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Identification and characterization of a TFIID-like multiprotein complex from *Saccharomyces cerevisiae*

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ABSTRACT **Although the mechanisms of transcriptional regulation by RNA polymerase II are apparently highly conserved from yeast to man, the identification of a yeast TATA-binding protein (TBP)–TBP-associated factor (TAF_{II}) complex comparable to the metazoan TFIID component of the basal transcriptional machinery has remained elusive. Here, we report the isolation of a yeast TBP–TAF_{II} complex which can mediate transcriptional activation by GAL4-VP16 in a highly purified yeast *in vitro* transcription system. We have cloned and sequenced the genes encoding four of the multiple yeast TAF_{II} proteins comprising the TBP–TAF_{II} multisubunit complex and find that they are similar at the amino acid level to both human and *Drosophila* TFIID subunits. Using epitope-tagging and immunoprecipitation experiments, we demonstrate that these genes encode bona fide TAF proteins and show that the yeast TBP–TAF_{II} complex is minimally composed of TBP and seven distinct yTAF_{II} proteins ranging in size from M_r = 150,000 to M_r = 25,000. In addition, by constructing null alleles of the cloned TAF-encoding genes, we show that normal function of the TAF-encoding genes is essential for yeast cell viability.**

The regulation of transcription of mRNA-encoding eukaryotic genes is a complicated process involving the modulation of chromatin structure, activities of upstream activators and repressors, and the concerted action of multiple components of the basal transcription machinery, including RNA polymerase II itself (1, 2). It is thought that the interaction of the TATA-binding protein (TBP), with the TATA-box promoter element is the first step in the formation of the RNA polymerase II preinitiation complex (PIC), and numerous studies have shown that PIC formation is subject to modulation by a variety of transcriptional regulators. However, the mechanisms by which these factors exert their effects are not yet fully understood. In metazoan systems, one basal factor that has been shown to be directly involved in mediating activation by upstream activators is the transcription factor TFIID, which is composed of TBP and TBP-associated factors (TAF_{II}s). Human and *Drosophila* TFIID complexes each contain at least eight TAF_{II}s, and the genes encoding a number of these have been cloned and sequenced (3–15). Several TAF_{II}s have been shown to interact directly with the activation domains of known transcriptional activator proteins (12–16), and these interactions are thought to be integral in some way to the transactivation process.

Although the mechanisms of transcriptional regulation are thought to be conserved from yeast to man, when we initiated our studies, a TFIID-like TBP–TAF_{II} multisubunit complex similar to human and *Drosophila* TFIIDs had not been directly biochemically identified from *Saccharomyces cerevisiae*, despite the genetic isolation of yeast genes which encode factors involved in mediating transcriptional activation (17, 18). Clearly if the mechanisms of transcriptional regulation are truly conserved between unicellular and multicellular organisms, then a TFIID-like TBP–TAF_{II} multiprotein complex containing coactivator activity should also exist in yeast. We previously showed that yeast TBP (yTBP), which is required for transcription by all three RNA polymerases (19, 20), is associated with at least nine distinct proteins ranging in size from ≈170 kDa to 25 kDa (21–23). We demonstrated that all of the components of the RNA polymerase III transcription factor TFIIIB, a known TBP–TAF complex, which includes the TAF_{III}70 protein Brf1p (also known as Tds4p and Pcf4p; refs. 24–26), were present in this TAF fraction. These data indicate that at least two of the TAFs of this protein fraction are RNA polymerase III specific. On the basis of these results, we therefore hypothesized that this TAF fraction might also contain RNA polymerase II-specific TAFs, particularly, a yeast multiprotein complex—i.e., TBP–TAF_{II}s—comparable to metazoan TFIID.

In this report, we describe the cloning and sequence of the genes encoding several of the yeast TAFs (yTAFs) in this TAF protein fraction and demonstrate that these yTAFs are, in fact, associated with TBP.¶ We also show that this TBP–TAF_{II} complex has the biochemical and genetic hallmarks of metazoan TFIIDs. Finally, we document that the genes encoding yeast TAF_{II}s are essential for yeast cell viability and discuss the potential interrelationships of this yeast TBP–TAF_{II} complex and yeast RNA polymerase II holoenzyme (27, 28) in transcriptional regulation. While this manuscript was in preparation, a report appeared by Reese *et al.* (29) that described a TBP–TAF_{II} complex which exhibits similar features to those described herein.

MATERIALS AND METHODS

Yeast Strains. YPH252 (30) is our standard wild-type yeast strain; gene disruptions were performed in diploid yeast strain SEY6210.5 (31). Protein extracts used for preparative yTAF protein purification by using anti-TBP IgG affinity chromatography were prepared from BJ5457 cells (21); hemagglutinin (HA)-tagged TBP–TAF_{II} protein complexes were prepared from haploid yeast strains derived from sporulated SEY6210.5-derived clones.

yTAF_{II} Purification and Protein Sequencing. Yeast TAF_{II} proteins were preparatively purified as detailed (21, 22) and sequenced (32), and the sequence information was analyzed

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Abbreviations: TBP, TATA-binding protein; TAF, TBP-associated factor; yTBP, yeast TBP; yTAF, dTAF, and hTAF, yeast, *Drosophila*, and human TAF, respectively; Ab, antibody; mAb, monoclonal Ab; WCE, whole-cell extract; HA, hemagglutinin.

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¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L40145).

via BLAST searches (33). The peptide sequences were as follows: yTAF$_{II}$150, (aa 165–174) KTTPGFQESV and (aa 991–1000) KQFLLDILVY; yTAF$_{II}$130, (aa 391–407) SLIEDVAEDWQWDEDMI, (aa 554–580) ESFSTSQDLTIGDTAPVYLMEYSEQTP, and (aa 789–820) SLITPEQISQVESMSQGLQFQEDNEAYNFDSK; yTAF$_{II}$90, (aa 22–63) NQRTNNAAGANSGQQPQQQSQGQSQQQGRSNGPFSASDLNRI and (aa 726–752) ATTEPSAEPDEPFIGYLGDVTASINQD; yTAF$_{II}$60, (aa 79–99) ALRVLNVEPLYGYYDGSEVNK, (aa 106–139) VNTSGGQSVYYLDEEEVDFDRLINEPLPQVPRLP, and (aa 236–243) ELQIYFNK. The numbers refer to amino acid position within the deduced protein sequences, with the N-terminal methionine given position 1.

TAF Gene Cloning. Intrapeptide PCR was performed by using degenerate oligonucleotides and either yeast genomic DNA or total yeast cDNA library DNA as template (34, 35), and appropriate-length PCR product fragments were sequenced. This information was used to obtain full-length clones of all the yTAF$_{II}$-encoding genes, which were sequenced by using standard dideoxynucleotide/chain termination methods. Details of the sequencing, cloning and construction, and verification of *taf*-null alleles are available on request.

Construction of HA-Tagged yTAF$_{II}$-Encoding Genes. Yeast expression plasmids encoding yTAF$_{II}$150, yTAF$_{II}$130, yTAF$_{II}$90, yTAF$_{II}$60, and yTBP or their triple HA-tagged variants (36) were constructed basically as described (22) by using standard methods for DNA manipulation. Our Tsm1p-encoding plasmid was kindly supplied by J. Haber (Brandeis University, Waltham, MA).

Immunological Methods. Analytical and preparative-scale immunoprecipitation and immunoblotting experiments were performed with yeast whole-cell extract (WCE) protein fractions as described (21, 22). Purification and elution of the HA-tagged TAF-containing complexes were achieved by using a solution of peptide (sequence GGYPYDVPDYAGGYPYDVPDYAGGYPYDVPDYAGG) in BA/300 (1 mg/ml) (22) containing 0.1% Nonidet P-40 and 100 μg of insulin per ml as carrier (HA epitope sequence underlined).

***In Vitro* Transcription Assays.** Transcription reactions were performed essentially as described (28, 37, 38). Reaction mixtures contained 50–100 ng of purified transcription factors yTFIIB, -E, -F, and -H; 100 ng of purified yeast core RNA polymerase II; and 100 ng each of template pSPGCN4CG (UAS$_{GCN4}$ driven) and pJJ470 (UAS$_{GAL4}$ driven), supplemented with 50 ng of recombinant yTBP and/or 100 ng of GAL4-VP16 and/or 50-100 ng of yTAFs.

RESULTS

Yeast TBP-TAF$_{II}$ Complexes Display TFIID-Like Coactivator Activity. To test whether our yTAF preparation (a typical yTAF polypeptide profile is shown in Fig. 1*A*) contained TFIID-like coactivator activity, we used a highly purified yeast *in vitro* transcription system to see if this protein fraction could mediate an RNA polymerase II transactivation event. We used purified GAL4-VP16 activator protein and essentially homogenous preparations of yeast TFIIB, -E, -F, and -H and core RNA polymerase II (28) for our transcription reconstitution assays (37, 38). As shown in Fig. 1*B*, the yTAF protein fraction was able to specifically mediate a modest but reproducible 2.5- to 3-fold activation by GAL4-VP16 without affecting transcription from the control GCN4-driven template. This result suggests that, as we hypothesized, our yeast TBP–TAF fraction does contain coactivator activity similar to that of metazoan TFIID.

Cloned Yeast TAF-Encoding Genes Show Marked Sequence Similarities to Metazoan TAF$_{II}$s. To characterize the yTAF$_{II}$s further, the genes encoding four of them were cloned and sequenced. Individual TAF proteins were isolated, and peptides derived from yTAF$_{II}$150, -130, -90, and -60 were sequenced (see *Materials and Methods* for sequences). A data

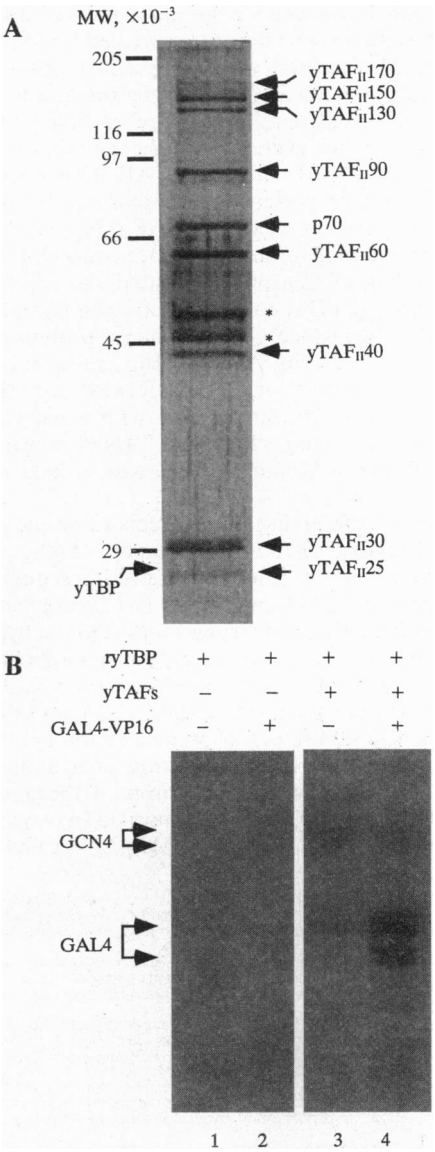

FIG. 1. Subunit composition and transcriptional activity of yTAF$_{II}$–TBP complex. (*A*) Silver-stained gel of proteins present in the yTAF fraction. yTBP and associated factors were purified by anti-TBP IgG affinity chromatography and fractionated by SDS/PAGE. The nine polypeptides that consistently coimmunopurify with yTBP are shown, and those thought to make up the RNA polymerase II-specific complex are labeled as yTAF$_{II}$s. Asterisks indicate polypeptides that are retained by a preimmune IgG-protein A affinity matrix. The abundant polypeptide species migrating with a mass of 70 kDa was previously thought (21) to be entirely Brf1p, but immunoblotting experiments using anti-Brf1p antibodies (ref. 24; kindly supplied by S. Hahn) suggest that although Brf1p migrates at this position, the amount of Brf1p is less than the strongly silver-stained 70-kDa species would indicate. Thus, the 70-kDa species has been designated p70. yTBP migrates slightly slower than yTAF$_{II}$25 and is difficult to visualize on these silver-stained gels, as neither yTBP nor yTAF$_{II}$25 stains well with silver. (*B*) Effect of the addition of the yTAF$_{II}$ fraction upon VP16-mediated transactivation. *In vitro* transcription reactions contained purified yTFIIB, yTFIIE, yTFIIF, yTFIIH, yeast core RNA polymerase II, and, as indicated, recombinant yTBP (ryTBP), yTAFs, or GAL4-VP16. Arrows indicate specific transcripts produced from the GCN4- (upper) and GAL4- (lower) driven templates. Phosphorimaging quantitation from this gel analysis for the UAS$_{GCN4}$-directed template is 1737, 1926, 3553, and 3394, for lanes 1–4, respectively, and for the UAS$_{GAL}$-directed template is 4096, 3844, 1917, and 11502, for lanes 1–4, respectively.

base search using the derived amino acid sequence information and the BLAST search algorithms (33) revealed that yTAF$_{II}$150 is encoded by the essential yeast gene *TSM1* (39) and that yTAF$_{II}$130 and yTAF$_{II}$60 are encoded by previously uncharacterized genes. Initial data base searches failed to find a match to yTAF$_{II}$90; however, after this gene was cloned and partially sequenced, we found that yTAF$_{II}$90 is encoded by ORFYBR1410, a gene present on chromosome II (40). Full-length clones of the genes encoding yTAF$_{II}$130, yTAF$_{II}$90, and yTAF$_{II}$60 were obtained as detailed in *Materials and Methods.* The gene encoding yTAF$_{II}$130 (designated *TAF130* for TBP-associated factor 130 kDa) consists of an open reading frame of 1066 amino acids, which could encode a protein of M_r = 120,685. The gene encoding yTAF$_{II}$90 (for consistency herein designated *TAF90* instead of ORFYBR1410) consists of an open reading frame of 798 amino acids with a deduced M_r = 88,960. The gene encoding yTAF$_{II}$60 (*TAF60*) consists of an open reading frame of 516 amino acids with a deduced M_r = 57,897.

Comparisons of the amino acid sequences predicted from the *TAF150* (*TSM1*), *TAF130, TAF90*, and *TAF60* genes with metazoan TAF-encoding genes reveal striking sequence similarities between the yTAFs and human and *Drosophila* TFIID subunits, a result which lends strong support to the hypothesis that the cloned yTAF genes encode actual subunits of a yeast TFIID multiprotein complex. yTAF$_{II}$150/Tsm1p is the yeast homolog of *Drosophila* TAF$_{II}$150 (dTAF$_{II}$150), and these two proteins are ≈50% similar at the amino acid level (41). *TSM1* was originally identified by the isolation of a temperature-sensitive mutant in a region of chromosome III located to the right of the *MAT* locus (39). At the amino acid level, yTAF$_{II}$130 is similar to both human TAF$_{II}$250 (hTAF$_{II}$250)/CCG1 and to dTAF$_{II}$230/250 (Fig. 2*A*). In the region of highest similarity, this yTAF is 78% similar to its metazoan counterpart (yTAF$_{II}$130 amino acids 440–830; refs. 3, 4, and 6). Thus, at this level of analysis, it appears that the N-terminal 60% of the metazoan TAF$_{II}$250 is conserved within yTAF$_{II}$130 sequences, a conclusion also reached by Reese *et al.* (29).

On the basis of overall length and sequence similarity, yTAF$_{II}$90 appears to be the yeast homolog of dTAF$_{II}$80 (Fig. 2*B*). yTAF$_{II}$90 is 62% similar to dTAF$_{II}$80 at its C terminus (7). The deduced amino acid sequence of yTAF$_{II}$90 also contains potential β-transducin (WD40) repeats like its metazoan counterpart. These repeats have been proposed to play a key role in directing protein–protein interactions between other proteins. These sequences in yTAF$_{II}$90 could be important in mediating specific protein–protein interactions with other TAF$_{II}$s, RNA polymerase II, basal factors, and/or other transcriptional regulatory proteins, either activators or repressors of transcription, as exemplified by the Tup1p/Ssn6p-α2 system (42). *TAF90* was also cloned, sequenced, and noted to resemble dTAF$_{II}$80 by Reese *et al.* (29). yTAF$_{II}$60, the homolog of dTAF$_{II}$60 and hTAF$_{II}$70 (9), has an overall acidic charge and may contain a leucine-zipper motif in its leucine-rich C-terminal region (Fig. 2*C*). The N terminus of yTAF$_{II}$60 is 47% similar to both dTAF$_{II}$60 and hTAF$_{II}$70.

Yeast TAF$_{II}$-Encoding Genes Are Essential for Yeast Cell Viability. To test whether the *TAF* genes were essential for yeast cell viability, large regions within plasmid clones carrying each yTAF-encoding gene (*TAF130, TAF90*, or *TAF60*) were deleted and replaced with *TRP1* sequences (43). The disrupting fragments were then purified and individually introduced into diploid yeast strains (44). The resulting Trp prototrophs were sporulated, and tetrads were dissected. In all three cases,

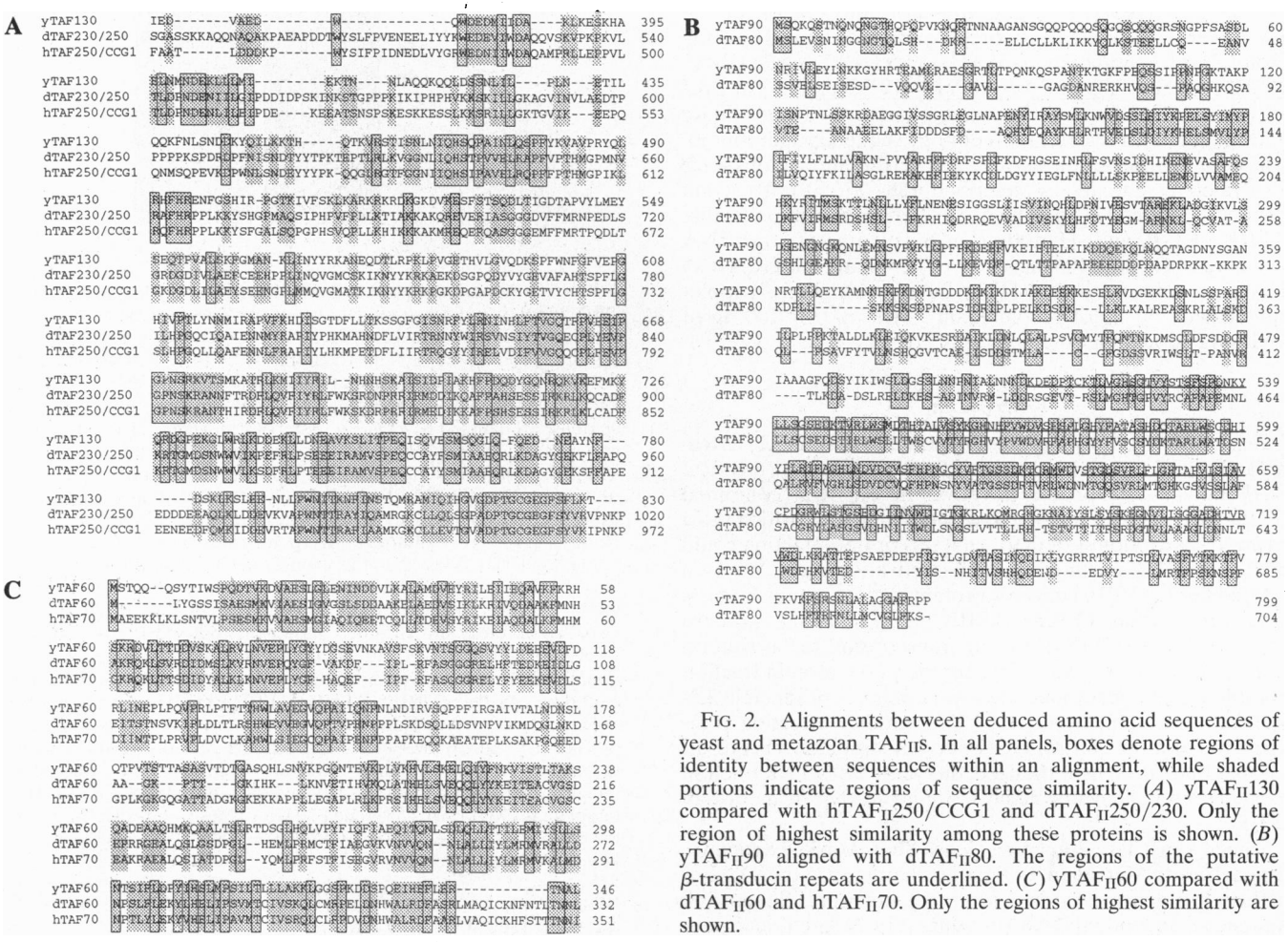

FIG. 2. Alignments between deduced amino acid sequences of yeast and metazoan TAF$_{II}$s. In all panels, boxes denote regions of identity between sequences within an alignment, while shaded portions indicate regions of sequence similarity. (*A*) yTAF$_{II}$130 compared with hTAF$_{II}$250/CCG1 and dTAF$_{II}$250/230. Only the region of highest similarity among these proteins is shown. (*B*) yTAF$_{II}$90 aligned with dTAF$_{II}$80. The regions of the putative β-transducin repeats are underlined. (*C*) yTAF$_{II}$60 compared with dTAF$_{II}$60 and hTAF$_{II}$70. Only the regions of highest similarity are shown.

viability segregated $2^+:2^-$ with no Trp^+ spores being recovered, indicating that each cloned *TAF* gene is essential for viability (data not shown). To obtain viable haploid yeast strains containing the individual disrupted *TAF* genes, a *URA3*-based plasmid containing the appropriate *TAF* gene was introduced into the corresponding diploid disrupted strain, followed by sporulation and tetrad dissection. Those tetrads which gave rise to four viable spores were isolated and analyzed for phenotype. In each case, germinated spores that were Trp^+ were also Ura^+ (data not shown). Further, cells which were Ura^+Trp^+ were also 5-fluoroorotic acid sensitive (45). *TSM1*, which encodes yTAF$_{II}$150, is known from the work of others to be an essential yeast gene (39). Together, these genetic experiments clearly indicate that TAF function is crucial for cell viability.

Yeast TAF-Encoding Genes Encode Bona Fide TAFs. To unequivocably show that the four genes we have identified actually encode yTAFs, we performed a series of coimmunoprecipitation experiments to show that our TAF$_{II}$s consistently copurify with yTBP and vice versa. To accomplish this, we engineered the DNA sequences encoding three copies of the influenza virus HA epitope (aa sequence YPYDVPDYA) into the *TAF150, TAF130, TAF90*, and *TAF60* genes, as well as into the gene encoding yTBP. The epitope-tagged yTAF$_{II}$130-, yTAF$_{II}$90-, yTAF$_{II}$60-encoding genes, as well as the epitope-tagged yTBP-encoding gene, each resident on a *HIS3, CEN/ARS* plasmid, were then separately exchanged for the corresponding *URA3*-marked, plasmid-borne, wild-type yTAF- or yTBP-encoding genes by using the plasmid shuffle technique (45) in strains carrying null chromosomal mutations of the cognate genes. The chromosomal copy of the gene encoding yTAF$_{II}$150 was also engineered to express this same epitope tag (as in ref. 22). The five resulting strains thus contained only the epitope-tagged version of these genes. Protein fractions prepared from these yeast strains were used in immunoprecipitation experiments (22), along with a wild-type yeast control strain which did not contain any HA-tagged genes. Using affinity-purified polyclonal rabbit anti-yTBP antibodies (Abs) for immunoprecipitations and the 12CA5 monoclonal Ab (mAb) (which recognizes the HA epitope) as the antibody for immunodetection, we found that HA-yTAF$_{II}$150, HA-yTAF$_{II}$130, HA-yTAF$_{II}$90, and HA-yTAF60 each coimmunoprecipitated with yTBP (Fig. 3*A*). Control immunoprecipitation reactions showed that, as expected, no 12CA5-reactive proteins were immunoprecipitated from the nontagged yeast cell extract, while HA-TBP was immunoprecipitated but only from the strain expressing the HA-tagged TBP (Fig. 3*A*, left two lanes). In the converse experiment, by using 12CA5 mAb for immunoprecipitation, followed by anti-yTBP Ab for immunodetection, yTBP coimmunoprecipitated with all of the HA-tagged TAF$_{II}$s: HA-yTAF$_{II}$150, HA-yTAF$_{II}$130, HA-yTAF$_{II}$90, and HA-yTAF$_{II}$60 (Fig. 3*B*), while the control immunoprecipitation/detection reactions again generated the predicted precipitation/reactivity patterns (see Fig. 3*B*, left two lanes). Repeating this experiment with these same yeast WCEs—(i.e., immunoprecipitation with either anti-TBP Ab or 12CA5 mAb) but using anti-Tsm1p IgG (TAF$_{II}$150; ref. 41) for immunodetection (data not shown) indicates that these four proteins (TAF$_{II}$150, TAF$_{II}$130, TAF$_{II}$90, and TAF$_{II}$60) appear to be associated with TBP in a distinct macromolecular complex, as suggested by our previous data (refs. 21–23; see Figs. 1*A* and 3*C*). The results of these experiments indicate that

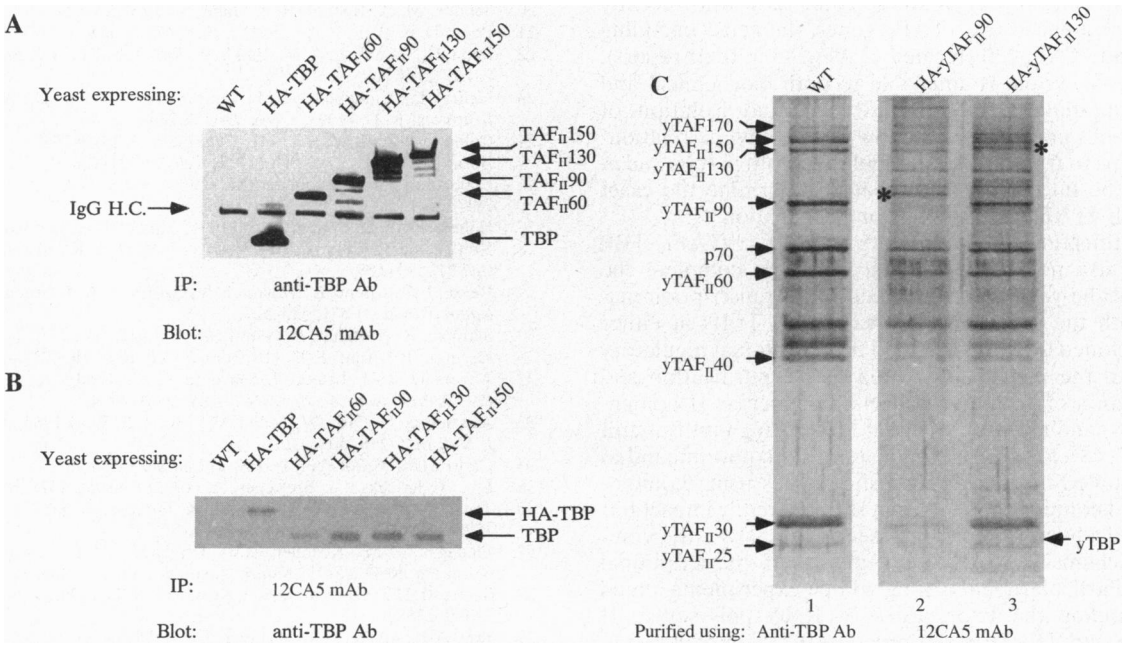

FIG. 3. Immunological characterization of the yTAF$_{II}$–TBP complex. (*A*) yTAF proteins TAF$_{II}$ 150, -130, -90, and -60 are associated with TBP in yeast WCEs. WCEs were prepared from yeast strains expressing either no epitope-tagged proteins (WT; strain YPH252; lane 1) or epitope-tagged proteins: TBP (lane 2), yTAF$_{II}$60 (lane 3), yTAF$_{II}$90 (lane 4), yTAF$_{II}$130 (lane 5), or yTAF$_{II}$150 (lane 6). TBP and any associated proteins were immunoprecipitated (IP) with affinity-purified anti-yTBP Abs. Immunoprecipitated proteins were fractionated by SDS/PAGE and blotted, and proteins containing the HA-epitope were detected by immunoblotting with mAb 12CA5, as detailed in *Materials and Methods.* Arrows and labels indicate epitope-tagged yTAF$_{II}$150, yTAF$_{II}$130, yTAF$_{II}$90, yTAF$_{II}$60, or TBP or IgG heavy chain (H.C.). (*B*) yTBP coimmunopurifies with epitope-tagged yTAFs. The same WCEs used for the experiment presented in *A* were again used for immunoprecipitation, except that the precipitating Ab was mAb 12CA5 and the immunodetection Ab was affinity-purified anti-TBP IgG, as indicated. TBP and HA-tagged TBP are shown by arrows and labels. Note that HA-tagged TBP migrates more slowly than non-tagged, WT TBP, as expected. (*C*) Comparison of the TAF$_{II}$ content of yTAF$_{II}$–TBP complexes immunopurified from WT and HA-tagged yeast strains. Immunopurified TAF proteins were fractionated by SDS/PAGE and detected by silver staining. On the left are the designations of yTAF$_{II}$s as shown in Fig. 1*A*. Lane 1 contains yTAFs purified by using polyclonal anti-yTBP Ab. Lanes 2 and 3 display the TAF proteins purified by using 12CA5 mAb and extracts from yeast strains expressing either HA-tagged yTAF$_{II}$90 or HA-tagged yTAF$_{II}$130, respectively. In lanes 2 and 3, asterisks denote the epitope-tagged yTAF$_{II}$s which migrate more slowly than their nontagged counterparts in lane 1, due to the additional amino acids of the HA epitope.

these proteins are all complexed with TBP and thus by definition are bona fide yTAFs.

The "Core" Yeast TBP–TAF_{II}-Complex Appears to Be Composed of TBP and Seven TAF_{II}s. To attempt to determine the overall subunit composition of the yeast TBP–TAF_{II} multisubunit complex, mAb 12CA5 was used to preparatively immunopurify HA-yTAF_{II}130- and HA-yTAF_{II}90-containing complexes, as detailed in *Materials and Methods.* SDS/PAGE analyses of these immunopurified TAF-complexes (Fig. 3*C*) demonstrated that polypeptides yTAF_{II}150, yTAF_{II}90, yTAF_{II}60, yTAF_{II}40, yTAF_{II}30, and yTAF_{II}25 all copurified with HA-yTAF_{II}130, while polypeptides yTAF_{II}150, yTAF_{II}130, yTAF_{II}60, yTAF_{II}40, yTAF_{II}30, and yTAF_{II}25 all coimmunopurified with HA-yTAF_{II}90. Additional polypeptides, presumably contaminants (but see refs. 14 and 15), appear to coimmunoprecipitate (purify) with these yTAF preparations. These data again support the idea that both the *TAF130* and *TAF90* genes encode bona fide yTAF_{II}s and, more important, demonstrate that the components of a core yTAF_{II}–TBP complex are, at a minimum, the seven yTAF_{II}s of M_r = 150,000, 130,000, 90,000, 60,000, 40,000, 30,000, and 25,000 characterized in this report. TBP stains poorly with silver and is difficult to visualize in Fig. 3*C* (but see Fig. 3 *A* and *B*).

DISCUSSION

The fact that *S. cerevisiae* contains what appears to be a TFIID complex and that four of the yeast TAF_{II}s are homologous to both human and *Drosophila* TFIID subunits at both the functional and structural levels provides further compelling evidence that the mechanisms of transcription are indeed conserved from yeast to man (1, 2, 46). All our data are consistent with the report of Reese *et al.* (29), who recently cloned and sequenced two yTAF_{II} genes, the genes encoding yTAF_{II}90 and yTAF_{II}130 (termed yTAF_{II}145 in their report). Since the yeast system is amenable to both biochemical and genetic manipulation, the identification and isolation of yTAF_{II} proteins and genes will allow us to study the relationship of TAF_{II}s to transcriptional regulation both *in vitro* and *in vivo.* It will be interesting to ultimately determine the exact roles of each yTAF_{II} in transcriptional regulation.

Our identification and characterization of a yTAF_{II}–TBP complex is also notable because it serves to complete the definition of the yeast RNA polymerase II transcription machinery. With the availability of yeast basal TFIIs in either purified or cloned form and purified transcriptional regulatory proteins, and the recent description of the purification and characterization of both a yeast RNA polymerase II holoenzyme (27, 28) and a yeast chromatin remodeling multisubunit complex (47, 48), at long last the materials are now in hand to perform detailed *in vitro* mechanistic studies using homologous purified components. This fact should greatly impact our ability to quickly and precisely dissect and analyze the complicated mechanisms of RNA polymerase II transcriptional regulation. Particularly interesting will be experiments aimed at understanding the interactions of RNA polymerase II holoenzyme and TFIID in the processes of promoter recognition and transactivation. We have shown previously (23) that the yTAF_{II} complex and the yeast RNA polymerase II (TFIIF-containing) holoenzyme complex share yTAF_{II}30 as a constituent subunit. The exact significance of this observation remains to be elucidated; however, this result argues for a level of protein–protein interaction among components of the basal RNA polymerase II transcription machinery that could lead to the recognition of previously unanticipated mechanisms for gene regulation.

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