MOLECULAR AND CELLULAR BIOLOGY, Jan. 1995, p. 534–539 Vol. 15, No. 1
0270-7306/95/$04.00+0

The Yeast TATA-Binding Protein (TBP) Core Domain Assembles with Human TBP-Associated Factors into a Functional TFIID Complex

QIANG ZHOU† AND ARNOLD J. BERK*

Molecular Biology Institute and Department of Microbiology and Molecular Genetics, University of California, Los Angeles, Los Angeles, California 90024-1570

Received 23 May 1994/Returned for modification 10 August 1994/Accepted 13 October 1994

In mammalian and *Drosophila* cells, the central RNA polymerase II general transcription factor TFIID is a multisubunit complex containing the TATA-binding protein (TBP) and TBP-associated factors (TAFs) bound to the conserved TBP carboxy-terminal core domain. TBP also associates with alternative TAFs in these cells to form general transcription factors required for initiation by RNA polymerases I and III. Although extracts of human HeLa cells contain little TBP that is not associated with TAFs, free TBP is readily isolated from yeast cell extracts. However, recent studies indicate that yeast TBP can also interact with other yeast polypeptides to form multiprotein complexes. We established stable human HeLa cell lines expressing yeast TBP and several yeast-human TBP hybrids to study TBP-TAF interactions. We found that the yeast TBP core domain assembles with a complete set of human TAFs into a stable TFIID complex that can support activated transcription in vitro. The fact that the yeast TBP core, which differs from human TBP core in ~20% of its amino acid residues, has the structural features required to form a stable complex with human TAFs implies that *Saccharomyces cerevisiae* probably contains TAFs that are structurally and functionally analogous to human TAFs. Surprisingly, the nonconserved amino terminus of yeast TBP inhibited association between the yeast core domain and human TAFs.

Initiation of transcription by RNA polymerase II requires the TFIID factor, which recognizes the TATA promoter element and nucleates the assembly of an initiation complex containing several other general transcription factors and RNA polymerase II (pol II). In higher eukaryotes, TFIID is a multisubunit protein complex consisting of the TATA-binding protein (TBP) and its associated polypeptides, TBP-associated factors (TAFs) (7, 29, 35). Isolated TBP can function with the other pol II general transcription factors in formation of a preinitiation complex as well as in basal-level in vitro transcription from a pol II promoter, but the TAFs associated with TBP in the TFIID complex are required for transcriptional stimulation by activator proteins (7, 29, 35). Initially identified as a TATA-binding factor essential for pol II transcription, TBP has been demonstrated to be required for transcription by pol I and pol III (11).

The carboxy-terminal 180 amino acids of TBP are phylogenetically conserved and form a protease-resistant domain that binds to TATA elements and mediates basal-level pol II transcription in vitro (13, 18, 22). The amino-terminal portions of TBP, however, vary in length and show little or no conservation among widely divergent eukaryotes. The conserved carboxy-terminal domain of yeast (*Saccharomyces cerevisiae*) TBP is approximately 80% identical to its human counterpart. Molecular genetic studies of *S. cerevisiae* revealed that the nonconserved amino-terminal domain of yeast TBP is not required for viability (5, 9, 23, 25, 36). Consistent with this result, human TAFs required for activated pol II transcription were found to interact with the conserved carboxy-terminal core domain of human TBP (34).

The crystal structures of TBP from *Arabidopsis thaliana* (21) and from *S. cerevisiae* (4) revealed that the TBP conserved carboxy-terminal core domain folds into a novel, symmetric DNA-binding structure composed of two topologically identical halves of 88 to 89 amino acid residues. These two halves, related by an approximate twofold axis, form a saddle-shaped domain that binds to the minor groove of TATA box DNA through the underside of the saddle (16, 17). On the other hand, the TBP amino-terminal domain is connected to one side of the saddle-shaped conserved core, on the top of the saddle. Interestingly, most of the amino acid differences between the human and yeast TBP conserved carboxy termini map to the top and sides of the TBP molecular saddle, which are postulated to interact with other transcription factors (21). Human TBP has been shown to respond to acidic activators in conjunction with the other components of the yeast transcription machinery in vivo (27), although it is unable to functionally replace the endogenous yeast TBP as the sole source of TBP in yeast cells (5, 9).

In contrast to human and *Drosophila* TBP, yeast TBP was thought to exist as a monomeric unit without tightly associated factors (3, 12, 26). However, isolated yeast TBP, like its human and *Drosophila* counterparts, can mediate only basal-level but not activated pol II transcription (1, 15). It appears that additional factors are required for yeast TBP to support activated transcription. Recent studies suggest that a major portion of yeast TBP is associated with other proteins to form complexes in yeast extracts (24). Some of the yeast TBP-associated polypeptides have been identified as subunits of TFIIIB required for pol III transcription. More recently, the *Drosophila* $TAF_{II}150$ was cloned and shown to be highly homologous to an essential yeast gene, *TSM1*, which was found associated with TBP in yeast extracts (31).

* Corresponding author. Mailing address: Molecular Biology Institute, University of California, Los Angeles, CA 90024-1570. Phone: (310) 206-6298. Fax: (310) 206-7286. Electronic mail address: berk@ewald.mbi.ucla.edu.

† Present address: Center for Cancer Research, E17-529, MIT, Cambridge, MA 02139.

To determine whether yeast TBP, with its core domain different from the human TBP core at approximately 20% of all residues, can bind a full set of human TAFs or perhaps only some of the TAFs, we stably expressed an epitope-tagged form of yeast TBP in HeLa cells. Our results indicate that the conserved core domain of yeast TBP assembles with human TAFs into a functional TFIID complex and the nonconserved amino-terminal domain of yeast TBP inhibits this interaction.

MATERIALS AND METHODS

Construction of plasmids. A gene encoding the 12CA5 HA1 epitope (MGY PYDVPDYAV) (8) fused to residues 2 to 240 of yeast TBP was synthesized by PCR amplification of a yeast TBP clone (26). The amplified DNA fragment was inserted into the *Eco*RI site of pSRαMSVtkneo (20) to create pLTReyyyTBP. A similar procedure was followed to fuse the 12CA5 epitope to residues 58 to 240 of yeast TBP to create plasmid pLTReyyTBP for production of the amino-terminally truncated yeast TBP (−yyTBP). To synthesize genes encoding epitope-tagged human-yeast TBP hybrids hyyTBP, yhhTBP, yhyTBP, yyhTBP, hhyTBP, and hyhTBP, the 12CA5 epitope was appended to the second codon of each TBP hybrid by PCR amplification of yeast expression constructs 4, 3, 7, 6, 12, and 11, respectively (5). The amplified fragments were subsequently cloned into the retroviral expression vector.

Construction of HeLa cells expressing wild-type yeast TBP and various human-yeast TBP hybrids. HeLa cell lines expressing wild-type yeast TBP and various human-yeast TBP hybrids were established as described previously (34, 35). Clones were expanded in medium containing 250 μg of G418 per ml and screened by Western immunoblotting of total-cell protein with monoclonal antibody 12CA5. Cell lines were usually maintained in suspension in S-MEM plus 5% calf serum and 250 μg of G418 per ml in a volume of 500 ml. Cells from 5 to 10 liters of cultures were harvested and used for preparation of various TFIID complexes.

Affinity purification of TFIID complexes containing various human-yeast TBP hybrids. Nuclear extracts were prepared from 5 to 10 liters of various cell lines expressing distinct human-yeast TBP chimeras and then fractionated by phosphocellulose chromatography as described previously (6). A 1-ml portion of the undialyzed D (1.0 M KCl) fraction, containing ~2.5 mg of protein per ml, was incubated with 0.1 ml of drained beads of protein A-Sepharose covalently coupled to monoclonal antibody 12CA5 with rotation at 4°C for 6 to 8 h. Further purification steps were performed as described for the eTFIID complex (35).

Transcription assays. To analyze the transcriptional activity of TFIID (hhhTBP), TFIID(hyyTBP), and yeast TBP (yyyTBP) in vitro, 100 ng of template plasmid $pG_5E1BCAT$ (19) was incubated with partially purified general transcription factors in a 50-μl in vitro transcription reaction mixture as described previously (35). $pG_5E1BCAT$ contains five Gal4-binding sites inserted upstream of the adenovirus E1B TATA box and initiation region. Transcripts were quantitated by primer extension as described previously (26) with primers described by Boyer and Berk (2).

RESULTS

Yeast TBP interacts poorly with human TAFs. A series of human-yeast hybrid TBPs were analyzed in this study (see Fig. 2). Each hybrid is designated by a three-letter code indicating the species origin of each of the three regions constituting the hybrids. The first letter in the nomenclature of the chimeras indicates the origin of the sequence N-terminal to the conserved carboxy-terminal core domain. Human-yeast hybrid TBPs containing chimeric carboxy-terminal core domains were further specified by the second and third letters. The fusion of yeast and human TBP sequences to create TBP hybrid core domains was chosen at residue 172 in yeast TBP or residue 266 in human TBP (5), a position that divides the TBP core structure into approximately N-terminal and C-terminal halves (crossover within helix H1′ of the TBP saddle [21]). For example, the yhyTBP chimera is composed of the N-terminal domain of yeast TBP and a hybrid core domain with its N-terminal half containing the human-specific residues and its C-terminal half containing the yeast-specific residues. All the TBPs expressed from the retroviral vector were tagged with a 9-amino-acid residue sequence at their amino terminus that contains an epitope recognized by monoclonal antibody 12CA5 to identify and purify the TBP-containing complexes (8). Where it is helpful, the presence of this epitope tag is designated by the letter e.

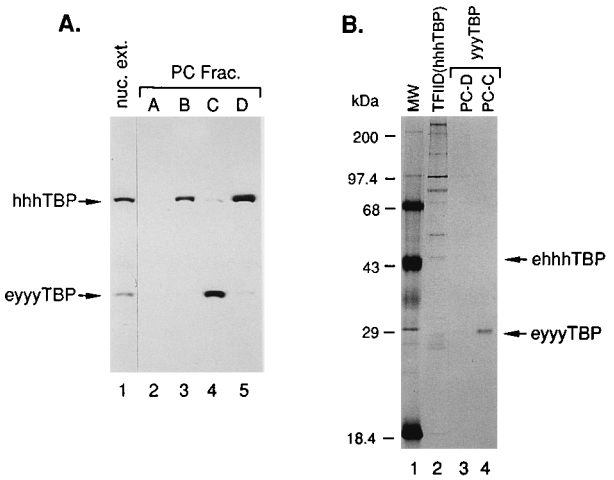

FIG. 1. Yeast TBP interacts very poorly with human TAFs. (A) Nuclear extract (nuc. ext.) (40 μg, lane 1) prepared from eyyyTBP-expressing cell line 4-2 as well as phosphocellulose fractions (PC Frac.) A, B, C, and D (6) (15 μg each, lanes 2 to 5) of this extract were subjected to SDS-polyacrylamide gel electrophoresis and Western blotting with both monoclonal antibody 12CA5 and polyclonal anti-human TBP rabbit serum. The positions of eyyyTBP recognized by 12CA5 and the endogenous human TBP (hhhTBP) recognized by the rabbit antiserum are indicated. (B) Comparison of immunoaffinity purified human TFIID(hhhTBP) complex with eyyyTBP from phosphocellulose C and D fractions of eyyyTBP-expressing cell nuclear extract. A silver-stained SDS–12% polyacrylamide gel is shown. Lanes: 1, molecular mass (MW) markers; 2, wild-type human TFIID(hhhTBP) eluted with HA1 peptide from a monoclonal antibody 12CA5 affinity column incubated with the phosphocellulose D fraction of LTRα3 cell extract (35); 3 and 4, epitope-tagged yeast TBP (eyyyTBP) immunoaffinity purified from the phosphocellulose D and C fractions, respectively, of nuclear extract prepared from eyyyTBP-expressing cell line 4-2.

To determine if yeast TBP can interact with human TAFs, a gene encoding an epitope-tagged yeast TBP (eyyyTBP) was stably introduced into HeLa cells by using a retroviral vector (35). Nuclear extract was prepared and fractionated by chromatography on a phosphocellulose column (6), and the resulting fractions were analyzed by Western blotting with both monoclonal antibody 12CA5, which recognizes eyyyTBP, and polyclonal anti-human TBP antibody, which recognizes the endogenous hhhTBP (Fig. 1). The highest concentration of hhhTBP was present in the D fraction (0.5 to 1.0 M KCl fraction; Fig. 1, lane 5). A significant amount of human TBP was also present in the B fraction (0.1 to 0.3 M KCl, lane 3), probably in the form of the TFIIIB complex required for pol III transcription (11) and B-TFIID of unknown function (30). In contrast to the endogenous hhhTBP, most of the epitope-tagged yeast TBP (eyyyTBP) was found in the C fraction (0.3 to 0.5 M KCl fraction, lane 4), while only a small fraction of eyyyTBP (less than 10%) was present in the D fraction (lane 5). This is the fraction in which isolated human TBP prepared free of TAFs from *Escherichia coli* elutes from phosphocellulose (22, 33a). Therefore, the elution profile of eyyyTBP suggests that the TAF(s) responsible for the relatively salt-stable interaction of the human TFIID complex with the phosphocellulose matrix was probably not associated with the majority of the yeast TBP protein in the extract.

To determine whether eyyyTBP present in the phosphocellulose C and D fractions associated with any human TAFs, eyyyTBP-containing complexes in these two fractions were purified by affinity chromatography on a 12CA5 monoclonal antibody column. The eluted fractions (Fig. 1B, lanes 3 and 4)

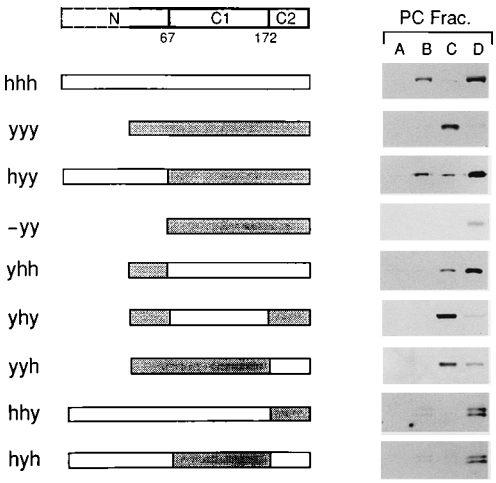

FIG. 2. Wild-type human and yeast TBPs and various yeast-human TBP hybrids are detected in different phosphocellulose fractions. The primary structures of wild-type human TBP (hhh), yeast TBP (yyy), amino-terminally deleted yeast TBP (−yy), and human-yeast TBP hybrids hyy, yhh, yhy, yyh, hhy, and hyh are shown. Yeast and human TBP sequences are represented by shaded and open boxes, respectively. Each hybrid is designated by a three-letter code indicating the species origin of each of the three regions composing the hybrids. The position of the junctions where yeast and human sequences were fused is indicated at the top of the panel, with the numbers referring to amino acid residues in yeast TBP. Quantitative Western blotting analysis of wild-type human and yeast TBPs and various TBP hybrids present in different phosphocellulose fractions is illustrated to the right of the TBP structures. Equal amounts of protein (60 μg per lane for −yy and 15 μg per lane for the rest) from phosphocellulose fractions (PC Frac.) A, B, C, and D were subjected to SDS-polyacrylamide gel electrophoresis followed by Western blotting with monoclonal antibody 12CA5. For TBP hybrids hhy and hyh, the blot was probed with polyclonal anti-human TBP rabbit serum, which recognized both the epitope-tagged TBP hybrids (the upper bands of the doublets) and the endogenous human TBP (the lower bands).

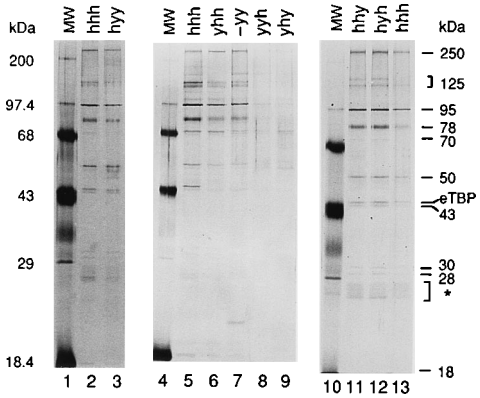

FIG. 3. Comparison of immunoaffinity purified TFIID complexes containing wild-type human TBP and various human-yeast TBP hybrids. TFIID complexes, purified from phosphocellulose D fractions of nuclear extracts prepared from cells expressing wild-type human TBP and various human-yeast TBP hybrids, were subjected to SDS-polyacrylamide gel electrophoresis followed by silver-staining analysis. Lanes 1, 4, and 10 contain molecular mass (MW) markers. Lanes 2, 5, and 13 contain human TFIID complex with wild-type human TBP (hhh) isolated from LTRα3 cells (35). The 21-kDa polypeptide in lane 7 near the bottom of the gel is the epitope-tagged amino-terminal-truncated yeast TBP (−yyTBP). The yhhTBP, yyhTBP, and yhyTBP polypeptides in lanes 6, 8, and 9 are difficult to see in this reproduction. An asterisk (*) marks the position of the light chain of 12CA5 antibody.

were compared with affinity-purified human TFIID (lane 2) by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and silver staining. As observed previously, purified human TFIID complex contained ehhhTBP and at least nine major TAFs (35). In contrast, purified eyyyTBP from the phosphocellulose C fraction appeared to be a single polypeptide without stably associated human TAFs (lane 4). The concentration of eyyyTBP in the D fraction was so low that it was difficult to purify sufficient quantities to characterize its TAF composition with great certainty. The small amount of yeast TBP present in the D fraction (<10% of the total yeast TBP in the nuclear extract) may have been associated with at least some human TAFs, since $TAF_{II}250$ and $TAF_{II}95$ were faintly visible (lane 3). A more darkly silver-stained gel showed that many polypeptides, some of which showed similar electrophoretic mobility to that of human TAFs, were present at low concentrations in this fraction (data not shown). These results indicate that >90% of the eyyyTBP present in stably transformed HeLa cells does not form a stable complex with human TAFs.

The amino-terminal domain of yeast TBP inhibits association of the core domain with human TAFs. To identify the differences in amino acid sequences between human and yeast TBPs responsible for their different affinities for human TAFs, we tested the abilities of various yeast-human TBP hybrids to interact with TAFs when the TBPs were stably expressed in HeLa cells. While the core domains of the yeast and human TBPs are 80% identical, the amino-terminal domains of human and yeast TBPs are completely divergent. Therefore, we first analyzed the TAF-binding ability of ehyyTBP, an epitope-tagged hybrid TBP consisting of the human TBP N-terminal domain and the yeast TBP core domain (Fig. 2). Nuclear extract was prepared from HeLa cells expressing ehyyTBP, fractionated by phosphocellulose chromatography, and analyzed by Western blotting with monoclonal antibody 12CA5 (Fig. 2). The chromatographic behavior of hyyTBP was quite different from that of wild-type yeast TBP (yyyTBP) and similar to that of wild-type human TBP (hhhTBP) (Fig. 2). A multisubunit protein complex containing ehyyTBP affinity purified from the phosphocellulose D fraction contained each of the TAFs found associated with the wild-type human TBP (Fig. 3, lanes 2 and 3). The presence of two additional polypeptides just below $TAF_{II}50$ was not observed consistently.

These results indicate that unlike the majority of wild-type yeast TBP, which does not interact with human TAFs, hyyTBP containing the core domain of yeast TBP and the amino-terminal domain of human TBP is capable of interacting with human TAFs. Since human TAFs interact with the TBP core domain (34), two possible explanations for the interaction between hyyTBP and human TAFs seemed likely: either the unique amino-terminal domain of human TBP stabilized TBP-TAF interactions when attached to the yeast TBP core domain, or the amino-terminal domain of yeast TBP interfered with yeast TBP-TAF interactions. To distinguish between these two possibilities, a cell line expressing a yeast TBP mutant (−yyTBP) with a deletion of nearly the entire yeast TBP amino-terminal domain was generated. In contrast to the full-length yeast TBP (yyyTBP), most of the N-terminally truncated yeast TBP (−yyTBP) was present in the phosphocellulose D fraction, similarly to wild-type human TBP (Fig. 2). The −yyTBP was also detected in the phosphocellulose B fraction after a prolonged exposure of the Western blot (data not shown). The elution profile of −yyTBP suggests that it had become associated with the TAF(s) responsible for the salt-stable interaction of the TFIID complex with phosphocellulose. Indeed, a multisubunit protein complex purified from the phosphocellulose D fraction contained a complete set of human TAFs (Fig. 3, compare lanes 5 and 7). Taken together,

these results demonstrate that the core domain of yeast TBP can form a stable complex with human TAFs and that the nonconserved amino-terminal domain of yeast TBP inhibits the interaction of the yeast TBP core domain with human TAFs.

Yeast-specific residues in the TBP core are required for the yeast TBP N terminus to inhibit association with human TAFs. To determine whether the inhibitory effect of the yeast TBP N-terminal domain is specific for the interaction of the yeast TBP core domain with human TAFs, a cell line expressing eyhhTBP, which contains the human TBP core domain fused to the yeast TBP N-terminal domain, was established. Western blotting with 12CA5 (Fig. 2) indicated that unlike wild-type yeast TBP, a higher concentration of yhhTBP was detected in the phosphocellulose D fraction than in the C fraction of nuclear extract prepared from HeLa cells stably expressing the chimeric protein. Furthermore, a multisubunit protein complex containing yhhTBP purified from the D fraction contained a complete set of human TAFs (Fig. 3, lane 6). These results indicate that although the yeast TBP amino-terminal domain inhibits the association of the yeast TBP core domain with human TAFs, it has a much weaker effect on the interaction between the human TBP core domain and TAFs.

Additional yeast-human TBP hybrids containing chimeric carboxy-terminal core domains were analyzed (Fig. 2). Phosphocellulose fractions prepared from stably transformed HeLa cells expressing these chimeric yeast-human TBPs were analyzed by Western blotting with monoclonal antibody 12CA5 to detect the epitope tag on each of the chimeric proteins (Fig. 2). Both yhyTBP and yyhTBP were found predominantly in the C fraction, with a small amount also found in the D fraction. When the D fractions from both cell lines were subjected to 12CA5 immunoaffinity purification, small amounts of human TAFs were found associated with both yhyTBP and yyhTBP (Fig. 3, lanes 8 and 9). More yhyTBP and yyhTBP appeared to complex with TAFs than did wild-type yeast TBP, suggesting a slight increase in their affinities for human TAFs. Nevertheless, neither yhyTBP nor yyhTBP could interact with human TAFs as well as did yhhTBP, which contains an intact human TBP core domain. Taken together, these results indicate that the yeast TBP N-terminal domain inhibits TAF binding by the yeast core domain more strongly than by the human core domain, with the hybrid core domains exhibiting intermediate properties.

TAF association was also analyzed for TBPs with chimeric core domains associated with the human N-terminal domain. Both hyhTBP and hhyTBP were found mostly in the phosphocellulose D fraction, cofractionating with the endogenous human TBP. This was demonstrated by Western analysis with an anti-human TBP rabbit antibody (Fig. 2) that recognizes both the endogenous human TBP (lower bands of the doublets) and TBP hybrids (upper bands), which migrate more slowly because of the additional ~1,000 Da contributed by the epitope tag. In addition, multisubunit TFIID complexes containing either hhyTBP (Fig. 3, lane 11) or hyhTBP (lane 12) and a complete set of TAFs were isolated from cells expressing these two TBP hybrids, indicating that both proteins are capable of assembling human TAFs into TFIID complexes.

Multisubunit TFIID complex containing hyyTBP supports *trans* activation by Gal4-AH. Reconstituted transcription reactions were carried out as described previously (35) to test whether the multisubunit TFIID complex containing hyyTBP and a complete set of human TAFs could support *trans* activation by Gal4-AH (10) in conjunction with other human general transcription factors and RNA polymerase II. The yyyTBP affinity purified from the phosphocellulose C fraction was also analyzed, as well as TFIID containing wild-type human TBP as a positive control. The template, $pG_5E1BCAT$ (19), contained five Gal4-binding sites cloned upstream of the adenovirus E1B TATA box and initiation region.

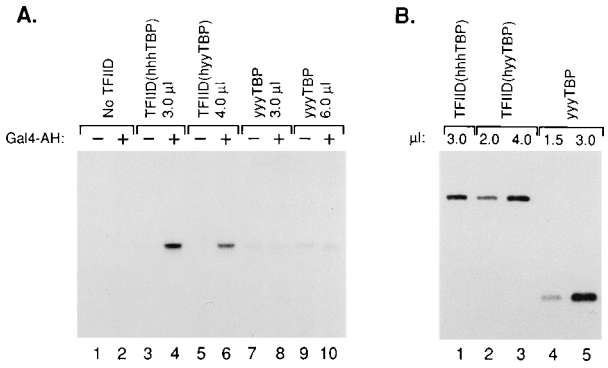

FIG. 4. TFIID complex containing hyyTBP [TFIID(hyyTBP)] supports *trans* activation by Gal4-AH. (A) Reconstituted in vitro transcription reactions were carried out in the absence (−) or presence (+) of Gal4-AH with the indicated sources of TFIID activity. The template contains five Gal4-binding sites upstream of the adenovirus E1B TATA box and initiation region. Epitope-tagged yeast TBP (yyyTBP) was affinity purified from the phosphocellulose C fraction of the nuclear extract prepared from cell line yyy4-2. Transcripts were isolated and analyzed by primer extension assays. (B) The amounts of wild-type TFIID (hhhTBP), TFIID(hyyTBP), and yeast TBP (yyyTBP) included in the transcription reactions in panel A were analyzed by Western blotting with monoclonal antibody 12CA5, which recognizes the epitope-tagged yeast TBP as well as the epitope-tagged hhhTBP or hyyTBP subunit present in the TFIID complexes.

In reactions lacking any source of TFIID activity, no specific transcription could be detected (Fig. 4A, lanes 1 and 2). Yeast TBP (yyyTBP) purified from the phosphocellulose C fraction supported detectable levels of basal transcription (lanes 7 and 9) in the absence of Gal4-AH, but no *trans* activation by Gal4-AH (lanes 8 and 10). This was expected since yeast TBP present in the phosphocellulose C fraction was not associated with TAFs which are required for *trans* activation (Fig. 1). On the other hand, addition of the multisubunit complex [TFIID (hyyTBP)] containing hyyTBP and its associated TAFs to the reactions supported transcription activation by Gal4-AH (lanes 5 and 6). The amount of transcript produced was approximately half that observed with a comparable amount (Fig. 4B) of the TFIID(hhhTBP) fraction containing wild-type human TBP (Fig. 4A, compare lanes 4 and 6). These results indicate that the yeast TBP core domain can support activated transcription when it is associated with human TAFs in the hyy chimeric TBP molecule.

DISCUSSION

We analyzed interactions between human TAFs and both yeast TBP and yeast-human TBP chimeras to gain insights into TAF-TBP interactions. We found that the conserved carboxy-terminal core domain of yeast TBP can assemble with human TAFs into a TFIID complex that is capable of supporting *trans* activation in vitro. Full-length yeast TBP, however, did not associate efficiently with human TAFs in stably transformed HeLa cells, because of the inhibition of TAF binding by the yeast TBP nonconserved N-terminal domain. TAFs required for activated transcription were shown previously to interact with the human TBP conserved core domain (34). We found here that the core domain of yeast TBP, which differs from the human TBP core domain at ~20% of all residues, nonetheless binds the full set of human TAFs when the inhibitory N-terminal domain is deleted. This conservation of function adds

to a growing list of observations suggesting that *S. cerevisiae* also makes use of TAFs that make conserved interactions with the yeast TBP core domain to mediate transcription by RNA polymerase II. Polypeptides associated with yeast TBP have been described previously (24). The deduced primary amino acid sequence of a *Drosophila melanogaster* TAF, $TAF_{II}150$, revealed a strong similarity to the essential yeast gene TSM-1, which was shown to behave like a TAF and interact with yeast TBP in vivo (31). Since the core domain of yeast TBP interacts with the full set of human TAFs, it is very likely that there are yeast TAFs in addition to TSM-1 which are structurally and functionally similar to *D. melanogaster* and human TAFs.

Yeast-human TBP hybrids bound either all of the TAFs or none of the TAFs. No partial TFIID complexes were observed in which only some of the TAFs were bound. This "all-or-none" mode of interaction between TBP and TAFs probably reflects an important architectural feature of the TFIID complex. We (34) and others (28, 32) have previously found that the two largest human TAFs ($TAF_{II}250$ and $TAF_{II}125$) contact TBP directly. *D. melanogaster* $TAF_{II}30\alpha$ was also recently shown to bind directly to TBP (33). These TAFs plus the remaining TFIID-TAFs probably associated with each other tightly so that interference with the binding of one TAF by the yeast TBP N terminus interferes with binding of the entire TAF complex.

Keaveney et al. (14) recently described a transient-transfection assay that allowed them to assay the ability of TBPs expressed from transfected genes to support activated transcription in mammalian cells. Full-length yeast TBP supported *trans* activation in their assays, suggesting that it was interacting with endogenous human TAFs. The apparent discrepancy between their results and the studies reported here probably results from significant differences in the transient-transfection assay compared with the assay presented here involving stably transformed cells. Expression of yeast TBP from the strong simian virus 40 promoter on a replicating plasmid in COS cells probably resulted in much higher levels of expression than in our stably transformed cells, which expressed yeast TBP at levels comparable to the very low level of endogenous TBP. In our stably transformed HeLa cells, ~10% of the yeast TBP associated with human TAFs. Yeast TBP might be better able to compete with human TBP for binding to TAFs when expressed at significantly higher levels in the transient-transfection assay.

The inhibition of TAF binding by the yeast TBP N-terminal domain was largely overcome when it was associated with the human core domain. Yeast-human TBP hybrid core domains bound TAFs to intermediate levels when linked to the inhibitory yeast N terminus. The ability of the human core domain to overcome inhibition of TAF binding by the yeast N terminus probably results from additional contacts between the human TAF complex and the human TBP core domain compared with the yeast core domain. It is also possible that the yeast TBP N terminus interacts with the yeast core domain, masking sites in the core that interact with TAFs. The yeast N terminus might interact with and mask the yeast core domain more strongly than the human core. Examination of crystal structures of yeast and *A. thaliara* TBP bound to TATA box DNA shows that the TBP amino-terminal domain is connected to one side of the saddle-shaped conserved carboxy-terminal core domain, on the top of the saddle (16, 17). Since TAF binding is inhibited by the yeast N-terminal domain, it is likely that the TAF complex interacts with the surface of TBP near this region.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant CA25235 from the National Cancer Institute.

We are grateful to Kevin Struhl for sending us plasmids containing the yeast-human TBP coding regions. We thank Carol Eng for technical assistance.

REFERENCES

1. **Berger, S. L., W. D. Cress, A. Cress, S. J. Triezenberg, and L. Guarente.** 1990. Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell **61:**1199–1208.
2. **Boyer, T., and A. J. Berk.** 1993. Functional interactions of adenovirus E1A with holo-TFIID. Genes Dev. **7:**1810–1823.
3. **Buratowski, S. P., S. Hahn, P. A. Sharp, and L. Guarente.** 1988. Function of a yeast TATA element-binding protein in a mammalian transcription system. Nature (London) **334:**37–42.
4. **Chasman, D. I., K. M. Flaherty, P. A. Sharp, and R. D. Kornberg.** 1993. Crystal structure of yeast TATA-binding protein and model for interaction with DNA. Proc. Natl. Acad. Sci. USA **90:**8174–8178.
5. **Cormack, B. P., M. Strubin, A. S. Ponticelli, and K. Struhl.** 1991. Functional differences between yeast and human TFIID are localized to the highly conserved region. Cell **65:**341–348.
6. **Dignam, J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11:**1475–1489.
7. **Dynlacht, B. D., T. Hoey, and R. Tjian.** 1991. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. Cell **66:**563–576.
8. **Field, J., J.-I. Nikawa, D. Broek, B. MacDonald, L. Rodgers, I. A. Wilson, R. A. Lerner, and M. Wigler.** 1988. Purification of a *RAS*-responsive adenylyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. Mol. Cell. Biol. **8:**2159–2165.
9. **Gill, G., and R. Tjian.** 1991. A highly conserved domain of TFIID displays species specificity in vivo. Cell **65:**333–340.
10. **Giniger, E., and M. Ptashne.** 1987. Transcription in yeast activated by a putative amphipathic α helix linked to a DNA binding unit. Nature (London) **330:**670–672.
11. **Hernandez, N.** 1993. TBP, a universal eukaryotic transcription factor? Genes Dev. **7:**1291–1308.
12. **Horikoshi, M., C. K. Wang, H. Fujii, J. A. Cromlish, P. A. Weil, and R. G. Roeder.** 1989. Purification of a yeast TATA box-binding protein that exhibits human transcription factor IID activity. Proc. Natl. Acad. Sci. USA **86:**4843–4847.
13. **Horikoshi, M., T. Yamamoto, Y. Ohkuma, P. A. Weil, and R. G. Roeder.** 1990. Analysis of structure-function relationships of yeast TATA box binding factor TFIID. Cell **61:**1171–1178.
14. **Keaveney, M., A. Berkenstam, M. Feigenbutz, G. Vriend, and H. G. Stunnenberg.** 1993. Residues in the TATA-binding protein required to mediate a transcriptional response to retinoic acid in EC cells. Nature (London) **365:**562–566.
15. **Kelleher, R. J., P. M. Flanagan, and R. D. Kornberg.** 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. Cell **61:**1209–1215.
16. **Kim, J. L., D. B. Nikolov, and S. K. Burley.** 1993. Co-crystal structure of TBP recognizing the minor groove of a TATA element. Nature (London) **365:**520–527.
17. **Kim, Y., J. H. Geiger, S. Hahn, and P. B. Sigler.** 1993. Crystal structure of a yeast TBP/TATA-box complex. Nature (London) **365:**512–520.
18. **Lieberman, P. M., M. C. Schmidt, C. C. Kao, and A. J. Berk.** 1991. Two distinct domains in the yeast transcription factor IID and evidence for a TATA box-induced conformational change. Mol. Cell. Biol. **11:**63–74.
19. **Lillie, J. W., and M. R. Green.** 1989. Transcription activation by the adenovirus E1a protein. Nature (London) **338:**39–44.
20. **Muller, A. J., J. C. Young, A. M. Pendergast, M. Pondel, N. R. Landau, D. R. Littman, and O. N. Witte.** 1991. BCR first exon sequences specifically activate BCR/ABL tyrosine oncogenes of Philadelphia chromosome-positive human leukemias. Mol. Cell. Biol. **11:**1785–1792.
21. **Nikolov, D. B., S.-H. Hu, J. Lin, A. Gasch, A. Hoffman, M. Horikoshi, N.-H. Chua, R. G. Roeder, and S. K. Burley.** 1992. Crystal structure of TFIID TATA-box binding protein. Nature (London) **360:**40–46.
22. **Peterson, M. G., N. Tanese, B. F. Pugh, and R. Tjian.** 1990. Functional domains and upstream activation properties of cloned human TATA binding protein. Science **248:**1625–1630.
23. **Poon, D., S. Schroeder, C. K. Wang, T. Yamamoto, M. Horikoshi, R. G. Roeder, and P. A. Weil.** 1991. The conserved carboxy-terminal domain of *Saccharomyces cerevisiae* TFIID is sufficient to support normal cell growth. Mol. Cell. Biol. **11:**4809–4821.
24. **Poon, D., and P. A. Weil.** 1993. Immunopurification of yeast TATA-binding protein and associated factors: presence of transcription factor IIIB transcriptional activity. J. Biol. Chem. **268:**15325–15328.
25. **Reddy, P., and S. Hahn.** 1991. Dominant negative mutations in yeast TFIID define a bipartite DNA-binding region. Cell **65:**349–351.
26. **Schmidt, M. C., C. C. Kao, R. Pei, and A. J. Berk.** 1989. Yeast TATA-box transcription factor gene. Proc. Natl. Acad. Sci. USA **86:**7785–7789.

27. **Strubin, M., and K. Struhl.** 1992. Yeast and human TFIID with altered DNA-binding specificity for TATA elements. Cell **68:**721–730.
28. **Takada, R., Y. Nakatani, A. Hoffmann, T. Kokubo, S. Hasegawa, R. G. Roeder, and M. Horikoshi.** 1992. Identification of human TFIID components and direct interaction between a 250-kDa polypeptide and the TATA box-binding protein (TFIIDτ). Proc. Natl. Acad. Sci. USA **89:**11809–11813.
29. **Tanese, N., B. F. Pugh, and R. Tjian.** 1991. Coactivators for a proline activator purified from the multisubunit human TFIID complex. Genes Dev. **5:**2212–2224.
30. **Timmers, H. T. M., R. E. Meyers, and P. A. Sharp.** 1992. Composition of transcription factor B-TFIID. Proc. Natl. Acad. Sci. USA **89:**8140–8144.
31. **Verrijzer, C. P., K. Yokomori, J.-L. Chen, and R. Tjian.** 1994. *Drosophila* $TAF_{II}150$: similarity to yeast gene TSM-1 and specific binding to core promoter DNA. Science **264:**933–941.
32. **Weinzierl, R. O. J., B. D. Dynlacht, and R. Tjian.** 1993. Largest subunit of *Drosophila* transcription factor IID directs assembly of a complex containing TBP and a coactivator. Nature (London) **362:**511–517.
33. **Yokomori, K., J.-L. Chen, A. Admon, S. Zhou, and R. Tjian.** 1993. Molecular cloning and characterization of $dTAF_{II}30\alpha$ and $dTAF_{II}30\beta$: two small subunits of *Drosophila* TFIID. Genes Dev. **7:**2587–2597.
33a. **Zhou, Q., and A. J. Berk.** Unpublished observations.
34. **Zhou, Q., T. G. Boyer, and A. J. Berk.** 1993. Factors (TAFs) required for activated transcription interact with TATA box-binding protein conserved core domain. Genes Dev. **7:**180–187.
35. **Zhou, Q., P. M. Lieberman, T. G. Boyer, and A. J. Berk.** 1992. Holo-TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. Genes Dev. **6:**1964–1974.
36. **Zhou, Q., M. C. Schmidt, and A. J. Berk.** 1991. Requirement of acidic amino acid residues immediately amino-terminal to the conserved domain of Saccharomyces cerevisiae TFIID. EMBO J. **10:**1843–1852.