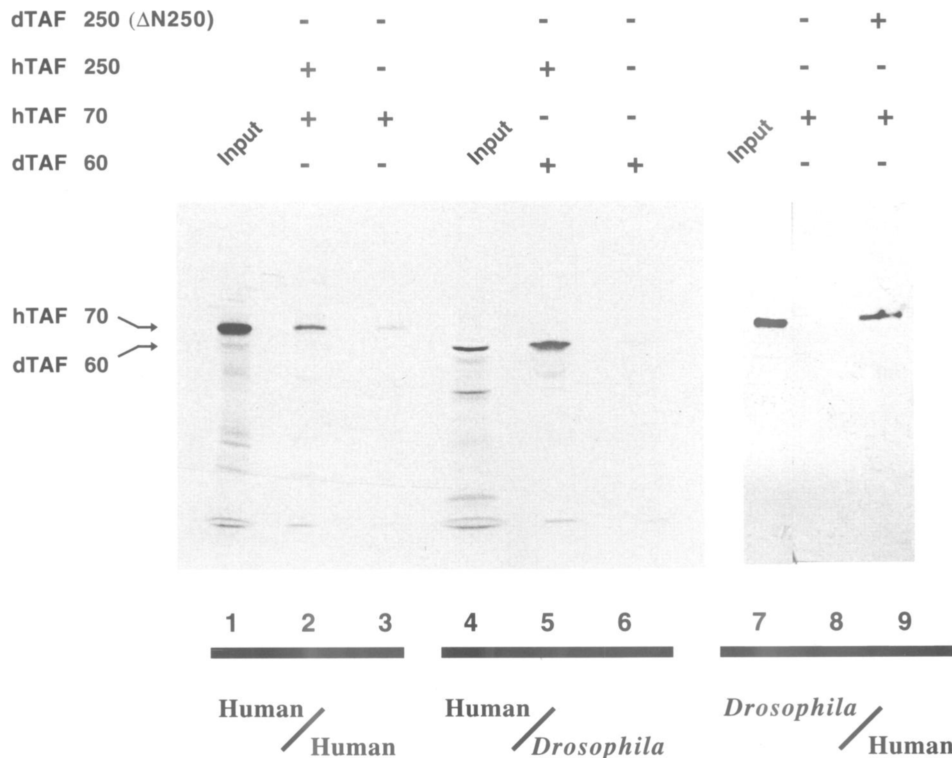

Fig. 4. Cross-species interactions between $TAF_{II}60/70$ and the largest subunit of TFIID. ^{35}S-radiolabelled $dTAF_{II}60$ or $hTAF_{II}70$ proteins generated by *in vitro* transcription/translation were incubated with either HA-tagged $hTAF_{II}250$ or *Drosophila* ΔN250 immobilized on beads with an anti-HA mAb [12CA5 (BAbCo)] or an anti-$dTAF_{II}250$ mAb, 30H9 (Weinzierl *et al.*, 1993), respectively. Binding of $dTAF_{II}60$ to $hTAF_{II}250$ and vice versa illustrates that the interaction between these TAFs is evolutionarily conserved.

similarly weak interaction was also observed between hTBP and $hTAF_{II}70$ (Figure 3C). Next we tested the ability of $dTAF_{II}60$ to interact with the largest subunit of the TFIID complex, $TAF_{II}250$. For the *Drosophila* experiments we have used ΔN250, a truncated version of $dTAF_{II}250$ (Weinzierl *et al.*, 1993), because this protein is expressed at a high level in baculovirus and appears to retain many, if not all of the functions thus far attributed to the full-length protein. In the presence of ΔN250 the recombinant $dTAF_{II}60$ is efficiently coimmunoprecipitated with anti-$dTAF_{II}250$ antibodies (Figure 3A). A triple complex containing dTBP, ΔN250 and $dTAF_{II}60$ also assembles efficiently. A similar set of experiments carried out with $hTAF_{II}70$ confirms that this protein binds efficiently to full-length $hTAF_{II}250$ in the absence (Figure 4) or presence of hTBP (Figure 3C). These results indicate that $dTAF_{II}60$ and $hTAF_{II}70$ interact most avidly with $TAF_{II}250$, although weaker interactions with TBP may contribute to the formation of a stable complex. We have previously demonstrated that $dTAF_{II}110$ also displays a high affinity for binding to ΔN250 (Weinzierl *et al.*, 1993). To show that $dTAF_{II}60$ and $dTAF_{II}110$ occupy distinct binding sites on ΔN250 we assembled a 'mini'-complex consisting of dTBP, ΔN250, $dTAF_{II}110$ and $dTAF_{II}60$. After immunopurification using anti-$dTAF_{II}250$ antibodies we observe a complex containing TBP and the three TAFs indicating that the observed TAF–TAF interactions are indeed mutually compatible (Figure 3B).

Evolutionary conservation of TAF–TAF interactions

Since we have both human and *Drosophila* proteins available, we also tested the cross-species binding properties of these two proteins to determine if TAF–TAF interfaces are conserved. Indeed, $dTAF_{II}60$ binds as efficiently to $hTAF_{II}250$ as $hTAF_{II}70$, and vice versa (Figure 4). This finding suggests that the conserved N-terminal two-thirds of $TAF_{II}60/70$ may be responsible for these conserved subunit contacts. We have previously established that *Drosophila* and human $TAF_{II}250$ can interact with either *Drosophila* or human TBP efficiently (B.D.Dynlacht and S.Ruppert, unpublished results). Thus, it seems evident that at least these three subunits of TFIID are interchangeable for complex formation between *Drosophila* and man.

$dTAF_{II}40$ is recruited into TFIID by $dTAF_{II}60$ and $hTAF_{II}70$

Finally, we tested the ability of $dTAF_{II}60$ and $hTAF_{II}70$ to interact with other members of the TFIID complex and found that at least one additional subunit interacts directly with this protein. Figure 5 documents the binding of recombinant $dTAF_{II}40$ (T.Hoey, unpublished; Goodrich *et al.*, 1994) to both $dTAF_{II}60$ and $hTAF_{II}70$. This interaction appears quite strong as complexes between $dTAF_{II}40$ and $dTAF_{II}60$ or $hTAF_{II}70$ can be detected either by coimmunoprecipitation, affinity chromatography or 'far Western' blotting (Kaelin *et al.*, 1992) of renatured protein (Figure 5 and data not shown). These results suggest that $dTAF_{II}60$ and its human counterpart, $hTAF_{II}70$, serve an important function in bridging contacts between subunits such as $dTAF_{II}40$ and $dTAF_{II}250$. Our findings also strengthen the proposal that the largest subunit of TFIID, $TAF_{II}250$, serves as the core subunit responsible for coordinating the interactions of the other TAFs, such as $TAF_{II}110$, $TAF_{II}60$ and TBP (Weinzierl *et al.*, 1993). These subunits, in turn, may provide the binding surfaces for other members of the complex such as $TAF_{II}40$ and $TAF_{II}80$ (Dynlacht *et al.*,

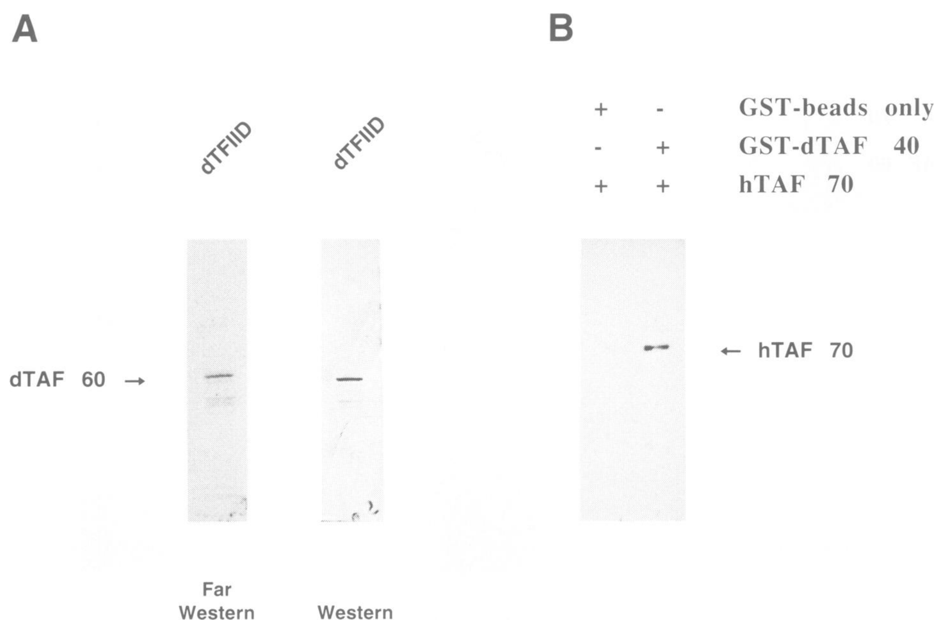

Fig. 5. Interaction of $dTAF_{II}60/hTAF_{II}70$ with another component of TFIID, $dTAF_{II}40$. (**A**) A 'far-Western' blot containing SDS–PAGE fractionated *Drosophila* TFIID complex components was probed after stepwise renaturation with ^{35}S-radiolabelled $dTAF_{II}40$ (T.Hoey, unpublished; J.Goodrich *et al.*, 1994). A single band, coinciding with the position of $dTAF_{II}60$ on a Western blot (arrow), is specifically detected after autoradiography, indicating a direct physical interaction between $dTAF_{II}40$ and $dTAF_{II}60$. (**B**) Glutathione beads (Pharmacia) were incubated with *Escherichia coli* extracts containing either glutathione-S-transferase (GST) alone or a GST–$dTAF_{II}40$ fusion protein. After incubating beads carrying equivalent amounts of either GST or GST–$dTAF_{II}40$ with a baculovirus extract containing recombinant $hTAF_{II}70$ the bound proteins were detected by SDS–PAGE and Western blotting with mAb 3B3 (anti-$TAF_{II}60$ or 70). $hTAF_{II}70$ is specifically retained on the beads carrying GST–$dTAF_{II}40$.

1993). Thus, with each new TAF that we are able to clone, express and characterize, a more complete picture of how the TFIID complex is assembled emerges. It will now be of great interest to search for potential interactions between TAFs such as $TAF_{II}60$, $TAF_{II}80$ and $TAF_{II}40$ and other components of the transcriptional apparatus, including basal factors and site-specific activators bound to DNA.

Materials and methods

Generation of polyclonal anti-TFIID antisera and λgt11 library screen

Mouse polyclonal antibodies directed against immunopurified *Drosophila* TFIID were prepared as described by Hoey *et al.* (1993). The serum was used to screen a size-selected (> 1.8 kb) λgt11 expression library prepared from *Drosophila* embryos (Zinn *et al.*, 1988). Positive clones were rescreened with monoclonal antibodies specific for $dTAF_{II}60$ (R.O.J. Weinzierl, unpublished) to select a cDNA encoding $dTAF_{II}60$ (λD6).

Protein microsequencing

Peptide sequence data were obtained from tryptic digests of immunopurified TAFs resolved by HPLC ($dTAF_{II}60$) or eluted from PVDF membranes ($hTAF_{II}70$).

Isolation of cDNA clones encoding $hTAF_{II}70$

A HeLa λZAPII cDNA library (Ruppert *et al.*, 1993) was screened under reduced stringency to identify clones cross-hybridizing to the cDNA encoding $dTAF_{II}60$ (λD6).

Baculovirus expression

cDNAs encoding full-length proteins were inserted into a baculovirus expression vector [pVL1393, Pharmingen, or derivatives thereof encoding a 16 amino acid HA epitope (S.Ruppert, unpublished)] and co-transfected into Sf9 cells with linearized baculovirus DNA (Pharmingen). Extracts derived from cells infected with the recombinant viruses were run on SDS–polyacrylamide gels and blotted. Recombinant proteins were detected with monoclonal antibody 3B3 ($dTAF_{II}60$) or anti-HA monoclonal antibody 12CA5 (obtained from BAbCo, Berkeley).

Preparation of cell extracts and proteins

Cell extracts from recombinant baculovirus-infected Sf9 cells were prepared as previously described (Dynlacht *et al.*, 1993; Weinzierl *et al.*, 1993). Briefly, infected Sf9 cells were resuspended in 0.1 HEMG-NDP (100 mM KCl, 25 mM HEPES, pH 7.6, 0.1 mM EDTA, 12.5 mM $MgCl_2$, 10% glycerol, 0.1% NP40, 1.5 mM DTT, 0.1 mM PMSF and 5 μg leupeptin/ml) and sonicated extensively. The soluble protein fraction present in the supernatant was used for the *in vitro* assembly reactions.

Preparation of ^{35}S-labelled proteins

^{35}S-labelled proteins were generated using the coupled transcription/translation reticulocyte system (Promega) using cDNAs subcloned into pBSK.

In vitro assembly/coimmunoprecipitation reactions

In vitro assembly reactions were carried out as previously described (Dynlacht *et al.*, 1993; Weinzierl *et al.*, 1993) using either baculovirus extracts or ^{35}S-labelled proteins as described in the corresponding figure legends.

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