Cloning and Characterization of an Essential Saccharomyces cerevisiae Gene, TAF40, Which Encodes yTAFII40, an RNA Polymerase II-specific TATA-binding Protein-associated Factor*

Edward R. Klebanow, David Poon, Sharleen Zhou‡, and P. Anthony Weil§

From the Department of Molecular Physiology and Biophysics, Vanderbilt University, School of Medicine, Nashville, Tennessee 37232-0615 and the Howard Hughes Medical Institute, Department of Molecular and Cellular Biology, University of California, Berkeley, California 94720

In this report we describe the cloning and initial characterization of TAF40, a gene that encodes a yeast TATA-binding protein-associated factor (yTAF) of \( M_r = 40,000 \). This gene has many similarities to other yTAFs described thus far in that it is present in a single copy per haploid genome, it is essential for viability, and the deduced protein sequence of yTAF40 exhibits similarity to previously described human and Drosophila TAFII40. Immunological studies confirm that yTAF40 protein is a subunit of a large multiprotein TATA-binding protein-TAF complex that contains a subset of the total number of the yTAFs present in yeast cell extracts. Transcription reactions performed using yeast whole cell extracts reveal that of the three nuclear RNA polymerases only RNA polymerase II function is abrogated when yTAF40 and associated proteins are immunodepleted from solution, indicating that the functionality of the multiprotein complex containing yTAF40 is RNA polymerase II-specific. By these criteria yTAF40 appears to encode a bona fide RNA polymerase II-specific TAF, and thus the protein that it encodes has been termed yTAFII40.

The TATA-binding protein (TBP) is required by all three nuclear RNA polymerases for transcription (Refs. 1–3; reviewed in Refs. 4–6). Inside the cell TBP exists complexed with a variety of TBP-associated factors (TAFs) (Refs. 1 and 7–14; see Refs. 15–17 for recent reviews). The collection of TAFs associated with TBP appear to dictate the polymerase specificity of a given TBP-TAF complex. Thus, the present day nomenclature of TAF40, TAF110, and TAF110 has arisen to allow demarcation of the RNA polymerase specificity of a given TAF. The exact number of TBP-TAF complexes that exist within the cell and the complement of TAFs that make up each specific complex are currently unclear and are actively being investigated by a number of laboratories.

TFIID is the TBP-TAF complex dedicated to RNA polymerase II (RNAPII) transcription. RNAPII is the polymerase responsible for the transcription of mRNA encoding genes. In the three major model systems studied to date (human, Drosophila, and yeast), the TFIID complex has been found to consist of TBP and 9–12 tightly associated TAFII polypeptides ranging in size from \( M_r = 250,000 \) to 15,000 (11, 13, 14, 18–27). Although all studies of TAFII function report an obligatory role for TBP in RNA polymerase II transcription, the exact role that the TBP-TAFII complex TFIIID plays in transcription and transcriptional regulation remains to be elucidated (28–31).

Studies of Saccharomyces cerevisiae TBP-TAF complexes by our lab and others have suggested the presence of a TFIIID complex that consists minimally of TBP and TAFII of 150, 130, 90, 60, 40, 30, and 25 kDa (14, 25, 27). Here we extend these findings by reporting the cloning and characterization of TAF40, the gene encoding the \( 40,000 \)-Da moiety of the yeast TFIIID complex. We find that the gene encoding yTAF40 is both single copy and essential for viability. The polypeptide sequence deduced for yTAF40 exhibits sequence similarity to two known metazoan TAFII: hTAFII28 (human) and dTAFII30β (Drosophila). In the studies reported here we demonstrate that yTAF40 is in fact a bone fide TBP-associated factor that is part of the yeast TFIIID complex. Further, by utilizing various in vitro transcription assays we show that the function of the yTAF40-containing TBP-TAF complex is RNA polymerase II-specific, thus the protein encoded by TAF40 has been termed yTAFII40.

MATERIALS AND METHODS

Yeast and Bacterial Strains—Protein extracts used for preparative yTAF protein purification via immunoadsorbent chromatography were prepared from BJ5457 cells (32). The yeast strains expressing either HA3-tagged TBP or the variously HA3-tagged yTAFs that were used for WCE preparation and coimmunoprecipitation studies have been described previously (25, 27, 33). The diploid yeast strain SEY26.0.3Δ (34), genotype MAT a/a leu2/leu2 ura3/ura3 his3/ his3 trp1/trp1 suc2/ suc2 ADE2/ade2 lys2/lys2/LYS2, was used as the starting strain for gene disruption experiments. Yeast strains yEK8, yEK31, and yEK200 (see below) have the following genotypes: yEK8, MAT a/a leu2/leu2 ura3/ura3 his3/ his3 trp1/trp1 suc2/ suc2 ADE2/ade2 lys2/lys2/LYS2 TAF40/taf40Δ::TRP1; yEK31, MAT a/a leu2/leu2 his3/ his3 trp1/trp1 suc2/ suc2 ade2/ade2 lys2/lys2/LYS2 TAF40Δ::TRP1; and yEK200, MAT a/a leu2/leu2 his3/ his3 trp1/trp1 suc2/ suc2 ade2/ade2 lys2/lys2/LYS2 TAF40Δ::TRP1 pRS316-Gal1-TAF40 cDNA; and yEK200, MAT a/a leu2/leu2 his3/ his3 trp1/trp1 suc2/ suc2 ade2/ade2 lys2/lys2/LYS2 TAF40Δ::TRP1 pRS316-HA-TAF40. All DNA subcloning manipulations were performed in Escherichia coli strain XL1-Blue (35).

yTAF40 Purification and Protein Sequencing—Yeast TAF proteins were preparatively purified by immunochromatography on columns containing anti-TBP IgG covalently coupled to protein A-Sepharose. Purified yTAFs (13, 25–27, 33) were separated by SDS-PAGE as described, transferred to a nitrocellulose membrane, and visualized by Ponceau staining, and the band corresponding to yTAF40 was excised and cleaved with trypsin. The resulting tryptic peptides were purified by high pressure liquid chromatography and sequenced. The sequences of four of the resulting peptides were determined. These peptides had...
Characterization of yTAF$_{14}^{90}$

The following sequences: peptide 1, K/RLLVTNLDKQTNRFVFRH; peptide 2, K/RQMDIQVISEDQDYVTXX; peptide 3, K/RTTFLGNSLLQSC; and peptide 4, K/RLQSDTLPNAYWR. BLAST searches (36) were used to scan data bases for protein sequence homologies, whereas Geneworks software (IntelliGenetics Inc.) was used to generate and format the DNA sequences.

**TAF40 Gene Cloning**—The gene encoding yTAF40 was cloned via a PCR strategy based upon the amino acid sequence of yTAF$_{40}$ tryptic peptide 2. Two degenerate primers with HindIII (upstream) and XhoI (downstream) restriction endonuclease recognition sites (upstream, GAGAAGGTTCGAAGTAGATTTCGCTCAC/ HindIII site underlined, and downstream, GAGAATCCTCGAGATTTCGCTCAC/ HindIII site underlined) were synthesized. In a first round of PCR, the downstream oligo was used together with an oligo (GCCACTCACTCCGCTTTCACAC) complementary to Gly1 promoter sequence downstream of the insertion site of cDNAs into vector pRS316-Gal1-cDNA (37), using total cDNA library DNA as the template. The products of this first PCR reaction were used as the template along with both degenerate oligonucleotides as primers in a second PCR reaction. The products of this second PCR reaction were subsequently digested with HindIII and XhoI, and the correct length (47 base pairs) product was cloned into HindIII/XhoI-digested pBSIKS$^{+}$ (Stratagene) for DNA sequence determination. From the determined nondegenerate sequence an oligonucleotide was designed (CGAAGGTTCGAAGTAGATTTCGCTCACAGAAGGTTCGAAGTAGATTTCGCTCAGAAGGTTCGAAGTAGATTTCGCTCAG), that was then used to obtain a yeast genomic DNA library (J.T. Celenza, personal communication) with the longest insert resulting from this library screening was sequenced. A 1.3-kilobase pair fragment of this CDNA clone was then 3'- and 5'-labelled and used to screen a yeast genomic DNA library (ATCC 77164) to obtain a full-length plasmid clone of the gene encoding yTAF40. The insert of the resulting plasmid was sequenced in entirety using standard dideoxy sequencing methods.

**Construction of a Yeast Strain Harkering a Chromosomal Null Mutation of TAF40**—Diploid strain SEY6210.5 was used as the parental strain for the disruption of TAF40. PCR was used to generate a knock-out DNA fragment containing 50 base pairs of TAF40 upstream sequences and 50 base pairs of TAF40 downstream sequences bracketing an intact TRP1 gene. This DNA fragment was gel-purified and used to generate, via homologous recombination (38), a TAF40 null allele by transforming strain SEY6210.5 to 5'thophosphoglutamate protoporphyrin; the resulting TRP$^{+}$ strain was called yEK8. Proper integration of the taf40::TRP1 disrupting fragment at the TAF40 locus was verified by genomic Southern blotting. One of the cDNA plasmids found to contain the full-length TAF40 CDNA termed pRS316-Gal1-TAF40 CDNA (see above) was then used to transform yEK8 to uracil protoporphyrin to produce the strain yEK9/pRS316-Gal1-TAF40. This strain was then sporulated, and the resulting tetrads were dissected onto galactose-containing plates. The resulting spores were germinated and subjected to phenotypic testing. One of the resulting spore clones derived from this dissection was termed yEK31. yEK31 has the relevant genotype of taf40::TRP1 ura3 leu2 pRS316-Gal1-TAF40 cDNA.

**Construction of a Yeast Strain Expressing HA$_{3}$-tagged yTAF40**—A plasmid expression system for HA$_{3}$-tagged yTAF40 was constructed as follows. The genomic 2646-base pair gene fragment was cloned into pBSIKS$^{+}$ (Stratagene). The EcoRI site in the TAF40 promoter region was eliminated by digesting with EcoRI and filling in these ends with E. coli Klenow DNA polymerase I and then religating the ends. Subsequently an EcoRI site was introduced just 5' to the TAF40 ORF ATG using site-directed mutagenesis (39) to create a HindIII site underlined. This plasmid termed pBSIKS$^{+}$-EcoRI-TAF40. A PCR fragment encoding three copies of the influenza hemagglutinin epitope (HA) tag (40) with EcoRI ends was inserted into the EcoRI site of pBSIKS$^{+}$-EcoRI-TAF40 to generate pBSIKS$^{+}$-HA$_{3}$-TAF40. An XbaI/HindIII fragment from this plasmid was then cloned into similarly digested pRS415 (Stratagene) to generate pRS415-HA$_{3}$-TAF40. The resulting plasmid was cotransformed, along with linearized baculovirus DNA (BaculoGold-Pharminogen) into SF-9 insect cells grown in TMN-FH medium (JRH Scientific). Three rounds of virus amplification were then carried out in SF-9 cells to generate high titer virus stocks. For large scale yTAF$_{40}$ protein production, virus at a multiplicity of infection of 5 was used to infect High-Five insect cells (Invitrogen) grown in spinner flasks using Ex-Cell 400 medium (JRH Scientific). Infected cells were harvested 64 h postinfection and then washed in ice-cold phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na$_{2}$HPO$_{4}$, 1.4 mM KH$_{2}$PO$_{4}$, pH 7.4) prior to making a lysate using a denaturing buffer (Buffer A, 6 M guanidine hydrochloride, 0.1 M Na$_{2}$HPO$_{4}$, 0.01 M Tris, pH 8.0). His$_{6}$TAF40 fusion protein was purified under denaturing conditions using nickel nitrilotriacetic acid (Qiagen) resin (1 ml of resin/500 ml of infected cells). After batchwise washing (Buffer B, 8 M urea, 0.1 M Na$_{2}$HPO$_{4}$, 0.01 M Tris, pH 6.3), the resin was loaded into a syringe column, and bound His$_{6}$TAF40 was eluted utilizing a pH jump (Buffer D, 8 M urea, 0.1 M Na$_{2}$HPO$_{4}$, 0.01 M Tris, pH 5.0). The approximate yield of purified protein was $\geq$1 mg of His$_{6}$TAF40 from 500 ml of infected high-Five insect cell culture. Protein purity (85%) was estimated by Coomassie staining of an SDS gel.

**Antibodies**—Polyclonal antibodies directed against yTAF40 were raised in rabbits by Bethyl Labs, Inc. (Montgomery, TX) using purified baculoviral expressed His$_{6}$TAF40 as antigen. Total rabbit IgG was purified from the resulting serum using a protein A-Sepharose (Sigma) chromatography (41). Anti-influenza virus hemagglutinin (anti-HA) monoclonal antibody, 12CA5, which reacts with the HA epitope (YPYDVPDYA), was purchased from Boehringer Mannheim.

**Immunodepletion Experiments**—Monoclonal anti-HA antibody 12CA5 cross-linked to protein A-Sepharose was used to deplete HA$_{3}$-yTAF40, and associated proteins from 100-$\mu$L aliquots of transcriptionally competent WCE were prepared from yeast strain yEK200 by the method of Woontner et al. (42). The procedures followed for the preparation of resins, and the immunodepletion reactions were as previously described (25). The supernatants from these immunodepletion reactions were frozen in 50-$\mu$L aliquots at $-70^\circ$C until assayed for both yTAF40 content and residual RNAP 1-, 2-, and 3-specific transcription activity.

**Immunoprecipitation and Immunoblotting**—Immunoprecipitation and immunoblotting was performed as described previously (25). Where indicated, blots were subjected to quantitation by exposing the developed blot to a Bio-Rad high intensity imaging screen, which was then analyzed using the Bio-Rad GS-250 Molecular Imager and Molecular Analyst Software.

**In Vitro Transcription Assays**—RNA polymerase I-, II-, and III-specific transcription assays were carried out as described previously (Refs. 25, 43, and 44, respectively). Specific transcript production was quantitated using a Bio-Rad Image as described above.

**RESULTS AND DISCUSSION**

**Cloning of the Yeast Gene Encoding yTAF40**—Using conventional and anti-TBP immunoaffinity chromatography we have been able to purify TAFs from yeast whole cell extracts (13, 25, 27, 33). A yTAF of M, 40,000 is readily visualized on silver-stained SDS gels from these preparations (e.g. see Fig. 1 in Ref. 25). Peptide sequence was obtained from this 40-kDa TAF as detailed under “Materials and Methods.” At the time when this sequence was obtained GenBank$^{26}$ and Saccharomyces Genome data base searches using the BLAST search algorithms (36) revealed that these sequences were novel.

In order to clone the gene encoding yTAF40, the amino acid sequence of one of the tryptic peptides derived from yTAF40 (peptide 2, K/RQMDIQVISEDQDYVTXX) was used to design degenerate oligonucleotide primer pairs for use in the polymerase chain reaction for amplification of the DNA encoding this peptide. Total yeast cDNA library DNA was used as the template for this PCR reaction. As detailed under “Materials and Methods,” two separate rounds of PCR amplification were performed to be obtained to perform significant quantities of the correct length product. This requirement for two separate rounds of PCR was probably due to the high degree of degeneracy of the oligonucleotides used as primers. The correct length PCR product was cloned, and the nucleotide sequence of the insert was determined. An oligonucleotide derived from this sequence was
then synthesized, labeled, and used as a probe to screen a yeast cDNA library (37) to obtain a full-length cDNA encoding yTAF40. Sequence information derived from positive clones from this screen allowed the preparation of a longer labeled probe, which was then used to screen a yeast genomic library. Sequencing of a positive genomic plasmid clone revealed an ORF of 1038 nucleotides (GenBank accession number U85960). As shown in Fig. 1 this sequence contained all four of the peptides obtained from the tryptic peptides derived from purified yTAF40 and deduced sequence of TAF40. 1741 nucleotides of the sequence containing TAF40 are shown. The yTAF40 ORF extends from nucleotides 352 to 1392. The amino acid sequences obtained from tryptic peptides derived from purified yTAF40 are underlined; translation initiation and termination codons are boxed; and the termination codon is indicated by the asterisk.

Sequence Similarity of yTAF40 to Other Metazoan TAFs—Upon searching more recent editions of sequence data bases with the yTAF40-deduced protein sequence several matches to yeast and other metazoan proteins were noted. The yTAF40 sequence matched \( p \approx 5.8 \times 10^{-200} \) to Protein Information Resource entry S55104, a previously uncharacterized yeast ORF.
Characterization of yTAF40

Interestingly yTAF40 also matched to PIR entries SS4780 (hTAFII28; p = 8.4e-11, 27% identity 67% similarity over yTAF40 residues 122–181) and B49453 (dTAFII30; p = 9.3e-11, 30% identity 68% similarity over yTAF40 residues 122–181). Several other weaker matches were also noted, the most notable being a match of yTAF40 residues 236–305 to human TFIIA α chain polypeptide sequences 280–349. Presented in Fig. 2 is an alignment of yTAF40 to both Drosophila TAFII30β (23) and human TAFII28 (19). Although overall identity between all three of these TAFs is relatively low (only 9% of yTAF40 sequence is identical to these other TAFs), in an 81-amino acid region between yTAF40 residues 111–192 there is 23% identity and 57% similarity among all three TAFs. It is important to note though that yTAF40 is significantly longer than either metazoan TAFII, thus overall identity must be somewhat low. The finding of similarity between yet another yTAF and metazoan TFIIID subunits at the amino acid sequence level is not surprising. In fact all of the yTAFs identified thus far display significant sequence similarity to metazoan TAFs. However, it remains to be determined if these similarities reflect true functional homology. Regardless, the finding that yTAF40 has sequence similarity to TAFII of other species is strong preliminary evidence that yTAF40 is in fact an RNA polymerase II-specific TAF. yTAF40 Is a Single Copy Essential Gene—Prior to performing gene disruption experiments, we examined TAF40 genomic DNA and RNA species to rule out the potential for multiple TAF40 encoding genes. Genomic DNA blots (not shown) indicated that TAF40 is present at a single copy per haploid genome, whereas RNA blots showed that only a single 1.1-kilobase RNA species anneals with TAF40-derived probes (not shown). The size of this mRNA is consistent with the size of the TAF40 ORF. When yeast strain yEK8 was sporulated and the resulting tetrads were dissected, viability segregated 2:2:2:2 as expected. These Trp+ Ura- spore clones were also unable to grow on 5-fluoroorotic acid containing medium (45) indicating a requirement for the TAF40 covering plasmid in the spore clones carrying the taf40Δ::TRP1 null allele. These genetic and biochemical analyses prove that TAF40 is an essential gene. With the exception of yeast TAF30 (ANC1/TFG3) (26), all of the other characterized yeast TAF-encoding genes (TAF170, TAF150, TAF130, TAF90, TAF60, and TAF25) are essential (25, 27, 33). That all of these TAFs are essential underscores the vital role that they must serve in yeast cell function.

yTAF40 Is a Bona Fide yTAF That Is Present in a Multiprotein Complex With TFIIID-specific TAFs—To show that yTAF40 is a bona fide TAF, we utilized the technique of communoprecipitation. If yTAF40 is truly TBP-associated, then it should be detectable in an immunoprecipitate of a WCE treated with antibodies directed against TBP. The converse should also be true in that TBP should be detectable in an anti-yTAF40 im-

![Figure 2. Alignment of the deduced amino acid sequences of yTAF40, dTAFII30β, and hTAFII28. Areas of sequence identity are contained within boxes. Gaps introduced to maximize alignment are indicated by shading. Although overall identity between these TAFs is only 9%, in the 81-amino acid region between yTAF40 residues 111 and 192, there is 23% identity and 57% similarity between these three sequences.](http://www.jbc.org/)

![Figure 3. yTAF40 communoprecipitates with TBP and TFIIID-specific TAFs. Top panel, both HA,TBP and HA,yTAF40 moieties are specifically immunoprecipitable from WCEs prepared from yeast strains expressing the relevant HA-tagged proteins. WCEs were prepared from yeast strains expressing the indicated HA-tagged versions of TBP or TAF. HA,yTAF40 proteins present in the WCEs were immunoprecipitated using anti-HA mAb 12CA5, and the immunoprecipitates were fractionated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane. Proteins containing the HA epitope were detected by blotting with anti-HA mAb 12CA5. The arrows and labels indicate the various epitope-tagged proteins. Bottom panel, yTAF40 is only detectable in anti-HA mAb immunoprecipitates derived from strains expressing HA-tagged proteins that are TFIIID-specific TAFs. Immunoprecipitates were prepared using anti-HA antibody from the WCEs used in the panel above. The immunoprecipitates were fractionated via SDS-PAGE and transferred to a membrane, but in this case the blots were probed with polyclonal anti-yTAF40 IgG. The arrows and associated labels indicate either native yTAF40 or the more slowly migrating HA,yTAF40 polypeptides.](http://www.jbc.org/)
Characterization of yTAF40

A 4\textsuperscript{-}mAb, 0.25; \textit{BUFFER} mAb and are as follows: relative to the amount found in the freshly thawed untreated WCE after detection. The signal on the immunoblot representing yTAF40 is indicated by the antibody (\textit{mAb}+HA PEPTIDE) prior to the addition of WCE to the mAb resin. To determine the extent of immunodepletion, 2 \mu l of the supernatants resulting after removal of immune complexes by centrifugation were compared with 2 \mu l of either freshly thawed WCE (\textit{WCE}) or WCE that had been incubated overnight, the length of time required for \textit{mAb} depletion (\textit{WCE} O/N). These samples were fractionated by SDS-PAGE, transferred to polyvinylidene difluoride, and immunoblotted using monoclonal anti-HA mAb 12CA5 IgG for detection. The signal on the immunoblot representing yTAF40 is indicated by the \textit{label} and \textit{arrow}. The amount of yTAF40 in each sample relative to the amount found in the freshly thawed untreated WCE after subtracting for background was determined using a Bio-Rad Imager and are as follows: WCE, 1.96; \textit{mAb}+HA PEPTIDE, 1.02; \textit{mAb}+\textit{BUFFER}, 0.25; \textit{mAb}, 0.25; \textit{WCE} O/N, 0.98. B, RNA polymerase I-specific transcription assay (25) to assess the effects of yTAF40 depletion on specific transcription. Results of the assay were scored by primer extension analysis. Following the transcription assay a constant amount of a 300-nucleotide \textsuperscript{32}P-labeled DNA fragment (\sim 1000 cpm) was included in all the subsequent steps of the analysis as a recovery control. The autoradiograms presented depict the 50-nucleotide extension product expected for the \textit{rRNA} primary transcript (\textit{labeled RNA Primary Transcript}). Also shown is the 300-nucleotide recovery control (\textit{Recovery Control}) from a representative experiment. After subtracting for background and normalizing to the recovery control the GS-250 Imager units obtained for the basal transcription reactions from each template were as follows: mock-depleted extract 400-nucleotide transcript, 1 \mu l of WCE 155 units, 3 \mu l of WCE 555 units, 5 \mu l of WCE 924 units; 320-nucleotide transcript: 1 \mu l of WCE 177 units, 3 \mu l of WCE 652 units, 5 \mu l of WCE 1161 units. The equivalent values for the depleted extract are: 400-nucleotide transcript: 1 \mu l of WCE 50 units, 3 \mu l of WCE 135 units, 5 \mu l of WCE 246 units; 320-nucleotide transcript: 1 \mu l of WCE 27 units, 3 \mu l of WCE 101 units, 5 \mu l of WCE 169 units. D, specific transcription by RNA polymerase III is not affected by depletion of yTAF40-containing complexes from WCE. Depleted and mock-depleted WCEs were assayed for specific RNA polymerase III transcription using a \textit{tRNA\textsubscript{au}}-encoded gene (50) as the template. The autoradiogram shown displays the size fractionated \textsuperscript{32}P-labeled products of the transcription assay with the primary \textit{tRNA\textsubscript{au}}-encoded gene transcript being indicated by the \textit{arrow} and \textit{label}. Increasing microliter amounts of 5, and 10 \mu l of the indicated WCEs were assayed. The number of GS-250 Imager units following correction for background for the mock-depleted extract were: 3 \mu l of WCE 1609 units; 5 \mu l of WCE 2921 units; and 10 \mu l of WCE 3566 units. For the depleted extract the data were as follows: 3 \mu l of WCE 1509 units; 5 \mu l of WCE 2561 units; and 10 \mu l of WCE 2726 units.
chemical studies suggested that yTAF40 is a subunit of a complex consisting minimally of yTAFs 150, 130, 90, 60, 30, and 25 and TBP (25, 27). As can be seen in Fig. 3 (lower panel, lanes 2–8 versus lane 1), yTAF40 is specifically detected in the immunoprecipitates formed using anti-HA mAb and WCEs derived from yeast strains expressing HA3-tagged TAFs 150, 130, 90, 60, 40, and 25 and TBP. Notably though, yTAF40 is not detected in immunoprecipitates formed from yeast strains expressing HA3-tagged Brf1p (yTAF170) (lane 9) or Mot1p (yTAF170) (lane 10). Both of these TAFs are components of distinct non-TFIID, TBP-TAF-containing complexes (33, 46–48). If the converse experiment is performed, that is immunoprecipitating yTAF170 (and associated polypeptides) from WCEs with polyclonal anti-yTAF40 antibodies and using anti-HA mAb for HA3-TAF and/or HA3-TBP immunodetection, the predicted and complementary result is obtained. In this case yTAF40 is found associated with yTAFs 150, 130, 90, 60, and 25 and TBP but not with either Mot1p or Brf1p (data not shown). That yTAF40 coprecipitates with each of the other known TAFs individually confirms our previous supposition that yTAF40 is in fact part of the yeast TFIID complex that contains all of these TAFs (13, 25, 27, 33).

**yTAF40 Containing Complexes Are Specific to RNA Polymerase II Function**—Having demonstrated that yTAF40 is a bona fide yTAF that is associated with previously characterized yTAF68, our next goal was to test if yTAF40 truly is a TFIID subunit. If this is true then yTAF40 functional activity should be RNA polymerase II-specific. The strategy that we took to test this idea again utilized coimmunoprecipitation. We immunodepleted a yeast WCE of yTAF40 and associated proteins and then used the depleted extracts for *in vitro* transcription assays to measure the ability of control, mock-depleted, and depleted extracts to support specifically initiated transcription by each of the three distinct nuclear DNA-dependent RNA polymerase systems. We reasoned that if yTAF40 was uniquely a subunit of TFIID, then only RNAP II-specific transcription should be altered in yTAF40-depleted WCEs.

Treatment of yeast WCE with the anti-HA mAb 12CA5 removed ~75% of the HA-tagged yTAF40 from the extract (Fig. 4A, compare lanes 3 and 4 with lanes 1, 2, and 5). This depletion was specific because when the anti-HA mAb was precubated with the HA peptide prior to mixing with WCE, this peptide-blocked mAb preparation failed to remove yTAF40 from the WCE (Fig. 4A, compare lane 2 with lanes 3 and 4 versus lane 2 compared with lanes 1 and 5). The ability to block the depletion was specific to the peptide because preincubation of the mAb with peptide buffer alone generated mAb that was fully able to deplete as effectively as untreated mAb alone (compare lanes 3 and 4, Fig. 4A). As a further control there was as much yTAF40 in WCE that underwent overnight incubation as there was in freshly thawed WCE (compare lanes 1 and 5 in Fig. 4A).

When the depleted extracts were tested for their ability to support transcription by RNA polymerase I (Fig. 4B) or RNA polymerase III (Fig. 4D), only minimal effects of yTAF40 depletion were observed. The depleted extract was as competent for specific RNAP I and RNAP III transcription as was the mock-depleted extract (i.e. HA-peptide blocked extract; Fig. 4B, compare lane 1 with lanes 2 and 3; Fig. 4D compare lanes 1–3 with lanes 4–6). In marked contrast, dramatic effects of depletion were observed when the depleted extract was tested for RNA polymerase II transcription. Basal transcription resulting from transcription from the G-minus cassette transcription template for the depleted extract was reduced to ~25% of that observed for the mock-depleted extract. These data clearly demonstrate that whether we deplete a yeast WCE of its TFIID-like TAF-TBP complex by removing yTAF40 (this report) or, as we previously demonstrated for yTAF25 (25), the result as expected is the same, only RNA polymerase II function is aborted. Furthermore, because yTAF40 is associated with yTAF25 (see Fig. 3, lane 8), these data further corroborate the idea that the yTAF25/yTAF40-containing TBP-TAF complex represents the major (if not the only) form of TFIID in yeasts. If this were not the case, then one would not expect such a large proportional decrease in basal RNA polymerase II-specific transcription upon removal of the complex by either yTAF25 or yTAF40 depletion.

In this report we have described the identification, cloning, and characterization of a gene termed *TAF40* that encodes a novel yeast TBP-associated factor, yTAF40. The deduced amino acid sequence of yTAF40 bears significant similarity to that of both human and *Drosophila* TAF ps. The single copy *TAF40* gene is essential for vegetative growth, a property this gene shares with other yeast TAF-encoding genes. We show that yTAF40 protein is TBP-associated and thus a bona fide TAF and that it is a subunit of a yeast TFIID multiprotein TBP-TAFII complex. Through this work we have added to the body of evidence supporting the existence of a discrete yeast TFIID complex by directly demonstrating that the yTAF40-containing TBP-TAF complex is specific to RNA polymerase II function. Further studies are needed to determine the exact composition and function of the TFIID complex.

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Edward R. Klebanow, David Poon, Sharleen Zhou and P. Anthony Weil

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