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The TFIID Components Human $TAF_{II}140$ and *Drosophila* BIP2 ($TAF_{II}155$) Are Novel Metazoan Homologues of Yeast $TAF_{II}47$ Containing a Histone Fold and a PHD Finger

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The RNA polymerase II transcription factor TFIID comprises the TATA binding protein (TBP) and a set of TBP-associated factors (TAF_{II}s). TFIID has been extensively characterized for yeast, *Drosophila*, and humans, demonstrating a high degree of conservation of both the amino acid sequences of the constituent TAF_{II}s and overall molecular organization. In recent years, it has been assumed that all the metazoan TAF_{II}s have been identified, yet no metazoan homologues of yeast $TAF_{II}47$ ($yTAF_{II}47$) and $yTAF_{II}65$ are known. Both of these $yTAF_{II}$s contain a histone fold domain (HFD) which selectively heterodimerizes with that of $yTAF_{II}25$. We have cloned a novel mouse protein, $TAF_{II}140$, containing an HFD and a plant homeodomain (PHD) finger, which we demonstrated by immunoprecipitation to be a mammalian TFIID component. $TAF_{II}140$ shows extensive sequence similarity to *Drosophila* BIP2 (dBIP2) ($dTAF_{II}155$), which we also show to be a component of *Drosophila* TFIID. These proteins are metazoan homologues of $yTAF_{II}47$ as their HFDs selectively heterodimerize with $dTAF_{II}24$ and human $TAF_{II}30$, metazoan homologues of $yTAF_{II}25$. We further show that $yTAF_{II}65$ shares two domains with the *Drosophila* Prodos protein, a recently described potential $dTAF_{II}$. These conserved domains are critical for $yTAF_{II}65$ function in vivo. Our results therefore identify metazoan homologues of $yTAF_{II}47$ and $yTAF_{II}65$.

Transcription factor TFIID is one of the general factors required for accurate and regulated initiation by RNA polymerase II. TFIID comprises the TATA binding protein (TBP) and TBP-associated factors (TAF_{II}s) (3, 24). A subset of TAF_{II}s are present not only in TFIID but also in the SAGA, PCAF, STAGA, and TFTC complexes (5, 16, 22, 33, 38, 53).

The function of TAF_{II}s has been studied using in vitro transcription systems which have provided evidence that they act as specific coactivators by interacting directly with transcriptional activator proteins (48). Such studies also indicated that TAF_{II}s contribute to promoter recognition and promoter selectivity (8, 10, 49). Genetic evidence obtained from TAF_{II} knockout and depletion experiments and temperature-sensitive (TS) mutants indicates that TAF_{II}s are involved in the control of gene expression in yeast and mammalian cells and that they act as antiapoptotic factors (11, 23, 37; for reviews, see references 2, 3, and 23).

TFIID has been characterized for *Drosophila*, humans, and yeast (7, 14, 40, 56). After its initial purification, many subunits were rapidly identified and the corresponding genes were cloned. Sequence comparisons allowed the identification of TAF_{II}s in each species sharing one or more conserved domains. Many of these domains correspond to structural motifs, such as the histone fold domain (HFD), the β-transducin repeat, and bromodomain, or catalytic domains, such as the histone acetyltransferase domain. These results indicate that the composition and organization of TFIID has been well conserved between yeast and humans (reviewed in reference 16).

In recent years, it has been assumed that all the mammalian TFIID components have been identified. Several observations, however, challenge this assumption. Characterization of highly purified yeast TFIID (yTFIID) has identified two novel TFIID subunits, $yTAF_{II}48$ (Mpt1) and $yTAF_{II}65$ (42, 45). While $yTAF_{II}48$ is the homologue of human $TAF_{II}135$ ($hTAF_{II}135$) in yeast (18, 42), no obvious metazoan homologues of $yTAF_{II}65$ have yet been identified. $TAF_{II}65$ contains an HFD which selectively heterodimerizes with $yTAF_{II}25$ (17). Furthermore, a second $yTAF_{II}$, $yTAF_{II}47$ (52), contains an HFD and selectively heterodimerizes also with $yTAF_{II}25$ (17). At present, no homologues of $yTAF_{II}47$ and $yTAF_{II}65$ in metazoans have been identified, yet a $yTAF_{II}25$ homologue has been well characterized for humans ($hTAF_{II}30$ [27]), and for *Drosophila*, two homologues have been identified (*Drosophila* $TAF_{II}24$ [$dTAF_{II}24$] and $dTAF_{II}16$ [19]). Metazoan TFIID must therefore contain a heterodimerization partner(s) for $hTAF_{II}30$, and $dTAF_{II}16$, and $dTAF_{II}24$, which would be considered homologues of $yTAF_{II}47$ and/or $yTAF_{II}65$.

In database searches with the $yTAF_{II}47$ HFD, we previously identified metazoan proteins with HFDs similar to that of $yTAF_{II}47$ (17). Here, we describe mouse $TAF_{II}140$ ($mTAF_{II}140$) and $hTAF_{II}140$, novel proteins containing an

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HFD with strong similarity to that of yTAF$_{II}$47 and a plant homeodomain (PHD) finger. We show that the mouse and human TAF$_{II}$140 HFDs selectively and directly heterodimerize with that of hTAF$_{II}$30. Immunoprecipitation experiments confirm that hTAF$_{II}$140 is a bone fide TAF$_{II}$ which can be immunopurified from HeLa cell extracts under stringent conditions with TBP and other identified hTAF$_{II}$s. Mammalian TAF$_{II}$140 shows high sequence similarity to the *Drosophila* protein BIP2 (bric-a-brac interacting protein 2), which also contains an HFD and PHD finger. We show that the dBIP2 HFD selectively and directly heterodimerizes with dTAF$_{II}$24 and that BIP2 is a component of dTFIID.

The *Drosophila* Prodos (PDS) is a protein essential for cell viability which has been shown to comprise an HFD which selectively heterodimerizes with dTAF$_{II}$16. Consequently, it has been proposed that PDS may be a dTFIID component (25). Here, we show that PDS shares sequence similarity with yTAF$_{II}$65 both in the HFD and in a second domain (designated the TAF$_{II}$65-PDS [TAP] domain [TAPD]) C terminal of the HFD. We show that the yTAF$_{II}$65 HFD and the TAPD are partially redundant functional domains required for yeast viability. The fact that PDS shares the HF and TAP domains which are required for yTAF$_{II}$65 function in vivo, along with its observed heterodimerization with dTAF$_{II}$16, indicates that PDS is the *Drosophila* homologue of yTAF$_{II}$65.

MATERIALS AND METHODS

Cloning of mTAF$_{II}$140 and hTAF$_{II}$140. The cDNA encoding mTAF$_{II}$140 HFD was isolated by reverse transcription (RT)-PCR using mouse F9 embryonal carcinoma cell RNA and oligonucleotide primers based on expressed sequence tag (EST) sequences. To isolate the full-length cDNA, an F9 embryonal carcinoma cDNA library was screened with the ^{32}P-labeled HFD fragment. A clone comprising the 5′ untranslated region (UTR) and amino acids 1 to 340 was isolated. A second overlapping clone encoding amino acids 238 to 747 was isolated by screening the same library with oligonucleotide probes derived from EST sequences. The 3′ end of the cDNA was cloned by RT-PCR using an oligonucleotide primer at amino acid 651 and primers in the 3′ UTR derived from EST sequences. The partial cDNA sequences were cloned in pBSK and sequenced. The three cDNAs were assembled by overlapping PCR into two partially overlapping fragments. In one fragment, a *Bam*HI site was introduced at nucleotide 1286 (GGCTCA-GGATCC) without changing the amino acid sequence. This fragment was cloned into the *Eco*RI-*Bam*HI sites of expression vector pXJ41 (54). A fragment from nucleotide 1286 to the end of the coding sequence was amplified with primers containing *Bam*HI and *Xho*I sites, and the resulting fragment was cloned into the pXJ41 vector containing the 5′ fragment, thus reconstituting the full open reading frame. The hTAF$_{II}$140 cDNA fragment containing the 5′ UTR and genes for amino acids 1 to 727 was cloned by screening a HeLa cell cDNA library with an oligonucleotide corresponding to genes for amino acids 54 to 72 of the HFD based on EST sequences. All constructs were verified by automated DNA sequencing, and further details are available upon request.

Preparation of antibodies and immunoprecipitations. Antibodies against human and mouse TAF$_{II}$140 were generated against a synthetic peptide corresponding to amino acids 108 to 127 of hTAF$_{II}$140 (as indicated in Fig. 2) coupled to ovalbumin. Monoclonal antibodies were generated as previously described (32). Two resulting monoclonal antibodies, 1C7 and 2F5, were used. The anti-BIP2 polyclonal antiserum was collected from rabbits immunized with a bacterially expressed glutathione *S*-transferase (GST)-fusion protein comprising amino acid residues 719 to 909 of the BIP2 protein. The anti-SAP130 and GCN5 antibodies were as previously described (M. Brand, J. G. Moogs, F. Lejeune, J. Stevenin, M. Oulad-Abdelghani, G. Almouzni, and L. Tora, submitted for publication). The anti-TBP monoclonal antibodies 2C1 and 3G3 and the anti-TAF$_{II}$ monoclonal antibodies were as previously described (6, 13, 27, 32, 35, 36). The polyclonal antisera against dTBP, dTAF$_{II}$16, dTAF$_{II}$24, and dTAF$_{II}$230 were as previously described (19, 28). Immunoprecipitations of HeLa cell nuclear extracts with monoclonal antibody 2C1 and elutions with the epitope peptide were performed as previously described (6, 27, 35, 36). Immunoprecipitations of *Drosophila* embryo extracts were performed as previously described (19). Western blottings were performed by standard techniques, and the proteins were detected using an ECL kit (Amersham) and autoradiography.

Two-hybrid and coexpression assays. All yeast and bacterial expression vectors were constructed by PCR using primers with the appropriate restriction sites, and constructs were verified by automated DNA sequencing. Details of constructions are available on request. LexA fusions were constructed in the multicopy vector pBTM116 containing the *TRP1* marker and the VP16 fusions were constructed in the multicopy vector pASV3 containing the *LEU2* marker (18), and assays were performed with the L40 strain as previously described (17, 50). Quantitative β-galactosidase assays on individual L40 transformants were determined as previously described (18). Reproducible results were obtained in several independent experiments, and the results of a typical experiment are shown in the figures.

Yeast complementation assays. Complementation assays were performed by the plasmid shuffle technique. Wild-type or mutated yTAF$_{II}$s were cloned in the multicopy pAS3 plasmid with a *LEU* marker. All yeast strains were transformed by the LiAc technique. Yeast strain YSLS58 that was used for plasmid shuffling of *TAF65* was derived from YSLS41 (45) by sporulation and tetrad dissection as previously described (17). For complementation assays, the indicated rescue plasmids were transformed and the wild-type TAF (URA3) plasmid was shuffled out by two passages on media containing 5-fluoroorotic acid. In all experiments, cultures were grown in yeast-peptone-dextrose unless selection was necessary, in which case cultures were grown in the appropriate selective synthetic dextrose (SD) medium.

Immunostaining of *Drosophila* embryos. The 0- to 2-h and 2- to 24-h embryos were dechorionated, and antibody staining and diaminobenzidine visualization were performed as previously described (44). The anti-BIP2 polyclonal antibody was diluted 1/3,000, and the peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG) was diluted 1/1,000.

Immunostaining of *Drosophila* salivary gland polytene chromosomes. Squashes of polytene chromosomes and antibody stainings were performed as previously described (57). The anti-BIP2 primary antibody was diluted 1/200. The Cy3-conjugated secondary antibodies were diluted 1/500. DNA was stained with Hoechst 33258.

Nucleotide sequence accession numbers. The sequences reported here have been assigned the following EMBL database accession numbers: mTAF$_{II}$140, AJ292189; hTAF$_{II}$140, AJ292190; dTAF$_{II}$155, AJ292191.

RESULTS

HFD of mTAF$_{II}$140 heterodimerizes with hTAF$_{II}$30. We previously reported the existence of a putative HFD at the N terminus of yTAF$_{II}$47 which is necessary and sufficient for yeast viability (17). This HFD shows strong sequence similarity to those of the hTAF$_{II}$135/ADA1/H2A family and mediates a selective heterodimerization with the yTAF$_{II}$25 HFD (17). Database searches with the yTAF$_{II}$47 HFD identified several metazoan proteins with potential HFDs located at their N termini (reference 17 and Fig. 1A).

To determine whether the HFD, which is identical in the human and murine proteins, would heterodimerize with hTAF$_{II}$30, two-hybrid experiments were performed in yeast by using LexA fusions of the hTAF$_{II}$30 HFD and VP16 fusions of the mouse protein (hereafter designated mTAF$_{II}$140). As previously described (17), strong interactions between the HFDs of yTAF$_{II}$47 and yTAF$_{II}$25 or hTAF$_{II}$30 were observed (Fig. 1B, lanes 1 and 5). Similarly, the mTAF$_{II}$140 HFD also interacted strongly with both the yTAF$_{II}$25 and hTAF$_{II}$30 HFDs (Fig. 1B, lanes 2 and 6). These interactions were abolished by mutation of residue W23 (W23R) in the L1 loop of mTAF$_{II}$140 HFD [mTAF$_{II}$140(1-88)m1] (Fig. 1B, lanes 3 and 7).

Direct heterodimerization of the mTAF$_{II}$140 and hTAF$_{II}$30 HFDs was tested by coexpression in *E. coli* (17, 18). When expressed alone, a GST fusion of the yTAF$_{II}$47 or mTAF$_{II}$140 HFDs was poorly soluble [Fig. 1C and D, lane 1, GST-yTAF$_{II}$47(1-81) and GST-mTAF$_{II}$140(1-88)]. In contrast,

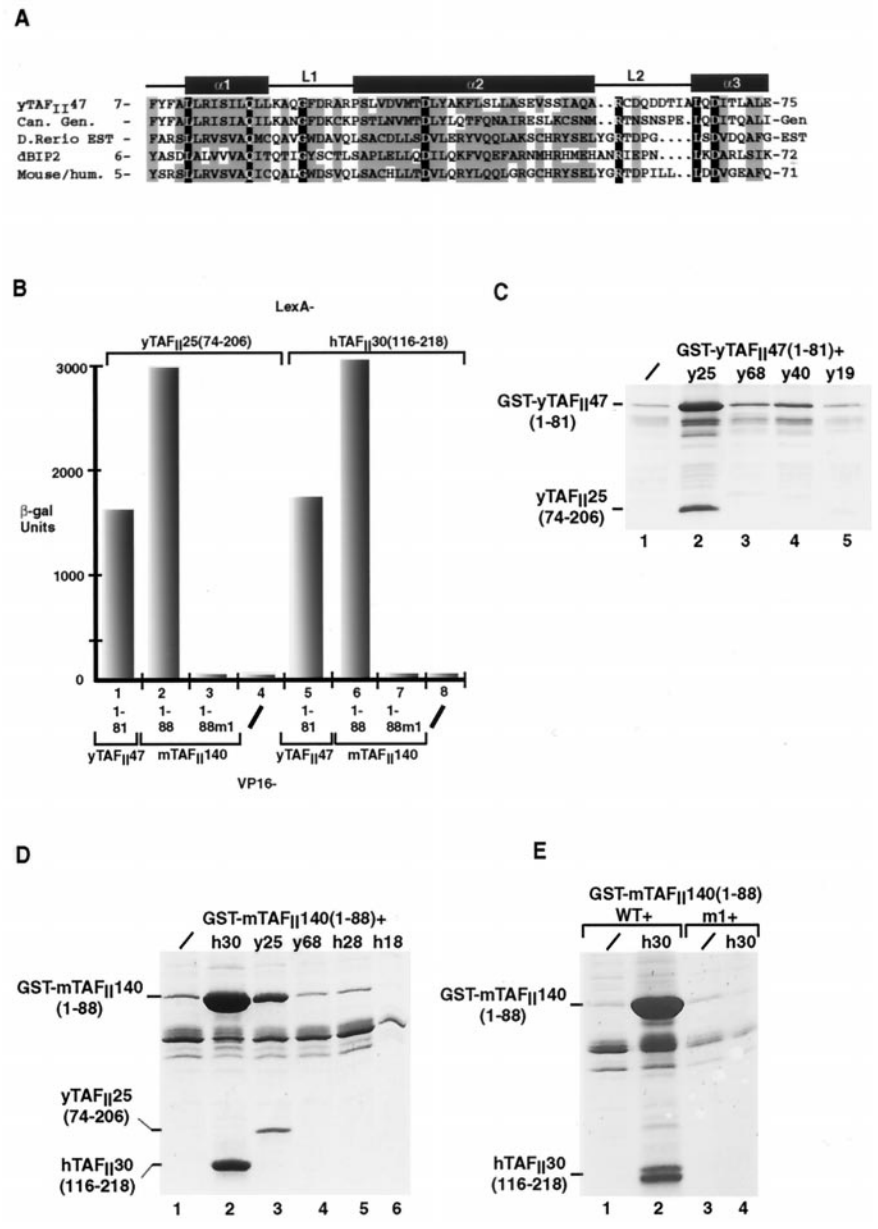

FIG. 1. Functional analysis of the HFD from a putative mouse and human yTAF$_{II}$47 homologue. (A) Alignment of the HFD sequence of yTAF$_{II}$47 with those of its putative *Candida albicans* (Can), zebrafish (*Danio rerio*), *Drosophila melanogaster* (dBIP2), and human and mouse homologues. The positions of the predicted α-helices and loops are indicated above the sequences. Identical amino acids are shown in white letters on a black background. Positions with conserved, mainly hydrophobic amino acids are boxed in gray. Amino acids were classified as follows: small residues, P, A, G, S, T; hydrophobic residues, L, I, V, A, F, M, C, Y, W; polar and acidic residues, D, E, Q, N; basic residues, R, K, H. Threonine residues are occasionally present in otherwise hydrophobic positions. The amino acid sequences shown without numbers are predicted from genomic (gen) or EST sequences. The accession numbers for the indicated sequences are as follows: *C. albicans*, 396380B03; zebrafish, GenBank accession no. AW343321fi76b06.y1; *Drosophila* BIP2, Q9XZU7; mouse, GenBank accession no. AA692266ur52c07. (B) Graphical representation of quantitative two-hybrid β-galactosidase assays. The LexA chimeras shown above the graph were tested with the VP16 chimeras shown below each column. −, negative controls with the VP16 domain alone. (C, D, and E) Coexpression of the yTAF$_{II}$47 and mTAF$_{II}$140 HFDs with those of other TAF$_{II}$s in *E. coli* cells. Bacteria were transformed to express the proteins shown above each lane, and following extract preparation, the soluble protein retained on glutathione-Sepharose beads was analyzed by SDS-PAGE and staining with Coomassie brilliant blue. /, GST-fusion was expressed alone. The locations of the GST-yTAF$_{II}$ and GST-mTAF$_{II}$ fusions and the retained yTAF$_{II}$25 and hTAF$_{II}$30 proteins are indicated.

when coexpressed with the yTAF$_{II}$25 HFD, GST-yTAF$_{II}$47 (1-88) was solubilized and formation of a heterodimeric complex was observed (Fig. 1C, lane 2, and reference 17). Similarly, GST-mTAF$_{II}$140(1-88) was also solubilized when coexpressed with the yTAF$_{II}$25 and hTAF$_{II}$30 HFDs, and the formation of a heterodimeric complex was observed in each case (Fig. 1D, lanes 2 and 3, and Fig. 1E, lane 2). Solubilization by the hTAF$_{II}$30 HFD and complex formation was abolished by the W23R mutation, which eliminated interaction in yeast (Fig. 1E, lane 4). No heterodimerization was seen, however, when

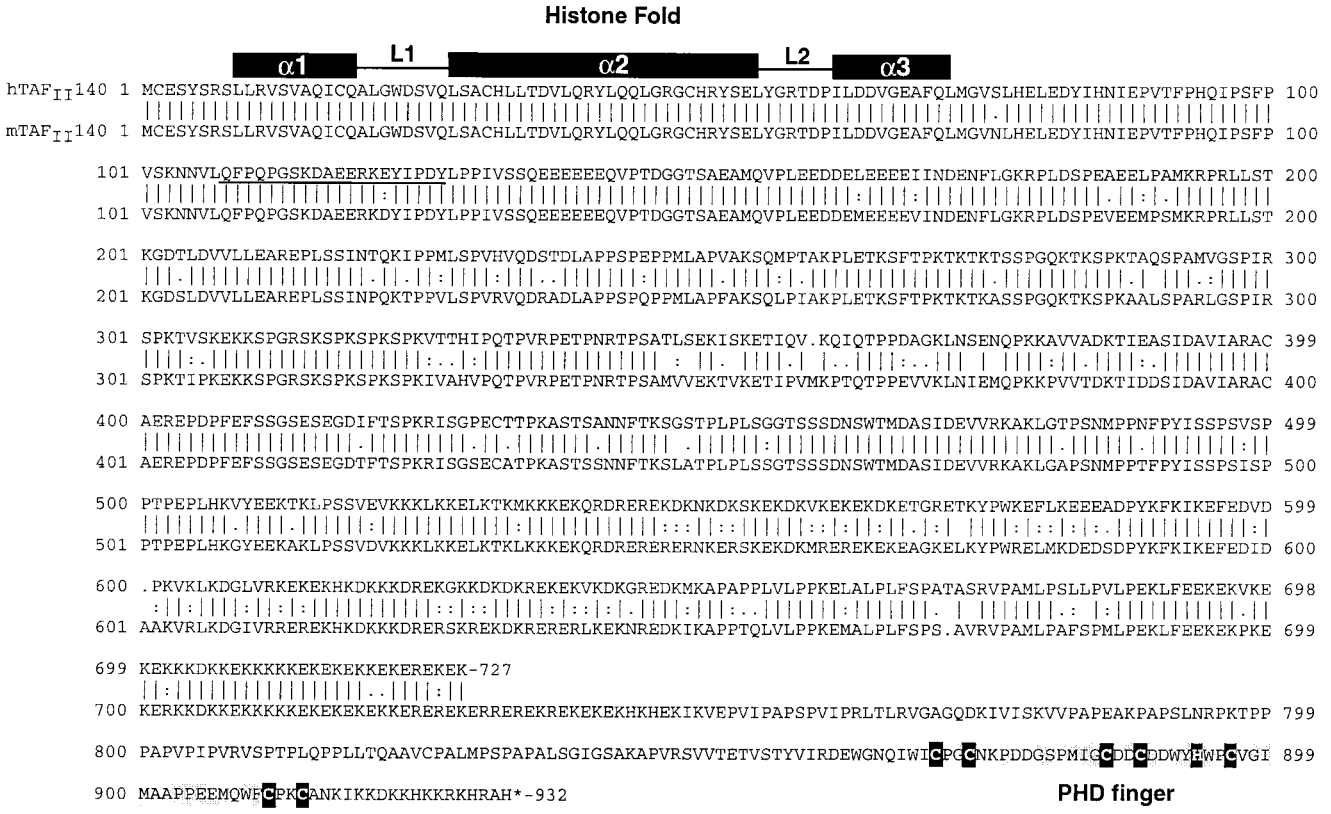

FIG. 2. The amino acid sequences of mTAF$_{II}$140 and hTAF$_{II}$140 are aligned. The positions of the HFD and PHD fingers are shown. In the PHD finger, the critical cysteine and histidine residues are shown in white letters. The amino acid sequences used to generate monoclonal antibodies are underlined. The alignment was generated using the GCG program.

the yTAF$_{II}$47 or mTAF$_{II}$140 HFDs were coexpressed with the HFDs of other TAF$_{II}$s (Fig. 1C, lanes 3 to 5, Fig. 1D, lanes 4 to 6, and data not shown).

Together, these results indicate that the HFD of mTAF$_{II}$140 selectively and directly heterodimerizes with hTAF$_{II}$30, indicating that it may be a novel component of mammalian TFIID.

TAF$_{II}$140 is a HeLa cell TFIID subunit. The full-length mTAF$_{II}$140 open reading frame was cloned by a combination of screening an F9 cell cDNA library and RT-PCR using information from overlapping clones in the EST databases (see Materials and Methods). Mouse TAF$_{II}$140 comprises 932 amino acids with the HFD located between amino acids 9 and 72 (Fig. 2). Interestingly, mTAF$_{II}$140 also possesses a single PHD finger of the type Cys4-His-Cys3 originally described for the HAT3.1, Polycomb-like, and HRX proteins (1, 47) (sometimes also called the leukemia-associated protein [LAP] domain [43]) at the C terminus of the protein. This PHD finger is preceded by a highly charged region comprising multiple lysine and glutamic acid residues and a region rich in proline and other small residues (Fig. 2).

A partial cDNA encoding the first 727 amino acids of hTAF$_{II}$140 was also isolated by screening a HeLa cell cDNA library. Comparison with mTAF$_{II}$140 showed that the HFD was identical in the two proteins which otherwise share strong sequence similarity (Fig. 2). Although we could not isolate a full-length clone for hTAF$_{II}$140, database searches identified a genomic clone and ESTs encoding a highly related PHD finger followed by a stop codon, as in mTAF$_{II}$140 (Fig. 4B). This provides strong evidence for the existence of an hTAF$_{II}$140 protein with an overall structure analogous to that of mTAF$_{II}$140.

Monoclonal antibodies were raised against a peptide of hTAF$_{II}$140 downstream of the HFD (Fig. 2, underlined), which is almost perfectly conserved between humans and mice. This monoclonal antibody recognizes a protein with a molecular mass of around 140 kDa in Cos cells transfected with an mTAF$_{II}$140 expression vector (Fig. 3A, lane 2). Although no endogenous protein was seen with small amounts of control untransfected Cos cell extract (Fig. 3A, lane 1), hTAF$_{II}$140 was clearly detected in more-concentrated HeLa cell nuclear extract (lane 3). The antibody also recognized a nuclear protein in HeLa cells in immunofluorescence experiments (data not shown).

To determine whether hTAF$_{II}$140 was a component of immunopurified TFIID, HeLa cell extracts were precipitated with the anti-TBP monoclonal antibody 2C1 (32) and the TFIID was eluted by using the 2C1 epitope peptide. HeLa cell TAF$_{II}$140 was clearly detected in the eluted TFIID (Fig. 3A, lane 4). HeLa cell TAF$_{II}$140 was also detected in immunopurified TFIID by using a second independent monoclonal antibody against the same peptide (Fig. 3B, lanes 2 and 3), while neither TBP nor hTAF$_{II}$140 was detected in control immunoprecipitations using the anti-Flag antibody (lane 1). Reprobing of the immunoblot shows that previously identified TAF$_{II}$s were present in the immunopurified TFIID as expected (Fig. 3B, lanes 4 to 6). It is possible that TAF$_{II}$140 was originally

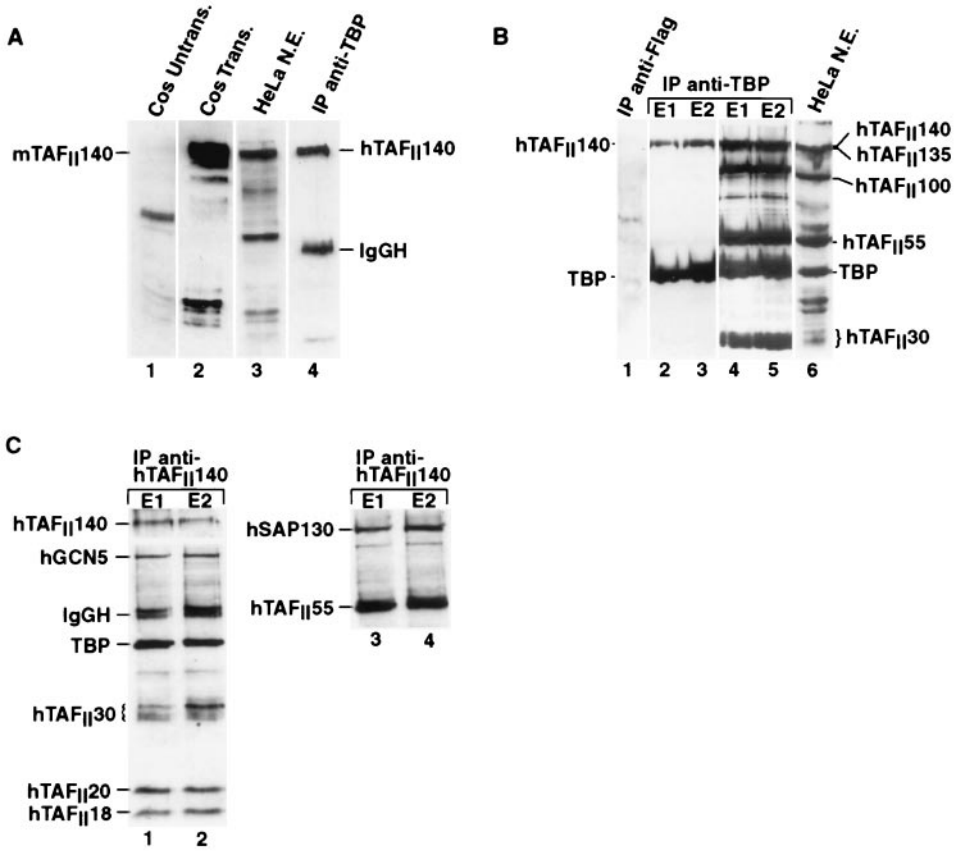

FIG. 3. Immunoprecipitations. (A) The origin of each extract is shown above each lane. Cos Untrans, 1 μg of untransfected Cos cell extract; Cos Trans, 1 μg of extract from Cos cells transfected with 5 μg of pXJ41-mTAF$_{II}$140; HeLa N.E., 15 μg of HeLa cell nuclear extract; IP anti-TBP, TFIID immunoprecipitated with monoclonal antibody 2C1 and eluted with the corresponding peptide. The positions of mTAF$_{II}$140 and hTAF$_{II}$140 are indicated along with that of residual 2C1 IgGH detected by the secondary conjugated antibody used in ECL. All lanes were probed with anti-TAF$_{II}$140 antibody 1C7. (B) The layout is the same as that in panel A, with the origin of the protein indicated above each lane. E1 and E2 indicate the first and second elutions of the 2C1 immunoprecipitation with the epitope peptide. The first lane shows a control immunoprecipitation with anti-Flag antibody m2. Lanes 1 to 3 were probed with the anti-TBP antibody 3G3 and the anti-hTAF$_{II}$140 antibody 2F5. Lanes 4 and 5 show the same blots that are in lanes 2 and 3, probed with additional antibodies; lane 6 shows HeLa cell nuclear extract incubated with all of the antibodies. (C) HeLa cell nuclear extract was precipitated with monoclonal antibody 1C7, and the precipitates eluted with the epitope peptide. The blot was probed with the antibodies used to detect the proteins indicated. In lanes 3 and 4, the blot shown in lanes 1 and 2 was reprobed with the indicated antibodies after inactivation of the first signal by prolonged incubation with ECL.

overlooked because it comigrates closely with hTAF$_{II}$135 (compare lanes 3 and 4).

The anti-hTAF$_{II}$140 antibodies were also used to immunoprecipitate HeLa cell nuclear extracts. TBP, TAF$_{II}$30, TAF$_{II}$20, TAF$_{II}$18, and TAF$_{II}$55 as well as other TFIID components (data not shown) were all coprecipitated with TAF$_{II}$140 (Fig. 3C, lanes 1 to 4). TAF$_{II}$30 is a component of the TFIID and TFTC complexes (5, 53). To determine whether TAF$_{II}$140 is also present in TFTC, we asked whether TFTC-specific components could also be immunoprecipitated with the anti-TAF$_{II}$140 antibody. Incubation of the immunoblots with antibodies against hGCN5 and SAP130 (Brand et al., submitted) indicated that both were coprecipitated (Fig. 3C). Therefore, the anti-TAF$_{II}$140 antibody precipitates both TFIID-specific subunits (TBP, hTAF$_{II}$18) and TFTC-specific subunits (SAP130, GCN5), indicating that it is present in both complexes.

BIP2 is a *Drosophila* TFIID subunit which heterodimerizes specifically with dTAF$_{II}$24. Database searches with mTAF$_{II}$140 show that it shares significant sequence similarity with the *Drosophila* protein BIP2 (Fig. 4A). BIP2 was first identified (J.-C. Pointud and J.-L. Couderc, unpublished data) in a two-hybrid screen using as bait the BTB (POZ) domain of bric-a-brac 1, a regulatory protein required for several morphogenetic processes in *Drosophila* development (21, 45). BIP2 contains an HFD in the N terminus and a PHD finger at the C terminus (Fig. 4A). The PHD fingers of mouse and human TAF$_{II}$140 and BIP2 are more closely related to each other than to PHD fingers from other proteins (Fig. 4B). The significant similarity with hTAF$_{II}$140 led us to ask whether BIP2 is a component of dTFIID.

We first determined whether the dBIP2 HFD would heterodimerize with those of dTAF$_{II}$16 or dTAF$_{II}$24. In yeast two-hybrid assays, the VP16-dBIP2 HFD chimera interacted strongly with the LexA-dTAF$_{II}$24 fusion, but only a weak interaction with dTAF$_{II}$16 was observed (Fig. 5A, lanes 1 and 2). In contrast, no significant interaction with the HFDs of yTAF$_{II}$25 or hTAF$_{II}$30 was observed (lanes 3 to 5). This indicates that the dBIP2 HFD can discriminate between the HFDs of dTAF$_{II}$16 and dTAF$_{II}$24. The selective interaction of the dBIP2 HFD with that of dTAF$_{II}$24 was also investigated by

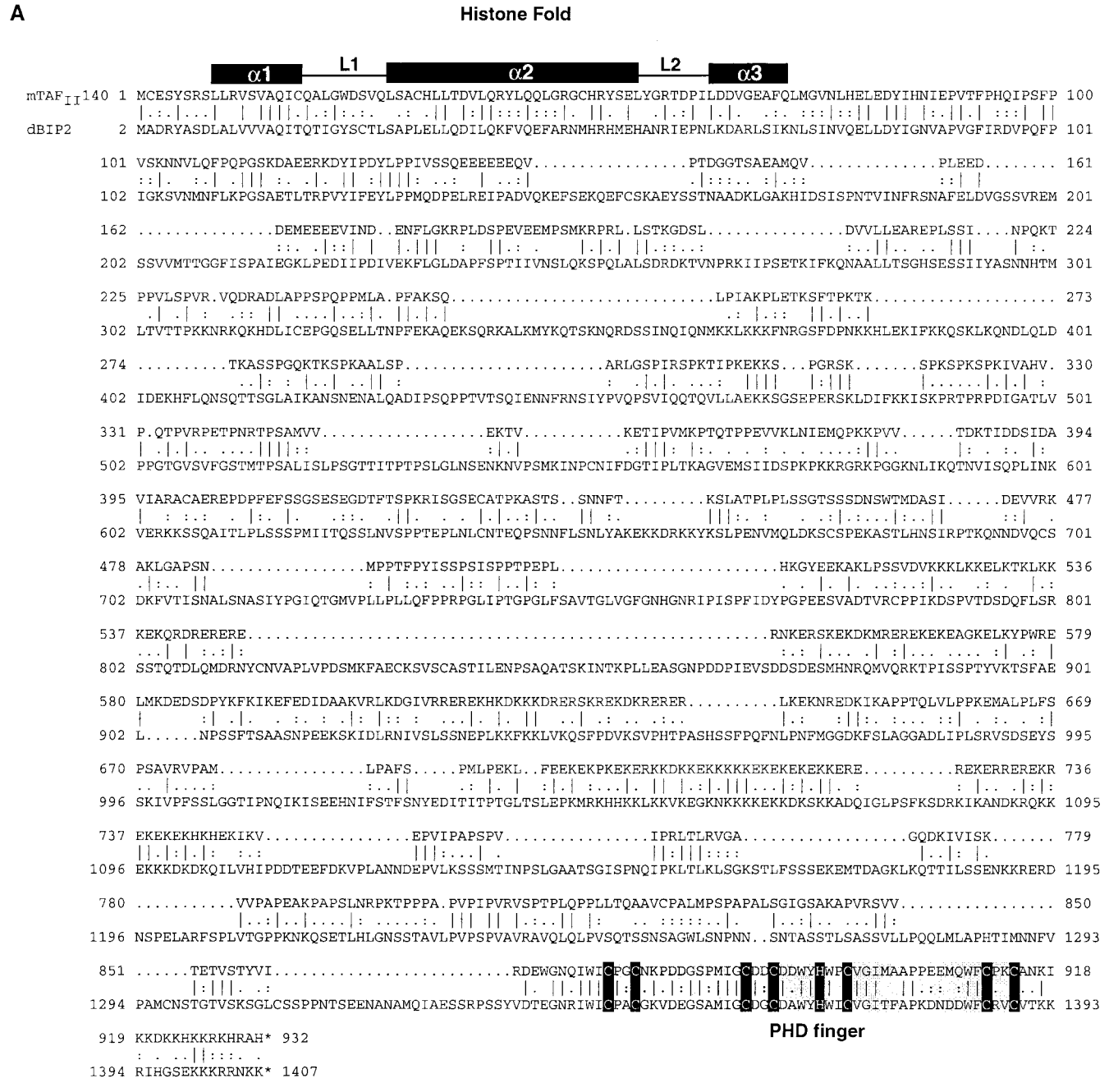

FIG. 4. (A) Alignment of the mTAF$_{II}$140 and dBIP2 amino acid sequences. The locations of the histone fold and PHD fingers are indicated. (B) Comparison of the PHD fingers of mTAF$_{II}$140, hTAF$_{II}$140, and dBIP2 with some of those previously characterized. Identical amino acids are shown in white letters against a black background. Critical cysteine and histidine residues are indicated (*).

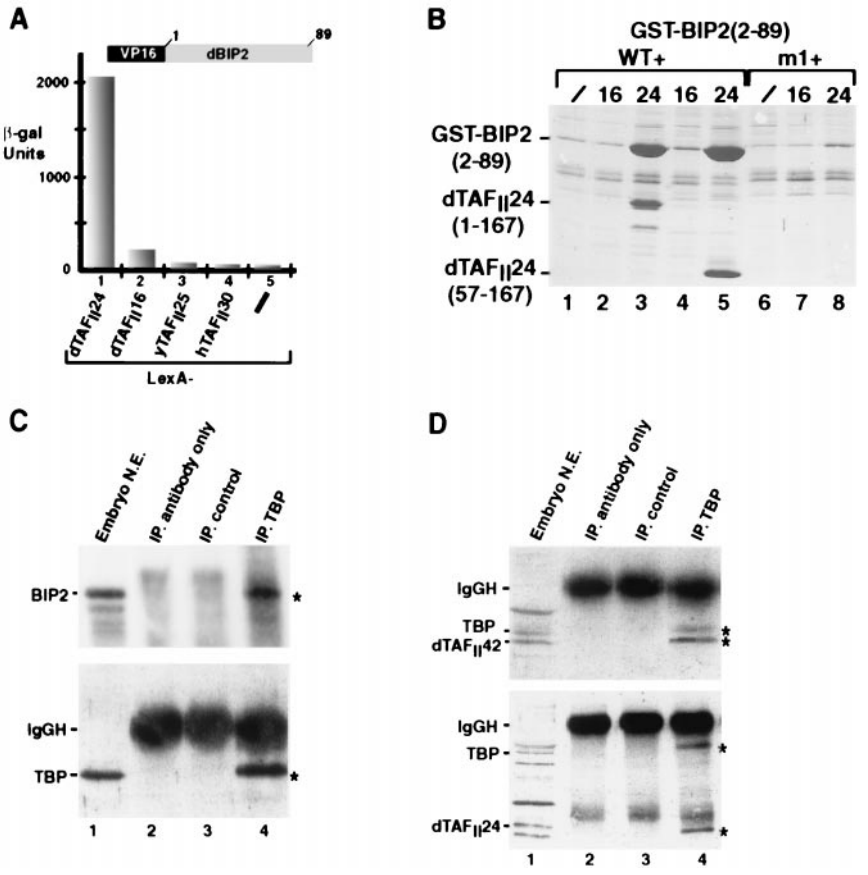

FIG. 5. (A) Graphical representation of quantitative two-hybrid β-galactosidase assays. The VP16-dBIP2 chimera is shown schematically above the graph and interactions with the LexA chimeras shown below each column are indicated. −, negative controls with the LexA domain alone. (B) Direct and selective dBIP2-dTAF$_{II}$24 heterodimerization. Bacteria were transformed to express the proteins shown above each lane, and following extract preparation, the soluble protein retained on glutathione-Sepharose beads was analyzed by SDS-PAGE and staining with Coomassie brilliant blue. /, the GST-fusion was expressed alone. For lanes 2 and 3, coexpression was performed with the full-length dTAF$_{II}$16 and dTAF$_{II}$24 proteins as indicated, while for lanes 4, 5, 7, and 8, coexpression was performed with the HFDs of the indicated proteins. The locations of the GST-BIP2 fusions and the dTAF$_{II}$24 proteins are indicated. (C and D) *Drosophila* embryo nuclear extract was precipitated with antisera against TBP (lane 4) or an irrelevant antiserum (raised against GST) (lane 3) as indicated. Lane 2 shows a control immunoprecipitation with the anti-TBP serum in the absence of added nuclear extract. *, locations of the precipitated proteins.

coexpression in *Escherichia coli* cells. GST-fusions of the BIP2 HFD were coexpressed with native dTAF$_{II}$16 and dTAF$_{II}$24. The GST-BIP2(2-89) fusion was solubilized by coexpression of full-length dTAF$_{II}$24, for which a complex of the two was retained on the resin, whereas with dTAF$_{II}$16, no solubilization nor heterodimerization was observed (Fig. 5B, lanes 2 to 3). Similarly, GST-BIP2(2-89) was solubilized by coexpression of the dTAF$_{II}$24 HFD(57-167), but not by the dTAF$_{II}$16 HFD (41-146) (lanes 4 and 5). The GST-BIP2-dTAF$_{II}$24 HFD heterodimerization was almost completely abolished by mutation of residues within the L1 loop [lane 8, G23R, Y24K, GST-BIP2 (2-89)m1]. These results indicate a direct and selective dBIP2-dTAF$_{II}$24 heterodimerization via their HFDs.

A polyclonal anti-dBIP2 serum was generated (see Materials and Methods). In immunostaining, the anti-dBIP2 serum, but not the preimmune serum, recognized a ubiquitously expressed nuclear protein throughout *Drosophila* embryogenesis (Fig. 6A and data not shown). This antibody also localized the dBIP2 protein on polytene chromosomes exclusively in decondensed regions, both puffs and interbands (Fig. 6B, arrowheads) which correspond to RNA polymerase II start sites in transcriptionally active chromatin (30, 46). In contrast, it is not found in the transcriptionally inactive heterochromatin around the centromere.

To determine whether dBIP2 is indeed a dTFIID subunit as suggested by its ability to interact with dTAF$_{II}$24, immunoprecipitation experiments were performed. The anti-dBIP2 serum recognized a protein with a mass of around 155 kDa in *Drosophila* embryo nuclear extracts (Fig. 5C, lane 1). The embryo extract was precipitated with an antiserum against dTBP, which has previously been shown to precipitate the dTFIID complex (19, 29). BIP2 was precipitated with the anti-dTBP sera (Fig. 5C, lane 4), while no immunoprecipitation was seen using an irrelevant antiserum (lane 3), and the signal did not result from a spurious cross-reaction with the anti-TBP serum (lane 2). As a positive control, dTBP, dTAF$_{II}$42, dTAF$_{II}$24, and dTAF$_{II}$230 were also selectively precipitated by the anti-TBP serum (Fig. 5C and D, lanes 4, and data not shown). These data indicate that dBIP2 (dTAF$_{II}$155) can be coimmunoprecipitated with dTBP and other dTAF$_{II}$s and hence is a subunit of dTFIID.

A functional domain of yeast TAF$_{II}$65 required for viability in yeast is shared with the *Drosophila* Prodos protein. The

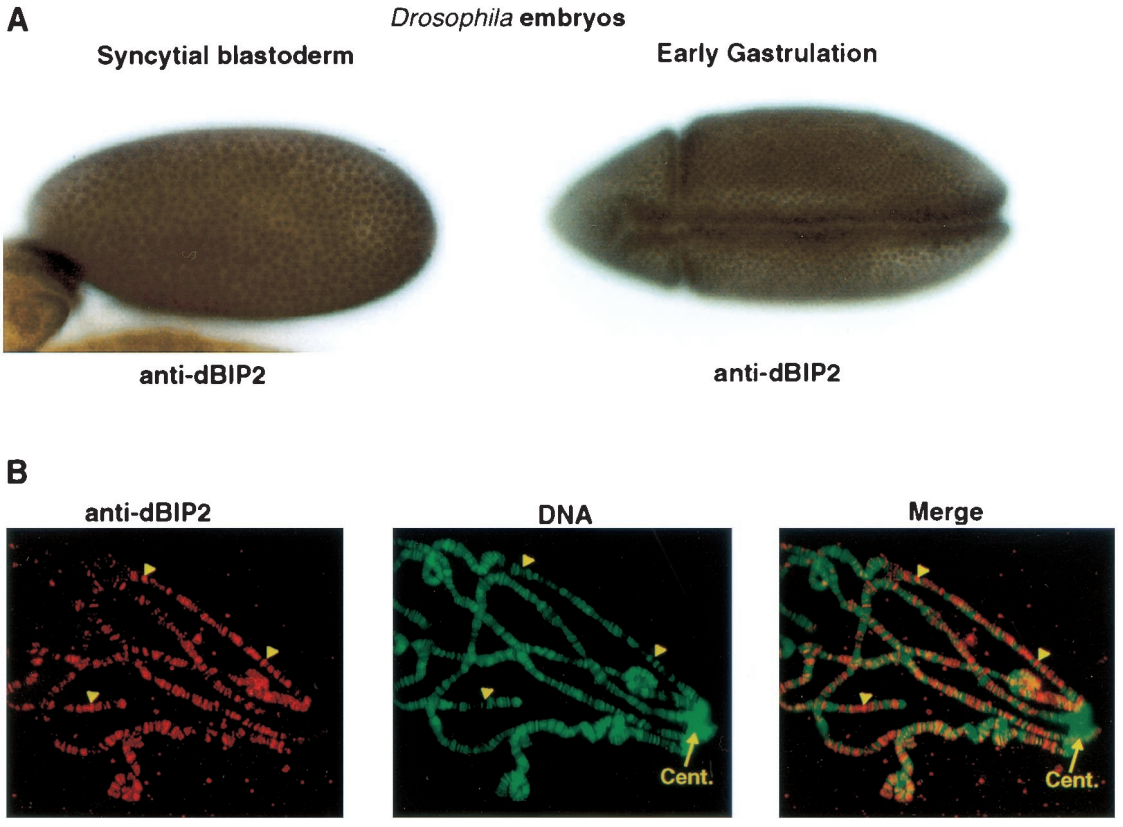

FIG. 6. (A) Immunodetection of dBIP2 in the nuclei of *Drosophila* embryos. The relevant stages are indicated. (B) In situ immunodetection of dBIP on salivary gland polytene chromosome spreads. The arrowheads indicate examples of interband regions staining strongly with the anti-dBIP2 serum. Cent., the centromeric region.

above results indicate that dBIP2 ($dTAF_{II}155$) is a TFIID component which selectively heterodimerizes with $dTAF_{II}24$. It has recently been reported that the PDS gene product contains an HFD and interacts specifically with $dTAF_{II}16$, leading to the idea that PDS is a TFIID subunit (25). Here, we show that dBIP2 ($dTAF_{II}155$) selectively heterodimerizes with $dTAF_{II}24$. $yTAF_{II}25$ can heterodimerize with either $yTAF_{II}47$ or $yTAF_{II}65$. Hence, if $dTAF_{II}155$ and $hTAF_{II}140$ are homologues of $yTAF_{II}47$, it is possible that PDS may be a homologue of $yTAF_{II}65$. We have previously shown that, in addition to the HFD, a second domain of $yTAF_{II}65$ located between amino acids 103 and 510 was essential for its function in yeast (17). As functional domains in TAF_{II}s are often evolutionarily conserved, we examined this region for similarities with PDS. Sequence comparisons indicate that $yTAF_{II}65$ shares significant sequence similarity with PDS not only in the HFD but also in the region C terminal to the HFD (Fig. 7A). Several highly conserved sequence motifs separated by insertions corresponding to potential loop regions in $yTAF_{II}65$ were observed. We therefore wished to determine whether this conserved domain (designated the TAPD) was essential for $yTAF_{II}65$ function.

Using the plasmid shuffle technique for yeast, we tested the ability of derivatives of $yTAF_{II}65$ with a deletion or mutation in the TAPD to complement the growth of a $yTAF_{II}65$ null strain. Deletion of the $yTAF_{II}65$ HFD does not abolish growth at 30°C but generates a temperature-sensitive phenotype (17), indicating that the region containing the TAPD contributes to $yTAF_{II}65$ function. We made further deletions to determine more precisely the location of this functional domain of $yTAF_{II}65$.

Deletion of amino acids downstream of 406 did not affect the ability of $yTAF_{II}65$ to rescue the growth of the null strain at 30°C, while a C-terminal truncation at amino acid 320 was unable to rescue growth [Fig. 8A, sections 2 to 4, summarized in Fig. 7B, compare $yTAF_{II}65$(103-406) and $yTAF_{II}65$(103-320)]. At the N terminus, a derivative beginning at amino acid 161 was able to complement growth, but a further deletion up to position 192, which deletes a highly conserved region of the TAPD, abolished function [Fig. 8A, sections 5 and 6, summarized in Fig. 7B, compare $yTAF_{II}65$(161-406) and $yTAF_{II}65$(192-510)]. As all of the viable constructs lacked the HFD, they showed a temperature-sensitive phenotype, being unable to complement at 37°C (data not shown). Therefore, a minimal TAPD between amino acids 161 and 406 is required for $yTAF_{II}65$ function.

The minimal domain described above comprises the most conserved regions of the TAPD. To further test the significance of the sequence similarity and to evaluate the relative contributions of the HFD and TAPD to $yTAF_{II}65$ function, we deleted or mutated one of the most conserved blocks between amino acids 170 and 200 in the context of wild-type $yTAF_{II}65$ and in the context of $yTAF_{II}65$ m1, where the HFD is mutated. When this region was deleted in the context of a wild-type HFD, complementation was observed [Fig. 8B, section 2, sum-

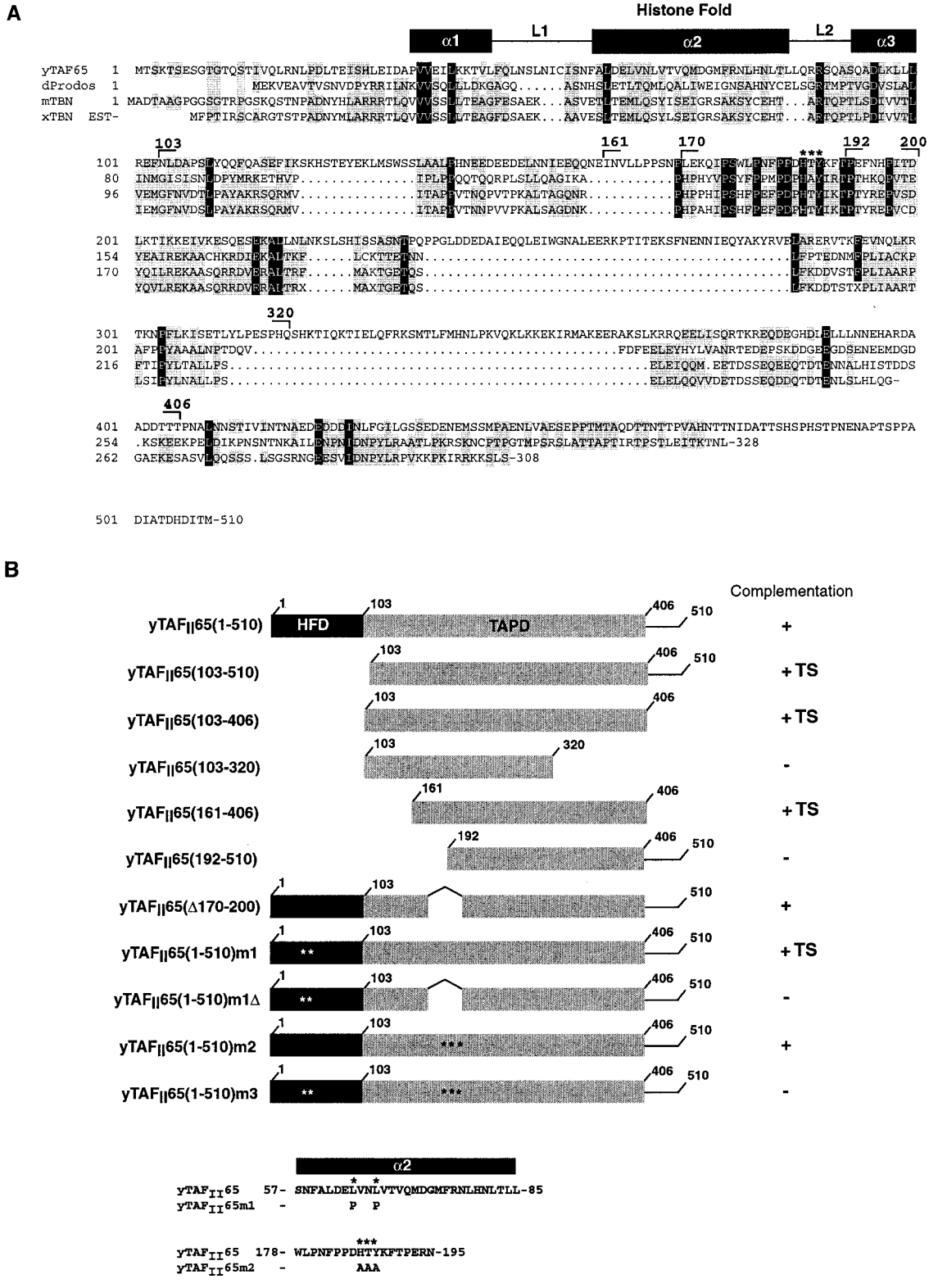

FIG. 7. (A) Comparison of the amino acid sequences of yTAF$_{II}$65, PDS, TBN, and a putative *Xenopus* TBN as described by Voss et al. (51). Identical amino acids are shown as white letters in a black background, and similar amino acids are boxed in gray. The location of the histone fold is indicated. The endpoints of the deletions in yTAF$_{II}$65 are indicated. The highly conserved HTY amino acids mutated in m2 are indicated (*). (B) The yTAF$_{II}$65 deletions are schematized along with their ability to rescue the growth of the yTAF$_{II}$65 null strain. The presence of mutations is indicated (*). The mutated amino acid sequences are shown at the bottom. TS, temperature sensitivity.

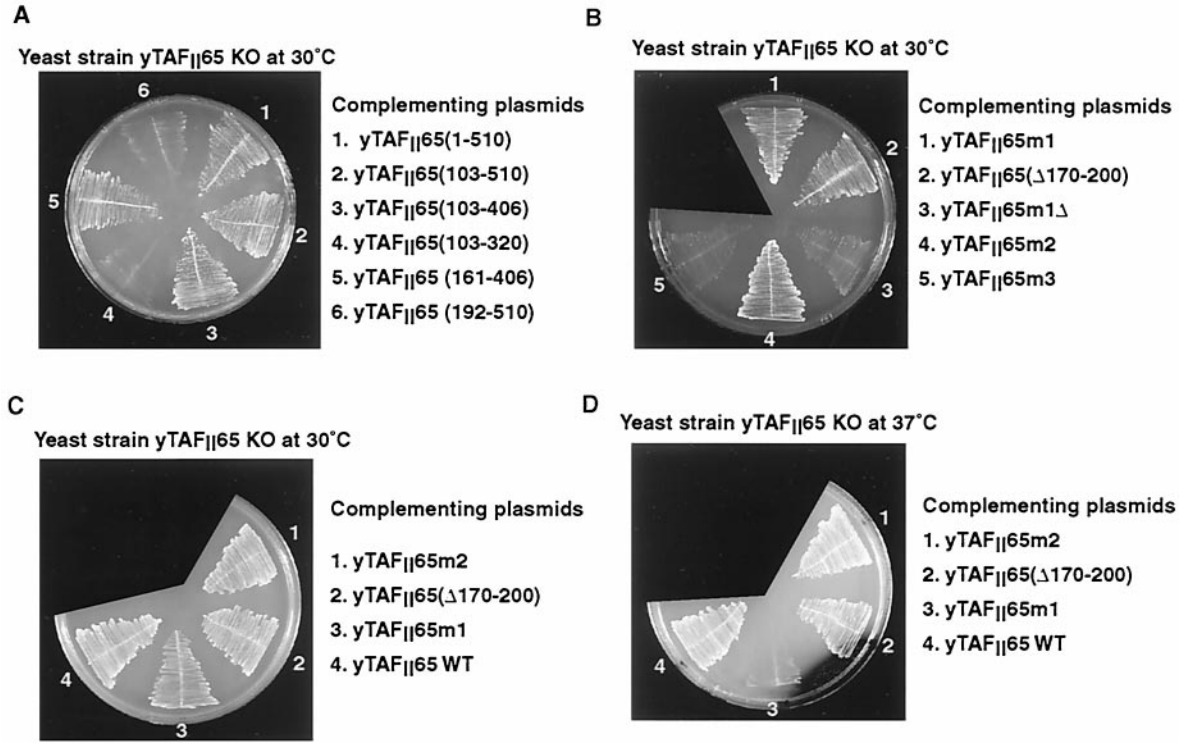

FIG. 8. The growth of yeast plated at the indicated temperatures is shown. The complementing plasmids used are next to the plates. WT, wild type.

marized in Fig. 7B, yTAF$_{II}$65(Δ170-200)]. In contrast, when the deletion was made in the presence of the mutated HFD, which by itself is viable at 30°C (Fig. 8B, section 1), no growth was observed [Fig. 8B, section 3, compare yTAF$_{II}$65(1-510)m1 with yTAF$_{II}$65(1-510)m1Δ]. Similarly, mutation of the highly conserved HTY residues within the region had no effect in the presence of the wild-type HFD but led to a loss of function in the context of a mutated HFD [Fig. 8B, sections 4 and 5, compare yTAF$_{II}$65(1-510)m2 and yTAF$_{II}$65(1-510)m3, summarized in Fig. 7B]. Hence, when the HFD is functional, the TAPD can be mutated, and likewise, when the TAPD is intact, the HFD is not absolutely required. These results indicate that yTAF$_{II}$65 contains two partially redundant domains. Nevertheless, the HFD seems to play a more important role since its mutation leads to a temperature-sensitive phenotype while mutation or deletion of the TAPD alone does not [compare yTAF$_{II}$65m1, yTAF$_{II}$65m2, and yTAF$_{II}$65(Δ170-200) in Fig. 8C and D].

DISCUSSION

TAF$_{II}$140 and BIP2 are novel metazoan TFIID subunits. We describe here mouse and human TAF$_{II}$140 and dBIP2 (dTAF$_{II}$155), novel components of the mammalian and *Drosophila* TFIID complexes. Both of these proteins contain an N-terminal HFD which selectively heterodimerizes with that of members of the yTAF$_{II}$25 family. The mouse and human TAF$_{II}$140 HFD heterodimerizes with those of both yTAF$_{II}$25 and hTAF$_{II}$30, whereas the dTAF$_{II}$155 HFD is much more selective, interacting only with that of dTAF$_{II}$24 and not with that of dTAF$_{II}$16. Most other organisms seem to have only one homologue of yTAF$_{II}$25. The existence of two closely related genes in *Drosophila* has allowed each one to evolve a more specialized role. For example, as previously reported (19), dTAF$_{II}$24 is associated with both the TFIID and the GCN5-containing complexes, while dTAF$_{II}$16 is present only in the TFIID complex. The differential presence of these proteins in the TFIID and GCN5-containing complexes imposes a specialization in partner choice. While yTAF$_{II}$47 and mouse and human TAF$_{II}$140 can heterodimerize with yTAF$_{II}$25 or hTAF$_{II}$30, the dTAF$_{II}$155 and PDS HFDs are much less promiscuous in their partners since there is a selective interaction of PDS with dTAF$_{II}$16 (25) and dTAF$_{II}$155 with dTAF$_{II}$24. Consequently, the specialization of the dTAF$_{II}$16 and dTAF$_{II}$24 HFDs has forced the dTAF$_{II}$155 and PDS HFDs to also evolve towards a restricted partner choice. This restriction contributes to the relative subunit compositions of the TFIID and GCN5-containing complexes.

TAF$_{II}$140 and dTAF$_{II}$155 also contain a PHD finger at the C terminus of the protein. The PHD finger is found in almost 300 proteins, most of which are nuclear (39). The majority of these proteins are involved in transcription and interactions with chromatin, such as the Polycomb-like family, and more recently the ATP-dependent chromatin remodeling factor ACF (1, 41). Mutations in the PHD fingers of several proteins have been associated with disease (for examples, see references 4 and 20). The structures of the PHD finger of the Williams-Beuren syndrome transcription factor (WSTF) and the KAP-1 corepressor have been recently solved by nuclear magnetic resonance and shown to comprise an interleaved zinc finger which binds two zinc ions (9, 39).

While the function of the PHD finger is unknown, it has

been suggested that it is involved in protein-protein interactions. This is the first time a PHD finger has been found in a TFIID subunit, and it may mediate TFIID interactions with chromatin or with chromatin-associated proteins. Its presence in TFIID is particularly intriguing since many of the factors in which it is found are associated with repressor activity, histone deacetylases, and interactions with heterochromatin (for an example, see references 15, 31, and 55). BIP2, however, is not localized on heterochromatin but is associated with transcriptionally active regions of polytene chromosomes. The PHD finger is not the only motif characteristic of chromatin-interacting proteins to be found in TFIID. $TAF_{II}250$ contains bromodomains, also found in many chromatin-interacting factors, which have recently been shown to mediate interaction with acetyl-lysine groups in the histone tails (12, 26). Hence, both the bromodomains and the PHD finger may be interfaces for directing TFIID to promoters within chromatin.

Immunoprecipitations demonstrate that both $hTAF_{II}140$ and $dTAF_{II}155$ are TFIID subunits, since they are coprecipitated under stringent conditions along with TBP and TAF_{II}s. It has been previously shown that $dTAF_{II}24$ is coprecipitated by the $dTAF_{II}16$ antisera and vice versa (19). The simplest interpretation is that both $dTAF_{II}24$ and $dTAF_{II}16$ are present in the same dTFIID complex containing one (or more) molecules of each. This TFIID would therefore contain both PDS and $dTAF_{II}155$. An analogous result was obtained with yeast, where $yTAF_{II}47$ can be precipitated from strains expressing a tagged version of $yTAF_{II}65$ (45). These data were supported by immunoelectron microscopy which showed the presence of multiple molecules of $yTAF_{II}65$, $yTAF_{II}47$, and $yTAF_{II}25$ in the same TFIID particle (C. Leurent, S. Sanders, V. Mallouh, P. A. Weil, D. B. Kirschner, L. Tora, and P. Schultz, submitted for publication).

In HeLa cells, $hTAF_{II}30$ is a component of both TFIID and TFTC. When HeLa cell nuclear extracts were immunoprecipitated with the anti-$TAF_{II}140$ monoclonal antibody, both TFIID-specific components, such as $TAF_{II}18$ and TBP, and TFTC-specific components, such as GCN5 and SAP130 (also ADA3 and SPT3; our unpublished data), were detected. The $TAF_{II}30$-$TAF_{II}140$ heterodimer therefore appears to be a component of both TFIID and TFTC. At first sight this may seem surprising since, in yeast, $yTAF_{II}47$ is TFIID specific and is not present in SAGA where the SAGA-specific ySPT7 heterodimerizes with $yTAF_{II}25$ (17). However, $hTAF_{II}135$ and $hTAF_{II}150$ are TFTC components, yet their yeast homologues, $yTAF_{II}48$ and $yTAF_{II}150$, are not present in SAGA. In this respect, the findings for yeast and mammalian cells are somewhat different and reflect the fact that TFTC and SAGA have similar, but not identical, compositions. The observation that $hTAF_{II}140$ is a TFTC component does not, however, rule out the existence of a hSPT7 or dSPT7 homologue, as we previously suggested (17).

The evolutionarily conserved TAPD is required for $yTAF_{II}65$ function in vivo. Here, we describe the novel TAPD present in $yTAF_{II}65$ and PDS. The presence of an HFD and the TAPD in PDS which is known to interact physically and functionally with dTFIID components (25) indicates that PDS is a $yTAF_{II}65$ homologue. These domains are also present in the mammalian protein TBN, further suggesting that it may also be a TAF_{II} (25, 51).

The TAP domain is located C terminal of the HFD, and in $yTAF_{II}65$ it is partially redundant with the HFD. In complementation experiments, deletion or mutation of the HFD does not abolish $yTAF_{II}65$ function when the TAPD is wild type. Similarly, the TAPD can be mutated or deleted without loss of function when the HFD is present. In contrast, simultaneous mutation of both leads to a loss of function. Hence, the HFD and the TAPD play redundant roles in $yTAF_{II}65$ function.

As the function of the $yTAF_{II}65$ HFD is to mediate heterodimerization with $yTAF_{II}25$ and hence the integration of $yTAF_{II}65$ into TFIID, it is likely that the TAPD is also an interface for interactions between $yTAF_{II}65$ and another TAF_{II}(s). In the absence of the HFD, the TAPD would allow $yTAF_{II}65$ to associate with TFIID and support growth and vice versa. Obviously, when both are mutated, it is no longer possible for $yTAF_{II}65$ to interact with the TFIID, and this is lethal for yeast. Note, however, that the HFD seems to be the more critical for $yTAF_{II}65$ function, since all the viable strains in which it was deleted had a temperature-sensitive phenotype, whereas the strains in which only the TAPD was mutated grow at 37°C. This is consistent with the fact that histone-like heterodimers are extremely stable and suggests that the interactions mediated by the TAPD are less so. Nonetheless, it is worth noting that the TAPD comprises several conserved sequence blocks, suggesting that it may be composite in nature. Thus, even when amino acids 170 to 200 are deleted or mutated, the remainder of the domain may act cooperatively with the HFD to allow growth at 37°C. We have previously reported that the temperature sensitivity resulting from mutation or deletion of the $yTAF_{II}65$ HFD could be selectively suppressed by overexpression of $yTAF_{II}68$ (17). This suggests that the TAPD may interact with $yTAF_{II}68$. Biochemical and structural analyses will be required to determine how the TAPD interacts with $yTAF_{II}68$ and/or other TFIID components.

In this study, we have proposed that BIP2 ($dTAF_{II}155$) and $hTAF_{II}140$ are homologues of $yTAF_{II}47$ due to the similarity in sequence of the HFDs and their ability to heterodimerize with the $yTAF_{II}25$ homologues in dTFIID and hTFIID and that PDS is a homologue of $yTAF_{II}65$ based on the shared HFD and TAPD. These results indicate a remarkable conservation of TFIID subunit composition. One notable exception to this is the presence of a PHD finger in $hTAF_{II}140$ and its absence in $yTAF_{II}47$. Nevertheless, in yeast, the bromodomains and kinase activity in $hTAF_{II}250$ are not present in $yTAF_{II}130$ ($yTAF_{II}145$) but are provided by the association of the Bdf1 and Bdf2 proteins with TFIID (34). Database searches with the $mTAF_{II}140$ and $hTAF_{II}140$ PHD finger revealed the presence of several yeast proteins with related PHD fingers. It is possible that, by analogy with Bdf1 and Bdf2, one of these PHD finger-containing proteins associates with yTFIID to provide the missing domain.

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