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Molecular cloning and analysis of two subunits of the human TFIID complex: hTAF$_{II}$130 and hTAF$_{II}$100

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ABSTRACT **Transcription factor TFIID is a multiprotein complex composed of the TATA box-binding protein (TBP) and multiple TBP-associated factors (TAFs). TFIID plays an essential role in mediating transcriptional activation by gene-specific activators. Numerous transcriptional activators have been characterized from mammalian cells; however, molecular analysis of the components of mammalian TFIID has been incomplete. Here we describe isolation of cDNAs encoding two TAF subunits of the human transcription factor TFIID. The first cDNA is predicted to encode the C-terminal 947 residues of the 130-kDa human TAF subunit, hTAF$_{II}$130. The second cDNA encodes the C-terminal 801 residues of the 100-kDa subunit, hTAF$_{II}$100. Recombinant TAFs expressed in human cells by transient transfections are capable of associating with the endogenous TAFs and TBP to form a TFIID complex *in vivo*. Protein binding experiments demonstrate that hTAF$_{II}$130, like its *Drosophila* homolog dTAF$_{II}$110, interacts with the glutamine-rich activation domains of the human transcription factor Sp1. Furthermore, hTAF$_{II}$130 shows reduced binding to the Sp1 mutants with impaired ability to activate transcription, suggesting a role for hTAF$_{II}$130 as a direct coactivator target for Sp1.**

Transcriptional regulation of the genes transcribed by eukaryotic RNA polymerase II is a complex process that requires concerted action of promoter-specific transcription factors and the general transcription machinery (reviewed in refs. 1–3). In response to extracellular and intracellular signals, promoter-specific transcription factors bind to specific target DNA sequences and affect transcription. Recent research has focused on the mechanisms by which the DNA-binding transcriptional activators modulate transcription initiation. A search for proteins that might serve as their targets has revealed a class of transcription factors termed coactivators that function as intermediary factors between DNA-bound transcription factors and the components of the basal machinery. One such target is TFIID, a multiprotein complex that consists of the TATA box-binding protein (TBP) and at least eight TBP-associated factors, or TAFs. TAFs have been shown to directly bind to selected activators and are thought to mediate activator-dependent stimulation of transcription (4–7).

The *Drosophila* dTAF$_{II}$110, one of the first TAF$_{II}$s to be cloned, interacts directly with the human transcription factor Sp1 (5). Subsequent *in vitro* transcription studies using a partial TFIID complex reconstituted with dTBP, dTAF$_{II}$250, dTAF$_{II}$150, and dTAF$_{II}$110 demonstrated that dTAF$_{II}$110 functions as a coactivator to mediate Sp1-dependent activation of transcription (4). Most importantly, it was shown that the interaction between dTAF$_{II}$110 and Sp1 is mediated by the glutamine-rich activation domain of Sp1 and that mutations in this domain that decrease the ability of Sp1 to activate transcription also decrease its ability to interact with dTAF$_{II}$110 (8), lending further support to the notion that dTAF$_{II}$110 serves as the coactivator for Sp1.

To compare the activities of human and *Drosophila* transcription factors, we set out to isolate the cDNA clones encoding a subset of human TAF proteins. Here we report the isolation and characterization of cDNAs encoding human TAF$_{II}$130 and human TAF$_{II}$100.

MATERIALS AND METHODS

Protein Preparation and Microsequencing. Nuclear extracts were prepared from 200–250 liters of α3 cells, a HeLa cell line stably transformed with hemagglutinin antigen (HA)-tagged human TBP (9), and TFIID complex immunopurified with α-HA and α-TBP antibodies (10). TBP and TAFs were eluted with HA peptide, resolved on a 6–12% gradient SDS/polyacrylamide gel, and blotted onto a poly(vinylidene difluoride) membrane. Specific bands corresponding to TAFs were visualized by staining with Ponceau S, excised, and digested with trypsin (11). Peptide sequences were obtained from the tryptic digests eluted from the membrane.

Preparation of Probes for cDNA Library Screening. Degenerate oligonucleotides were synthesized based on the microsequencing data and used in a PCR reaction to amplify the corresponding cDNA fragment from a library of cDNAs. In the case of hTAF$_{II}$130, two sets of degenerate PCR primers were designed corresponding to each end of a long peptide sequence (pep4, see Fig. 2*A*). An amplified product (40 nucleotides) was obtained in a PCR reaction using cDNA prepared from HeLa poly(A)$^+$ RNA. In the case of hTAF$_{II}$100, two peptides (pep5 and pep6, see Fig. 3) were found to be nearly identical in sequence to those found in dTAF$_{II}$80. Degenerate oligonucleotides designed from these peptides (which were 13 amino acids apart in dTAF$_{II}$80) were used to amplify a cDNA fragment that spanned between the two peptides. The cDNA fragments thus isolated were used as probes to screen multiple human cDNA libraries. Positive clones for both TAFs were obtained from screening λgt10 and λZAPII HeLa cell cDNA libraries (12) and the NTera2D1 line of human teratocarcinoma cDNA library (13).

***In Vivo* TFIID Complex Assembly Assay for Recombinant TAFs.** The procedure was carried out as described (14). hTAF$_{II}$100 and hTAF$_{II}$130 cDNAs were tagged with an HA epitope and transfected by calcium-phosphate coprecipitation into human embryonic kidney 293 cells under the control of a cytomegalovirus promoter. Mininuclear extracts were prepared from transfected cells (15) and TFIID immunopurified with a polyclonal α-TBP antibody. Proteins separated by

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Abbreviations: TBP, TATA-box binding protein; TAF, TBP-associated factor; HA, hemagglutinin antigen.

Data deposition: The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U75308 and U75309).

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SDS/PAGE were immunoblotted with the α-HA monoclonal antibody (12CA5) to detect for the presence of the recombinant protein.

***In Vitro* Protein Binding Studies.** Sp1 DNA affinity columns were prepared by coupling biotinylated oligonucleotides containing multiple Sp1 binding sites. Sp1 prepared from recombinant vaccinia virus-infected HeLa cells (4) was bound to the DNA affinity resin and incubated with *in vitro*-translated radiolabeled $hTAF_{II}130$. HA-tagged $hTAF_{II}130N/C$ was expressed from a pET expression vector in *Escherichia coli* by subcloning downstream from the HA sequence an *Nde*I–*Cla*I fragment derived from the central portion of $hTAF_{II}130$ (*Nde*I site introduced at residue 270 by PCR; *Cla*I present at residue 700). HA-$hTAF_{II}130N/C$ was purified and bound to an α-HA antibody resin. A control resin was prepared with an *E. coli* extract harboring the pET-HA expression vector alone. The resins were incubated with a crude HeLa cell nuclear extract (16) and proteins in the bound fraction were analyzed by SDS/PAGE and immunoblotting with α-Sp1 antibody to detect for binding of the endogenous Sp1.

Yeast Two-Hybrid Methods. The *Nde*I–*Cla*I fragment of $hTAF_{II}130$ was subcloned in-frame into the yeast pAS1 vector (17) to generate G4-$hTAF_{II}130N/C$. Plasmids expressing fusions of Sp1 to the acidic activation domain of GAL4 (8) were cotransformed with G4-$hTAF_{II}130N/C$ into yeast strains (Y153 and Y526) and assayed for β-galactosidase activity (5). For studies using the mutant Sp1 constructs, pGAD plasmids carrying deletions or a linker substitution mutation (M37) in Sp1 domain Bc (8) were cotransformed with a pEG202 plasmid (18) expressing $hTAF_{II}130N/C$ into the yeast strain W303 (18). Quantitative β-galactosidase assays (19) were performed in triplicate.

RESULTS

Cloning of the cDNAs Encoding Human $TAF_{II}130$ and $TAF_{II}100$. To obtain the cDNAs corresponding to the subunits of the human TFIID complex we immuopurified TFIID from HeLa α3 cells and separated the components by SDS/PAGE (Fig. 1). Multiple tryptic peptides were obtained from the protein bands corresponding to $hTAF_{II}130$ and $hTAF_{II}100$. Microsequencing of the purified peptides allowed us to design degenerate oligonucleotides for PCR amplification of the corresponding cDNA sequences. Four independent cDNA clones were isolated for $hTAF_{II}130$ from the λZAPII HeLa cDNA library. The longest $hTAF_{II}130$ cDNA (clone 3a2) was 4.2 kb in length with 2.8 kb of open reading frame encoding 947 amino acids (Fig. 2) and 1.4 kb of 3′ untranslated sequence. The 5′ end of the remaining three cDNAs were found to be ≈1 kb downstream of the 5′ end of clone 3a2. Based on the Northern blot analysis and comparison of the gel mobility of the *in vitro*-translated recombinant product and the native protein, clone 3a2 appears to be missing the N-terminal ≈100 amino acid residues of $hTAF_{II}130$ (data not shown). Multiple attempts to obtain the 5′ end of the cDNA have failed because of the extraordinarily high GC content of the gene in this region (80% G+C in the first 1 kb of cDNA). However, we are confident that the unusual cDNA sequence in clone 3a2 is genuine since we have isolated genomic $hTAF_{II}130$ clones that include this region (data not shown).

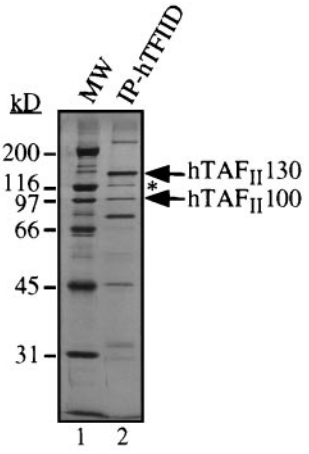

FIG. 1. TFIID complex immunopurified from HeLa cells. $hTAF_{II}$s and hTBP were separated by SDS/PAGE and stained with silver. Positions of $hTAF_{II}130$ and $hTAF_{II}100$ are indicated. A 110-kDa $hTAF_{II}$ protein immunologically related to $hTAF_{II}130$ is indicated by an asterisk (*). We have obtained one peptide from this protein whose sequence is found in the predicted amino acid sequence of $hTAF_{II}130$, suggesting that the 110-kDa $hTAF_{II}$ is a derivative or a breakdown product of $hTAF_{II}130$.

Multiple independent cDNAs were also isolated for $hTAF_{II}100$ from the λgt10 HeLa cell library and the NTera cDNA library. The longest cDNA obtained from the NTera library was 2.4 kb in length, and included an open reading frame encoding 801 amino acids (Fig. 3) with a very short 3′ untranslated sequence of 19 nucleotides. In the recently reported cDNA sequence of $hTAF_{II}100$, the authors suggest that a methionine equivalent to residue 2 in our predicted amino acid sequence (Fig. 3) is the first codon of the $hTAF_{II}100$ protein (20). However, neither their reported cDNA sequence nor ours contain an in-frame stop codon upstream of the methionine in question. Since the N-terminal amino acid sequence obtained from sequencing the endogenous $hTAF_{II}100$ protein is different from the N-terminal sequence predicted by the cloned $hTAF_{II}100$ (S. Zhou and R. Tjian, personal communication), this methionine (position 2 in Fig. 3) may not be the N-terminal methionine of $hTAF_{II}100$. We have also made multiple attempts to extend the current $hTAF_{II}100$ cDNA to include the complete 5′ end. Since the translated product of the cDNA is similar in size to the endogenous protein, the missing cDNA sequence is probably very short.

The identity of the cDNA clones was confirmed by the presence of multiple peptide sequences obtained from microsequencing (Figs. 2*A* and 3). In addition, the cDNAs were translated *in vitro* and the protein products found to react with monoclonal antibodies raised against native human TAF_{II}s (data not shown). Comparison of the sequences of $hTAF_{II}130$ and the previously reported *Drosophila* $TAF_{II}110$ (5) is shown (Fig. 2 *B* and *C*). Two regions (designated I and II) share a high degree of sequence similarity, suggesting that $hTAF_{II}130$ is a true homolog of $dTAF_{II}110$. Region I (residues 449–528) is an 80 amino acid stretch within the central domain of $hTAF_{II}130$ that shares 46% identity (68% similarity) with $dTAF_{II}110$. Interestingly, a data base search found a similar region present within the putative transcription factor ETO/MTG8 that is associated with a chromosomal translocation in acute myelogenous leukemia (21, 22). *Nervy*, a *Drosophila* homolog of *ETO*, has also been cloned and shares extensive sequence similarity (31% identity over 71 residues) to the corresponding region I of $dTAF_{II}110$ (23). These findings suggest that region I represents a domain that has been conserved through evolution in multiple proteins involved in transcriptional regulation.

Region II (residues 689–947) located near the C terminus of $hTAF_{II}130$ also shows a high degree of sequence similarity with $dTAF_{II}110$ (55% identity, 72% similarity over 259 amino acid residues). This region of $dTAF_{II}110$ was shown to interact with $dTAF_{II}30\alpha$ (24). Besides regions I and II, the remaining $hTAF_{II}130$ and $dTAF_{II}110$ show little similarities except for multiple glutamine-rich domains present in both TAF_{II}s. Finally, the N-terminal 200 amino acids of $hTAF_{II}130$ are rich in glycine, proline, and alanine residues. This region is absent from the N terminus of $dTAF_{II}110$, suggesting a function unique to $hTAF_{II}130$.

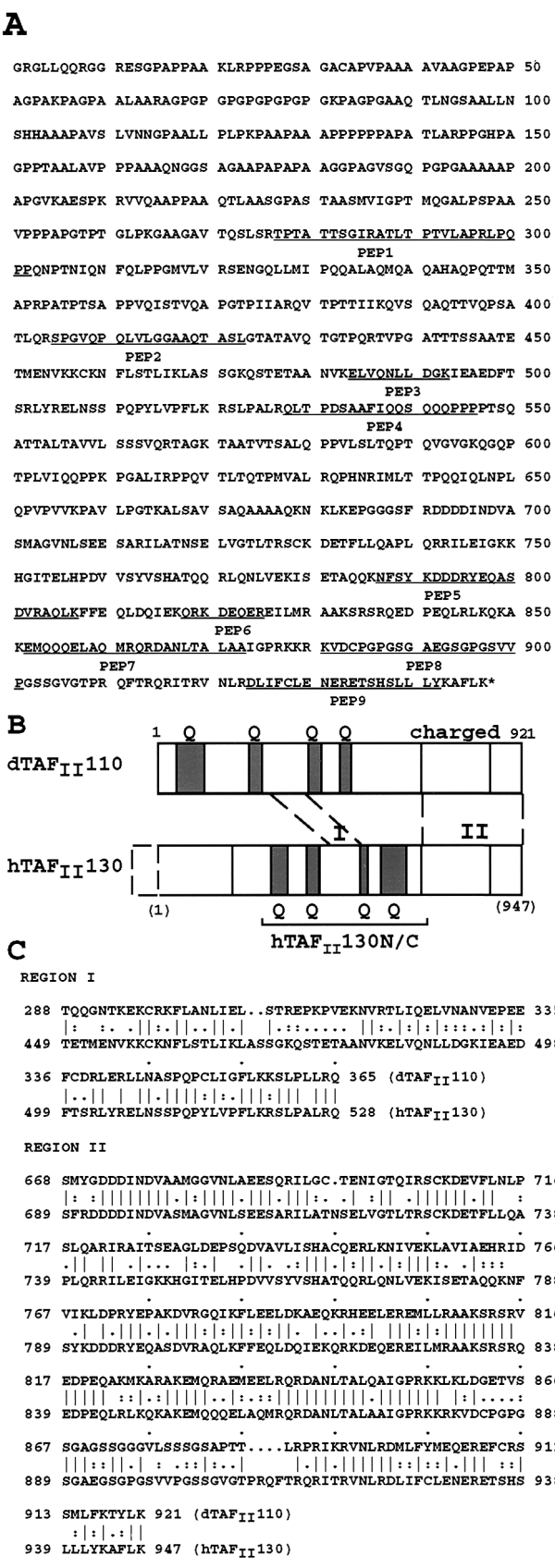

FIG. 2. (*A*) Predicted C-terminal 947 amino acid sequence of hTAF$_{II}$130. Peptides obtained from microsequencing of immunopurified hTAF$_{II}$130 protein are underlined. (*B*) Schematic comparison of hTAF$_{II}$130 with *Drosophila* dTAF$_{II}$110. Region I (residues 449–528): 68% sequence similarity (46% identity) over 80 residues. Region II (residues 689–947): 72% similarity (55% identity) over 259 residues as determined by the BESTFIT sequence analysis program (Genetics Computer Group, Madison, WI). Glutamine-rich regions (presence of 20–30% glutamines in hTAF$_{II}$130) are shaded and denoted by Q. hTAF$_{II}$130N/C subdomain used in protein binding assays is indicated by a bracket. Dotted box represents the predicted missing N-terminal region. (*C*) Amino acid sequence alignment of conserved residues within regions I and II of dTAF$_{II}$110 and hTAF$_{II}$130.

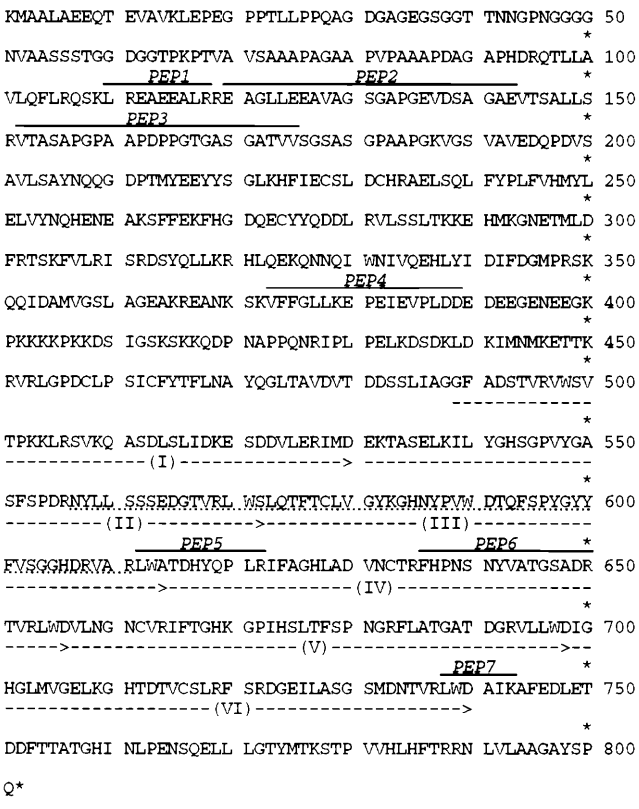

FIG. 3. Predicted C-terminal 801 amino acid sequence of hTAF$_{II}$100. Peptide sequences obtained from microsequencing the endogenous hTAF$_{II}$100 are indicated above the amino acid sequence. The dotted line below residues 557–611 represents 55 amino acids missing from a spliced variant of hTAF$_{II}$100 isolated from the NTera cDNA library. The six WD repeats are indicated by the dotted arrows.

The predicted sequence for hTAF$_{II}$100 (Fig. 3) is very similar to the sequence of *Drosophila* TAF$_{II}$80 (25, 26) and yeast TAF$_{II}$90 (27, 28), again indicating that they are true homologs. The C-terminal 730 amino acids of hTAF$_{II}$100 share 44% identity (65% similarity) with dTAF$_{II}$80 and 35% identity (57% similarity) with yTAF$_{II}$90. All three TAFs contain at least six WD repeats (Fig. 3) that are found in proteins of diverse biological function. The recently solved crystal structure of the β-subunit of the G protein transducin suggests that proteins containing WD repeats form β-propellers (29, 30). Interestingly, we have isolated at least one spliced form of hTAF$_{II}$100 from the NTera cDNA library that is missing one and a half WD repeats (Fig. 3); however, its significance has not been determined.

Recombinant hTAF$_{II}$100 and hTAF$_{II}$130 Become Stably Incorporated into the TFIID Complex in Transfected 293 Cells. To determine whether the near full-length cDNAs are capable of inclusion in a TFIID complex *in vivo*, the cDNAs encoding hTAF$_{II}$100 and hTAF$_{II}$130 tagged with an HA epitope at the N terminus, and HA-tagged full-length hTAF$_{II}$70 as a control (14), were individually expressed in human 293 cells by transient transfection. Nuclear extracts were prepared from transfected cells and the TFIID complex immunopurified using an anti-TBP antibody. An immunoblot

of the immunopurified TFIID complex probed with α-HA antibody is shown (Fig. 4). The HA-ΔNhTAF$_{II}$100 polypeptide showed a similar mobility to HA-hTAF$_{II}$70 since the ΔNhTAF$_{II}$100 construct used in this experiment contained the C-terminal 704 residues of hTAF$_{II}$100 (lanes 2 and 3). Each HA-tagged polypeptide was recovered in the immunopurified TFIID fraction, demonstrating that the recombinant hTAF$_{II}$s were capable of being incorporated into a TFIID complex with the enodgenous TBP and TAFs *in vivo*.

hTAF$_{II}$130 Binds to Sp1 *In vitro*. TAFs are thought to serve as transcriptional coactivators by interacting with site-specific transcription activators as well as basal factors. It has been demonstrated that dTAF$_{II}$110 binds Sp1 and functions as a coactivator for Sp1 (4, 5). Since Sp1 is a human transcription factor, it is more relevant to determine whether human TAF$_{II}$130 can interact with Sp1, and this was addressed using a variety of protein–protein interaction assays. Recombinant Sp1 purified from HeLa cells was immobilized on a DNA affinity resin bearing tandem Sp1 binding sites (Fig. 5*A*). Incubation with *in vitro*-translated radiolabeled hTAF$_{II}$130 resulted in retention of hTAF$_{II}$130 by the Sp1 resin but not by the control resin (lanes 2 and 3).

To determine whether this association was mediated by the Sp1 activation domains, glutathione *S*-transferase pulldown experiments were performed using glutathione *S*-transferase fusion proteins expressed and purified from mammalian cells (data not shown). The results demonstrate specific binding of hTAF$_{II}$130 to the Sp1 B domain rather than to a control fusion. In a reciprocal experiment, the central domain of hTAF$_{II}$130N/C (containing residues 270–700) tagged with HA was expressed and purified from *E. coli* and immobilized on an α-HA antibody affinity column. Incubation of this matrix with *in vitro*-translated full-length Sp1 resulted in retention of Sp1 by HA-hTAF$_{II}$130N/C but not by the control resin prepared with an extract from *E. coli* harboring the expression

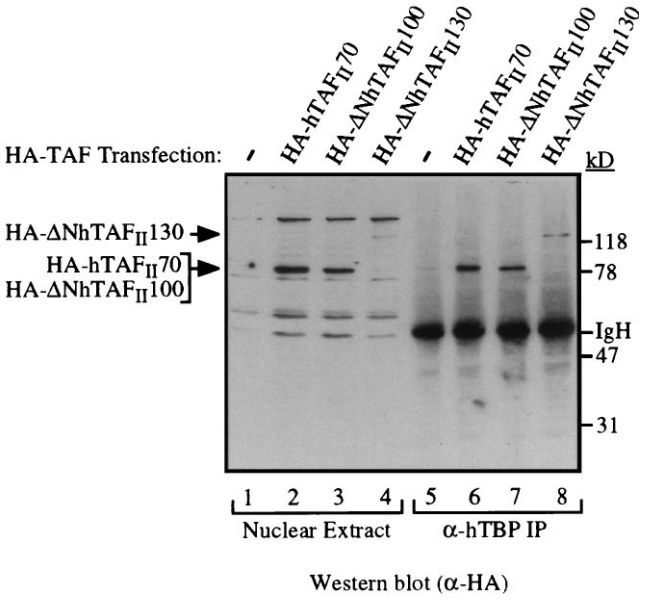

FIG. 4. Recombinant HA-tagged hTAF$_{II}$s can be stably incorporated into the TFIID complex *in vivo*. Nuclear extracts were prepared from 293 cells transfected with a control plasmid (lanes 1 and 5) or expression plasmids encoding HA-hTAF$_{II}$70 (lanes 2 and 6), HA-ΔNhTAF$_{II}$100 containing C-terminal 704 residues (lanes 3 and 7), HA-ΔNhTAF$_{II}$130 (C-terminal 947 residues, lanes 4 and 8) for 48 hours and TFIID was immunopurified using a polyclonal α-hTBP antibody. Presence of each recombinant hTAF$_{II}$ was detected by Western blotting using the α-HA antibody. Presence of endogenous hTAF$_{II}$s in immunopurified TFIID was detected by probing the same blot with a mixture of α-hTAF$_{II}$ monoclonal antibodies (data not shown).

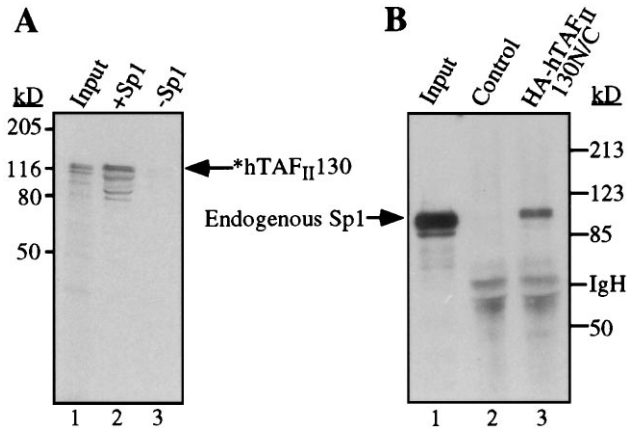

FIG. 5. Interactions between Sp1 and hTAF$_{II}$130 detected *in vitro*. (*A*) Radiolabeled hTAF$_{II}$130 was incubated with purified Sp1 bound to a DNA-affinity resin (lane 2) or a control resin prepared without Sp1 protein (lane 3). Input lane represents approximately 10% of each reaction. (*B*) Endogenous Sp1 was detected by Western blotting after incubating a crude HeLa cell nuclear extract (1.5 mg) with a resin containing immobilized HA-hTAF$_{II}$130N/C, expressed and purified from *E. coli* (lane 3), and a control resin (lane 2).

vector with an HA-tag (data not shown). The immobilized HA-hTAF$_{II}$130N/C was also mixed with a crude nuclear extract prepared from HeLa cells and found to retain endogenous Sp1, which was detected by Western blot analysis followed by incubation with a polyclonal α-Sp1 antibody (Fig. 5*B*, lanes 2 and 3). Together, these *in vitro* experiments argue for a direct protein–protein interaction between the activation domain B of Sp1 and the central domain of hTAF$_{II}$130.

hTAF$_{II}$130-Sp1 Interaction Domain Is Localized to the Central Glutamine-Rich Region of hTAF$_{II}$130 and Correlates with Transactivation by Sp1. In addition to the protein binding assays performed *in vitro*, the region of hTAF$_{II}$130 involved in interaction with Sp1 was also defined *in vivo* using the yeast two-hybrid system (Fig. 6*A* and data not shown). The Sp1 interaction domain was localized to a central region of hTAF$_{II}$130 that includes multiple glutamine-rich regions (20–30% glutamines). To examine the correlation between the ability of Sp1 to interact with hTAF$_{II}$130 and its ability to activate transcription, we tested subfragments of the Sp1 activation domain (8) using yeast two-hybrid experiments. For each Sp1 mutant, the results obtained using human TAF$_{II}$130 were consistent with the previous results for the interaction of Sp1 mutants and *Drosophila* TAF$_{II}$110 (Fig. 6 *B* and *C* and data not shown). Mutations in Sp1 B that reduce transcription also interfere with the binding to hTAF$_{II}$130. Mutations in the C-terminal half of Sp1 B domain have been shown to significantly decrease transactivation by Sp1 (8, 31). Therefore, we conclude that the ability of hTAF$_{II}$130 to bind to Sp1 correlates with the ability of Sp1 to activate transcription. As with dTAF$_{II}$110 (32), hTAF$_{II}$130 also interacted with the N-terminal domain of CREB that contains the Q2 activation domain but not with a derivative missing one-half of the Q2 domain (data not shown). In contrast, the C-terminal proline-rich activation domain of CTF (33) did not interact with hTAF$_{II}$130 (data not shown). These results suggest that hTAF$_{II}$130 interacts with the glutamine-rich activation domains present in Sp1 and CREB but not with the proline-rich activation domain of CTF.

DISCUSSION

Recent progress in uncovering the mechanisms of transcriptional regulation from eukaryotic promoters transcribed by RNA polymerase II has led to the notion that TFIID functions as an intermediary between site-specific transcriptional acti-

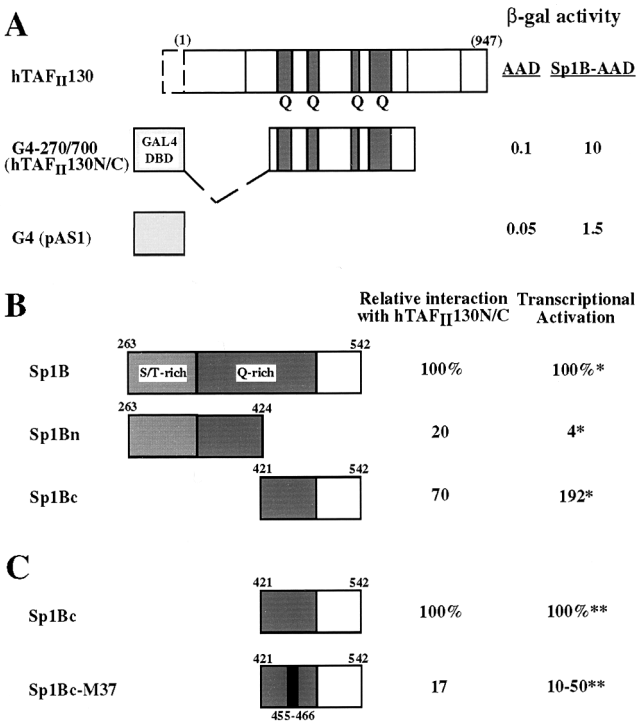

FIG. 6. (*A*) Sp1-hTAF$_{II}$130 interaction detected by yeast two-hybrid assay. hTAF$_{II}$130N/C fused to the GAL4 DNA binding domain (residues 1–147, lightly shaded) is shown schematically. β-Galactosidase activity measured from lysates of yeast cotransformed with AAD fusions are indicated on the right. (*B*) hTAF$_{II}$130 interacts preferentially with the C-terminal subdomain of Sp1 B that activates transcription in HeLa cells. Yeast plasmids containing the full-length Sp1 B domain (amino acids 263–542), or the subdivisions Bn (263–424), or Bc (421–542) fused to the acidic activation domain (8) were cotransformed with lexADBD-hTAF$_{II}$130N/C fusion. The resulting β-galactosidase activity measured relative to the activity of the full-length Sp1 B, is indicated on the right. *, Previously reported relative values of transcriptional activation by equivalent fusion constructs expressed and assayed in HeLa cells (8). (*C*) A linker substitution mutation (indicated by the black bar) in the C-terminal subdomain of Sp1 B reduces both interaction with hTAF$_{II}$130 and transcriptional activation in human cells. The yeast two-hybrid experiments were conducted as described for *B*. **, Transcriptional activation values measured in 293 cells by transient transfection were taken from (31) and S. Smale (personal communication).

vators and components of the basal transcription machinery. Direct interactions between specific activators and TAF subunit(s) in the TFIID complex have been demonstrated, suggesting that TAFs may make multiple contacts with different transcriptional regulators thereby integrating signals transmitted to the gene promoter from multiple sources (4–7). To investigate the role of each TAF subunit in mediating transcriptional activation, we have isolated and characterized cDNA clones encoding two human TAF proteins: hTAF$_{II}$130 and hTAF$_{II}$100. Our goal is to identify the mammalian activators that contact these human TAFs and to characterize TAF-activator interactions relevant to transcriptional regulation. This paper provides several lines of evidence that suggest that hTAF$_{II}$130 is the target of the glutamine-rich activation domain of Sp1.

When expressed in mammalian cells the recombinant hTAF$_{II}$130 and hTAF$_{II}$100 proteins were capable of stably associating with the endogenous TFIID complex, demonstrating that the isolated cDNAs encode bona fide hTAF$_{II}$s. The predicted C terminus of human TAF$_{II}$130 shares a high degree of sequence similarity with the C terminus of *Drosophila* TAF$_{II}$110, arguing that hTAF$_{II}$130 is the mammalian counterpart of dTAF$_{II}$110. Sequence comparisons between two homologs have revealed conserved and non-conserved regions in hTAF$_{II}$130. The conserved C-terminal domain might be involved in interaction with other TAFs and basal factors. For example, TAF$_{II}$250, TFIIA and dTAF$_{II}$30α have previously been shown to interact with dTAF$_{II}$110 (24, 34, 35).

Outside of the conserved C-terminal region, hTAF$_{II}$130 shows less sequence similarity to dTAF$_{II}$110; however, both proteins interact with the activation domain of Sp1, suggesting that they share structural similarities. The central domain of hTAF$_{II}$130 interacts with the B activation domain of Sp1 both in *in vitro* protein-binding assays and in the yeast two-hybrid assay. Most importantly, we observed a tight correlation between Sp1's ability to activate transcription in mammalian cells and its ability to interact with hTAF$_{II}$130, consistent with a role for hTAF$_{II}$130 in mediating Sp1-dependent transcriptional activation. These results are in agreement with previously reported interactions between the activation domain of Sp1 and dTAF$_{II}$110 (8).

In contrast, the most N-terminal portion of hTAF$_{II}$130 appears to be unique to this protein and might engage in some as yet unidentified function such as binding to promoter DNA. The *Drosophila* TAF$_{II}$150 has been shown to bind specifically to the initiator and downstream sequences of the adenovirus major late promoter and play a role in promoter selectivity (36). Indeed, TFIID immunopurified from HeLa cells shows extensive interaction with promoter DNA (9), consistent with additional DNA contacts involving components of TFIID. Interestingly, the mammalian homolog of dTAF$_{II}$150 is not tightly associated with TFIID (37); therefore, other TAFs such as hTAF$_{II}$130 in the tightly associated TFIID complex might be responsible for interaction with promoter DNA, thereby contributing to promoter selectivity and transcriptional regulation. Indeed, a recent study showed that a 135-kDa polypeptide present in immunopurified TFIID can be crosslinked to promoter DNA (38).

We have found that both Sp1 B and the N-terminal activation domain of CREB seem to interact through the central domain of hTAF$_{II}$130 (residues 270–700) in the yeast two-hybrid assay. This contrasts with the interaction between dTAF$_{II}$110 and the DNA binding/transactivation domain of the progesterone receptor, that requires more C-terminal domain of dTAF$_{II}$110 (39). Therefore, multiple activators may contact the same TAF protein via different structural domains. It has been demonstrated for the *Drosophila* transcriptional regulators Bicoid and Hunchback that two activators that target different TAFs in the TFIID complex function synergistically when bound to the same promoter, presumably by enhancing recruitment of TFIID and/or by increasing the stability of the preinitiation complex (40). These findings are significant in the context of naturally occurring promoters where site-specific regulators bind to multiple sites adjacent to or overlapping with other factor binding sites. Therefore, it will be important to determine the nature of protein–protein interactions between hTAF$_{II}$130 and those activators that contact hTAF$_{II}$130 including Sp1 and CREB.

Our results, together with those of others, suggest that Sp1 may use independent domains to make separate contacts with distinct TAFs to activate transcription. In addition to the interaction between the B activation domain and hTAF$_{II}$130 reported here, hTAF$_{II}$55 has been shown to contact a C-terminal portion of Sp1 consisting of the DNA binding domain and activation domain D (6). Interestingly, Smale and colleagues performed transient transfection studies with reporter constructs driven by synthetic core promoter elements and found that stimulation of initiator-containing core promoters by Sp1 occurred with the glutmaine-rich activation domains of Sp1 (31). By contrast, TATA box-mediated transcription required full-length Sp1. Therefore, they proposed that interaction with hTAF$_{II}$130 might function through the initiator element whereas interaction with hTAF$_{II}$55 might be addi-

tionally required for Sp1-dependent stimulation of transcription from the TATA box-containing promoters.

Recent findings suggest that $hTAF_{II}130$ may be a member of a family of related proteins. A cDNA clone has been isolated that encodes a B-cell specific form of $hTAF_{II}130$ that is present in substoichiometric amounts (41). It appears that no true homolog of $hTAF_{II}130$ exists in yeast, raising the possibility that $hTAF_{II}130$ is a TAF unique to higher eukaryotes. This is consistent with the reported observations that glutamine-rich activators such as Sp1 lack the ability to activate transcription when introduced into yeast while acidic activators activate transcription from a variety of host cells including yeast (42). $hTAF_{II}130$ and related proteins may have evolved to accommodate an increasingly large repertoire of transcriptional activators required to carry out complex programs of gene expression during cell growth and differentiation in higher eukaryotes.

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