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Distinct domains of $hTAF_{II}100$ are required for functional interaction with transcription factor TFIIFβ (RAP30) and incorporation into the TFIID complex

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TFIID is the DNA binding component of the RNA polymerase II transcriptional machinery and is composed of the TATA binding protein (TBP) and TBP-associated factors (TAF_{II}s). Here we report the characterization of a new human TAF, $hTAF_{II}100$, which is the human homologue of *Drosophila* $TAF_{II}80$ and yeast $TAF_{II}90$. $hTAF_{II}100$ interacts strongly with $hTAF_{II}250$, $hTAF_{II}55$ and $hTAF_{II}28$, less with $hTAF_{II}20$ and $hTAF_{II}18$, weakly with TBP and not at all with $\Delta NTAF_{II}135$ and $hTAF_{II}30$. Deletion analysis revealed that the C-terminal half of $hTAF_{II}100$, which contains six WD-40 repeats, is not required for incorporation into the TFIID complex. Our results suggest that $hTAF_{II}100$ can be divided into two domains, the N-terminal region responsible for interactions within the TFIID complex and the C-terminal WD repeat-containing half responsible for interactions between $hTAF_{II}100$ and other factors. An anti-$hTAF_{II}100$ antibody, raised against a C-terminal epitope, selectively inhibited basal TFIID-dependent *in vitro* transcription and the specific interaction between $hTAF_{II}100$ and the 30 kDa subunit of TFIIF (RAP30). We demonstrate that the $hTAF_{II}100$–TFIIF interaction supports pre-initiation complex formation in the presence of TFIID. Thus, this is the first demonstration that a TAF_{II} functionally interacts with a basal transcription factor *in vitro*.

Keywords: basal RNA polymerase II transcription/ $dTAF_{II}80$/$yTAF_{II}90$/TBP/WD repeats

Introduction

Accurate initiation of transcription by RNA polymerase II (Pol II) on TATA-containing promoters is nucleated by the multisubunit transcription factor TFIID, in which the DNA binding subunit is the TATA binding protein (TBP). Binding of TFIID is followed by the subsequent ordered interactions of the other basal factors (TFIIA, TFIIB, -IIE, -IIF, -IIH and -IIJ) and Pol II to yield a productive pre-initiation complex (PIC) (Buratowski *et al.*, 1989; Roeder, 1991; Zawel and Reinberg, 1995). TFIID, which consists of the TBP and up to 13 TBP-associated factors, called TAFs, has an M_r of ~700–750 kDa (Pugh and Tjian, 1991; Tanese *et al.*, 1991; Timmers *et al.*, 1992; Zhou *et al.*, 1992; Brou *et al.*, 1993a; Chiang *et al.*, 1993; Yokomori *et al.*, 1993; Jacq *et al.*, 1994; Mengus *et al.*, 1995). Recombinant TBP can replace TFIID for basal transcription from TATA-containing Pol II promoters in reconstituted transcription systems, but appears to be inefficient in supporting transcription from TATA-less promoters and in mediating activator-dependent transcription (Pugh and Tjian, 1992; Brou *et al.*, 1993a; Aso *et al.*, 1994). These observations suggest that Pol II TAFs (TAF_{II}s) function both to mediate the effect of transactivators and to recruit TFIID complexes to TATA-less promoters. Previously, we have shown by using chromatographic purification and immunoprecipitation that functionally distinct TFIID complexes composed of both common and specific TAF_{II}s exist in human HeLa cells (Brou *et al.*, 1993a,c; Jacq *et al.*, 1994; Mengus *et al.*, 1995). Recently, specific substoichiometric TAF_{II}s have been described from *Drosophila* that specify promoter selectivity (Hansen and Tjian, 1995). TAF_{II}s which are present in every TFIID complex were designated core TAF_{II}s (Jacq *et al.*, 1994).

In order to better understand the precise molecular mechanisms in which TAFs are involved, many of the *Drosophila* (d) and human (h) TFIID subunits have been cloned and characterized (Tjian, 1995). It has been demonstrated that certain TAF_{II}s can serve as co-activators by making direct contacts with enhancer binding proteins to direct transcriptional activation. Confirming our initial hypothesis (Tasset *et al.*, 1990; Brou *et al.*, 1993a,c), it was shown that different classes of activation domains contact distinct components of the TFIID complex to stimulate transcription. For example, $dTAF_{II}110$ serves as a co-factor for the glutamine-rich transcription factor Sp1, since $dTAF_{II}110$ interacts directly with Sp1 *in vitro* and mediates the activity of Sp1 in yeast, but does not bind to proline-rich or acidic activation domains (Hoey *et al.*, 1993; Gill *et al.*, 1994). $dTAF_{II}40$ and its human homologue, $hTAF_{II}31$, were found to interact functionally with the acidic activating domain of VP16, but not with the glutamine-rich activating domain of Sp1 or the proline-rich activating domain of CTF-1 (Goodrich *et al.*, 1993; Klemm *et al.*, 1995). In addition, $dTAF_{II}40$, $dTAF_{II}60$ and their human homologues, $hTAF_{II}31$ and $hTAF_{II}70$ respectively, interact with p53 and mediate p53 activation *in vitro* (Lu and Levine, 1995; Thut *et al.*, 1995). A functional interaction was also found between $hTAF_{II}30$, a specific TAF_{II} that is present only in a subpopulation of TFIID complexes, and the hormone binding domain of the human oestrogen receptor (Jacq *et al.*, 1994). In contrast, $hTAF_{II}55$ was found to interact *in vitro* with several distinct activators (Sp1, YY1, USF, CTF, E1A, Tat) possessing different types of activation domains

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(Chiang and Roeder, 1995). TAF_{II}s have also been shown to interact *in vitro* with general Pol II transcription factors (GTFs), possibly to help recruit GTFs into the pre-initiation complex (Goodrich *et al.*, 1993; Histake *et al.*, 1995; Klemm *et al.*, 1995). However, the functional significance of these interactions has yet to be demonstrated. The potential for multiple contacts between an activator and individual components of the basal transcription machinery may allow synergistic activation (see Discussion).

Here we report the isolation and characterization of a new human core TAF, $hTAF_{II}100$, which is the human homologue of $dTAF_{II}80$ (Dynlacht *et al.*, 1993; Kokubo *et al.*, 1993b) and yeast (y) $TAF_{II}90$ (Reese *et al.*, 1994). We demonstrate that the C-terminal half of $hTAF_{II}100$, which contains six complete WD-40 repeats originally described in the β subunits of G proteins (Fong *et al.*, 1986; Neer *et al.*, 1994), is not required for interaction with the TFIID complex in transfected cells. Co-immunoprecipitation assays indicate that $hTAF_{II}100$ interacts with $hTAF_{II}250$, $hTAF_{II}55$, $hTAF_{II}28$, $hTAF_{II}20$ and $hTAF_{II}18$, weakly with TBP and not at all with $\Delta NTAF_{II}135$ and $hTAF_{II}30$. In addition, an antibody raised against a C-terminal peptide selectively inhibited basal transcription in the presence TFIID. Moreover, we show that $hTAF_{II}100$ interacts specifically with the 30 kDa subunit of TFIIF (RAP30) and that this interaction is required during TFIID-dependent PIC formation *in vitro*.

Results

Molecular cloning of the 100 kDa subunit of the human TFIID complex

The human TFIID complex was purified from HeLa cell nuclear extract by three consecutive chromatographic steps followed by immunoprecipitation (Brou *et al.*, 1993a; Jacq *et al.*, 1994), and partial amino acid sequences of the 100 kDa subunit were determined as described previously (Brou *et al.*, 1993b). Degenerate oligonucleotides from both the amino- and the carboxy-terminal ends of the peptide sequence AFEDLETDDFTTATGHINLP (Figure 1A) were used for PCR-mediated amplification. A 60 bp DNA fragment was subcloned, sequenced and found to encode the internal portion of the peptide. This DNA sequence was then used to screen a HeLa cell random primed cDNA library. One of the cDNAs obtained contains an open reading frame encoding a 799 amino acid polypeptide (Figure 1A) with a calculated M_r of 87.9 kDa. However, both the endogenous and the recombinant proteins migrate in SDS–PAGE with an apparent M_r of 100 kDa. Consequently, this human TFIID subunit has been named $hTAF_{II}100$.

Comparison of $hTAF_{II}100$ with $dTAF_{II}80$ (Dynlacht *et al.*, 1993; Kokubo *et al.*, 1993b) and $yTAF_{II}90$ (Reese *et al.*, 1994) revealed that $hTAF_{II}100$ is the human homologue of the *Drosophila* and the yeast proteins. Overall, $hTAF_{II}100$ is 65% similar (44% identical) to $dTAF_{II}80$ and 57% similar (39% identical) to $yTAF_{II}90$ (Figure 1B). The C-terminal half of these proteins has a significant sequence homology to the WD-40 or β-transducin repeats originally described in the β subunit of guanyl nucleotide regulatory proteins, or G proteins (Fong *et al.*, 1986; Neer *et al.*, 1994). $hTAF_{II}100$ has six segments that show sequence similarity to the WD-40 repeat consensus sequence (Neer *et al.*, 1994). Note that the N-terminal 450 amino acid region of $hTAF_{II}100$ and the corresponding regions of $dTAF_{II}80$ and $yTAF_{II}90$ (see Figure 1B) have no significant similarities to any other known proteins.

To confirm that the isolated cDNA encoded a TAF_{II}, monoclonal antibodies (mAbs) were raised against peptides derived from the sequence of $hTAF_{II}100$ (numbered 1 and 2 in Figure 1B). These antibodies, mAb2D2 and mAb1TA (raised against peptide 1 and 2 respectively), have been used in previous studies to show that $hTAF_{II}100$ is present in affinity-purified hTFIID (Eberhard *et al.*, 1993; Jacq *et al.*, 1994; Rudloff *et al.*, 1994). In immunoprecipitation experiments, both of these anti-$hTAF_{II}100$ mAbs co-immunoprecipitated $hTAF_{II}100$, TBP and other TAF_{II}s (Figure 2 and data not shown). Note that the recombinant $TAF_{II}100$ overexpressed either in HeLa or Cos cells migrates in SDS–PAGE at a position identical to the endogenous human protein (data not shown). These results, together with the high sequence similarity to $dTAF_{II}80$ and $yTAF_{II}90$, confirm that the isolated cDNA encodes the 100 kDa protein component present in the TFIID complex.

The N-terminal half but not the WD-40 repeat-containing region of $hTAF_{II}100$ is required for intracellular interaction with the TFIID complex

Several reports suggested that the WD-40 repeats may play a role in protein–protein interactions (for review, see Neer *et al.*, 1994). To investigate whether the WD-40 repeats of $hTAF_{II}100$ are involved in protein interactions within the endogenous TFIID complex, various N- and C-terminally truncated mutants were created (Figure 3C) and cloned in the pXJ42 eukaryotic expression vector (Xiao *et al.*, 1991). The full-length $hTAF_{II}100$ and its N- and C-terminally truncated mutant polypeptides were tagged with the haemagglutinin antigen (HA) epitope at their N-termini (Figure 3C). HeLa cells were transiently transfected with the plasmids encoding the various HA-$TAF_{II}100$ derivatives. Note that all the mutants were expressed in the nuclei of the transfected HeLa cells as verified by immunocytochemistry (data not shown). Whole cell extracts (WCEs) were prepared from the transfected cells (Figure 3A lanes 1–6, and B lanes 1–3) and the TBP-containing complexes immunopurified with the anti-TBP mAb3G3 (Brou *et al.*, 1993a). The immunoprecipitated complexes were then separated by SDS–PAGE and analysed by Western blot. Anti-TBP, anti-$hTAF_{II}55$ (Figure 3A) and anti-$hTAF_{II}100$ (Figure 3B) antibodies were used to confirm that approximately the same amounts of TFIID complexes were immunoprecipitated (Figure 3A lanes 13–18, and B lanes 7–9). An antibody directed against the HA tag (mAb12CA5) was then used to test whether the overexpressed HA-$TAF_{II}100$ and its derivatives were able to be incorporated into the native hTFIID complex. The overexpressed recombinant full-length $TAF_{II}100$ incorporated into the endogenous TFIID complex (Figure 3A lane 7, and B lane 4), confirming the validity of this method. A truncated $TAF_{II}100$ mutant lacking the whole WD-40 repeat-containing region (ΔC418; Figure 3C) still maintained interactions with the TFIID complex (Figure 3A, lane 8). In contrast, but as expected, the mutant containing all WD-40 repeats but lacking the N-terminal

Fig. 1. (**A**) Nucleotide and amino acid sequences of the $hTAF_{II}100$ cDNA clone. The $hTAF_{II}100$ open reading frame extends from nucleotide 24 to 2422. The amino acid sequences determined by microsequencing of peptides obtained after tryptic digestion of the endogenous $hTAF_{II}100$ protein are boxed. (**B**) Comparison of the amino acid sequence of $hTAF_{II}100$ with that of *Drosophila* (d) $dTAF_{II}80$ (Dynlacht *et al.*, 1993) and yeast (y) $TAF_{II}90$ (Reese *et al.*, 1994). The arrows represent the complete WD-40 repeating units according to Neer *et al.* (1994). Identical amino acids in the three sequences are shown with bold characters. The peptides that were used to generate mAb2D2 and mAb1TA are boxed and labelled 1 and 2, respectively. The end points of the different C-terminal deletion mutants (ΔC) used in Figure 3 are indicated by the numbers over the sequence.

half of $hTAF_{II}100$ (ΔN418) was not incorporated into TFIID (Figure 3B, compare lanes 2 and 5). A further deletion of $hTAF_{II}100$ between amino acids 353 and 392 (ΔC353) considerably weakened the interaction(s) with the TFIID complex (Figure 3A, lanes 9 and 10), whereas the ΔC282 deletion totally abolished this interaction (lane

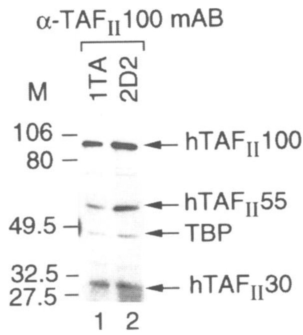
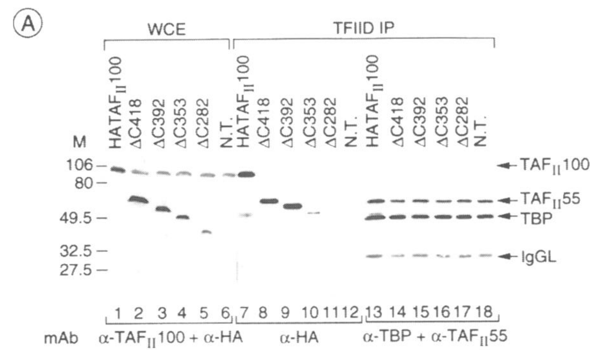


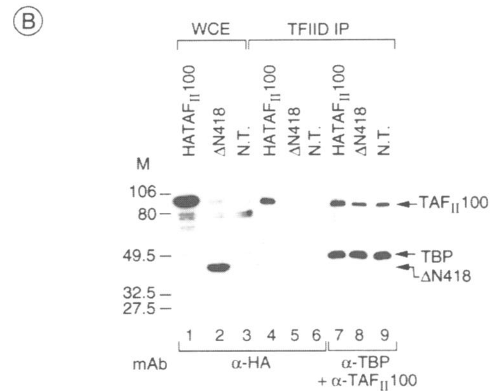
Fig. 2. The anti-$hTAF_{II}100$ mAbs co-immunoprecipitate TAF_{II}s and TBP from a HeLa cell nuclear extract (NE). $hTAF_{II}100$ was immunoprecipitated from 500 μl of NE with 10 μg of protein G-bound mAb1TA (lane 1) or mAb2D2 (lane 2). Beads were washed (see Materials and methods), boiled and $hTAF_{II}100$-bound proteins were analysed by Western blot with antibodies raised against TBP (mAb3G3; Brou *et al.*, 1993a), $hTAF_{II}55$ (mAb19TA), $hTAF_{II}30$ (mAb2F4; Jacq *et al.*, 1994) and $hTAF_{II}100$ (1TA).

11). These observations indicate that the region between amino acids 282 and 392, but not the WD repeat-containing region, is important for the incorporation of $hTAF_{II}100$ into the HeLa cell endogenous TFIID (Figure 3C; see also below).

Interaction of $hTAF_{II}100$ with individual components of the TFIID complex

To identify the subunits of TFIID that interact directly with $hTAF_{II}100$, the cDNAs encoding HA-$TAF_{II}250$, $\Delta NTAF_{II}135$, HA-$TAF_{II}100$, $hTAF_{II}55$, HA-$TAF_{II}30$, $hTAF_{II}28$, $hTAF_{II}20$, $hTAF_{II}18$ and hTBP were inserted in baculovirus expression vectors (see Materials and methods), and each TFIID subunit was expressed either alone or together with HA-$TAF_{II}100$ in SF9 cells. WCEs were made and the protein expression was tested by Western blot analysis using the appropriate antibodies raised against the human TAF_{II}s (see Figure 4A and C, lanes 1–4). The overexpressed $hTAF_{II}$s represent 5% or more of the total protein in WCEs of SF9 cells (data not shown). From these extracts, $hTAF_{II}100$ was immunoprecipitated either with an anti-HA mAb or with an anti-$hTAF_{II}100$ mAb, and bound proteins were analysed on Western blots (Figure 4B and C, lanes 5–6; summarized in Table IA). The extracts in which $hTAF_{II}100$ was not co-expressed served as negative controls for the immunoprecipitations. Since the overexpressed $hTAF_{II}$s in SF9 cell extracts greatly exceed (by at least 1000-fold) the endogeneous insect cell TAF_{II} concentrations, these interaction studies indicate that $hTAF_{II}100$ binds directly to $hTAF_{II}250$, $hTAF_{II}55$, $hTAF_{II}28$, $hTAF_{II}20$ and $hTAF_{II}18$ and suggest that $hTAF_{II}100$ may contact the same TAF_{II}s in the TFIID complex. Note that the same positive interactions were observed even when individually purified TAF_{II}s were incubated together before immunoprecipitation (data not shown). In contrast, no interaction was found between $hTAF_{II}100$ and $\Delta NTAF_{II}135$ (Figure 4C, lane 8) or $hTAF_{II}100$ and $hTAF_{II}30$ (Figure 4B, lane 10). The $hTAF_{II}100$–TBP interaction was weak when compared with the negative control, in which TBP bound non-specifically to the anti-HA antibody (Figure 4B, compare lanes 9 and 10); however, this interaction could also be observed when an anti-TBP mAb was used in the





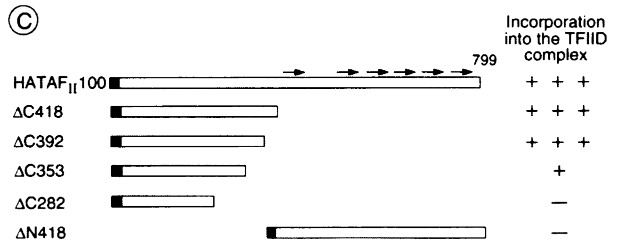


Fig. 3. The WD-40 repeats of $hTAF_{II}100$ are not required for its intracellular incorporation into the TFIID complex. (**A** and **B**) HeLa cells were either non-transfected (N.T.) or transiently transfected with expression vectors for HA-$TAF_{II}100$ (A and B, lanes 1). C-terminally truncated (ΔC) HA-$TAF_{II}100$ mutants (A, lanes 2–6) and N-terminally truncated (ΔN) HA-$TAF_{II}100$ mutant (B, lane 2). Whole cell extracts (WCEs) were made and the expression of the recombinant proteins was verified by Western blot using the α-$TAF_{II}100$ mAb (1TA) and α-HA mAb for (A), lanes 1–6 or the α-HA mAb for (B), lanes 1–3. From these WCEs, TBP-containing complexes were immunopurified (IP) and analysed by Western blot for the presence of the recombinant HA-$TAF_{II}100$ proteins in the TFIID complexes using the α-HA mAb (A, lanes 7–12, and B, lanes 4–6). The efficiency of the IP was verified by re-probing the same blots with (A) α-TBP mAb (3G3) and α-$TAF_{II}55$ mAb (19TA), and (B) 3G3 and α-$TAF_{II}100$ mAb (1TA). (**C**) Schematic diagram of the mutants used in (A) and (B). The numbers refer to amino acid positions in $hTAF_{II}100$ where the C-terminal mutants (ΔC) stop or where the N-terminal mutant (ΔN) starts. The small black box represents the HA tag. The WD-40 repeats are represented by arrows. The results of the $hTAF_{II}100$ incorporation into the TFIID complex using the ΔC and ΔN truncated mutants are summarized in a diagram.

immunoprecipitation (data not shown). These interactions were also observed in extracts of HeLa cells transiently overexpressing HA-$TAF_{II}100$ with one of the other TAF_{II}s (see Table IB and data not shown). The $hTAF_{II}100$ interaction study in transfected HeLa cells confirmed the results obtained by using the baculovirus overexpressed proteins (Table I), although the $hTAF_{II}100$ and TBP

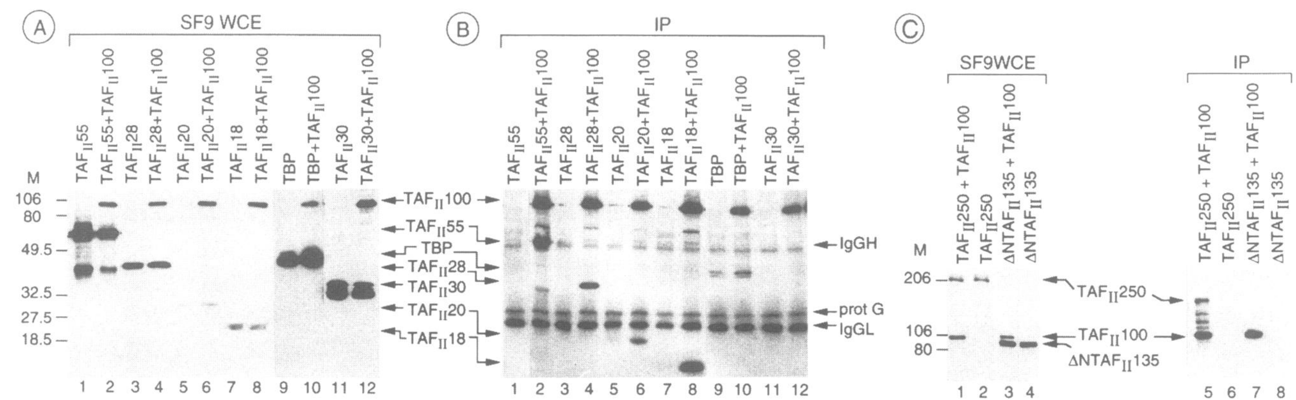


Fig. 4. Interaction of hTAF$_{II}$100 with individual components of the TFIID complex. (**A–C**) SF9 cells were co-infected with recombinant baculoviruses expressing TBP or TAF$_{II}$s either individually or in a pair with HA-TAF$_{II}$100 as indicated. Whole cell extracts (WCEs) were made and tested for protein expression by Western blot analysis with the appropriate mAbs raised against the different TAF$_{II}$s (A, lanes 1–12, and C, lanes 1–4). From these WCEs, TAF$_{II}$100 was immunoprecipitated (IP) using either the α-HA tag antibody 12CA5 (B, lanes 1–10, and C, lanes 7–8) or the α-TAF$_{II}$100 mAb2D2 (B, lanes 11–12, and C, lanes 5–6). TAF$_{II}$100-bound proteins were then analysed by Western blot with the appropriate mAbs. The positions of immunoglobulin heavy (IgGH) and light (IgGL) chains and protein G are indicated.

interaction was somewhat stronger in transfected HeLa cell extracts than in SF9 cell extracts (Table I, compare A and B).

An anti-hTAF$_{II}$100 antibody inhibits TFIID-dependent pre-initiation complex formation

To verify whether the anti-hTAF$_{II}$100 mAbs have an effect on Pol II transcription, a highly purified *in vitro* transcription assay was used in which the general Pol II transcription factors were either recombinant proteins overexpressed in *Escherichia coli* and purified (TFIIAα/β, TFIIAγ, TFIIB, TFIIEα and TFIIEβ) or extensively purified from HeLa cell nuclear extracts (TFIIF and RNA polymerase II). This system, when supplemented with either immunopurified TFIID or recombinant TBP, supports transcription from a negatively supercoiled DNA template containing the minimal adenovirus major late promoter (AdMLP) in the absence of TFIIH (Figure 5A, lanes 1 and 6; see also Parvin *et al.*, 1994). Increasing amounts of the two anti-hTAF$_{II}$100 mAbs, 1TA and 2D2, were pre-incubated with either TFIID (Figure 5A, lanes 2–5) or TBP (lanes 7–10) for 15 min at 25°C to allow the formation of antibody–antigen complexes. The template DNA and the missing general transcription factors were then added, incubated for 30 min and the transcription reactions started by addition of NTPs. Pre-incubation of TFIID with increasing amounts of anti-hTAF$_{II}$100 mAb (1TA) resulted in a 2- to 3-fold inhibition of basal transcription from the AdMLP (Figure 5A, lanes 2–3). No or only a weak inhibition was observed with the other anti-hTAF$_{II}$100 mAb (2D2), even at the highest amount tested (lane 5). In contrast, when TFIID was replaced by recombinant TBP, and thus hTAF$_{II}$100 was absent from the transcription reactions, neither of the anti-hTAF$_{II}$100 mAbs inhibited basal Pol II transcription (lanes 6–10).

Next, we carried out order-of-addition experiments to determine which step of the *in vitro* transcription process was inhibited by the antibody mAb1TA. The antibody was either pre-incubated with TFIID (–20 min) or added at different time points to the transcription mixtures containing the template DNA, TFIID and the other transcription factors but no NTPs (Figure 5B). Interestingly,

Table I. Comparison of the interaction pattern of hTAF$_{II}$100 with individual components of the TFIID complex in baculovirus co-infected SF9 cells (A) and in transiently transfected HeLa cells (B)

(A) Proteins interacting with TAF$_{II}$100 in SF9 cells		(B) Proteins interacting with TAF$_{II}$100 in HeLa cells	
TAF$_{II}$250	+++	TAF$_{II}$250	ND
ΔNTAF$_{II}$135	–	ΔNTAF$_{II}$135	ND
TAF$_{II}$55	+++	TAF$_{II}$55	+++
TAF$_{II}$30	–	TAF$_{II}$30	–
TAF$_{II}$28	+++	TAF$_{II}$28	+++
TAF$_{II}$20	++	TAF$_{II}$20	+
TAF$_{II}$18	++	TAF$_{II}$18	+
TBP	–/+	TBP	+

The averages of at least three independent experiments, similar to the one presented in Figure 4, are shown. +++, ++, +, -/+, – correspond to strong, moderate, weak, very weak (but detectable) and no interactions between hTAF$_{II}$100 and a given TAF$_{II}$ or TBP. ND: not determined.

transcriptional inhibition occurred only when the mAb1TA was added to the reaction mixtures before (–20 min; lane 3) or during (0 and 15 min; lanes 4 and 5) PIC formation. In contrast, the antibody had no effect on transcription after completion of PIC assembly (30 min; lane 6). These observations indicate that mAb1TA exerts its inhibitory effect on transcription at a step during PIC formation, but cannot inhibit initiation of transcription from the fully formed PICs. The selective inhibition of transcription initiation in the presence of TFIID by only one of the two antibodies suggests that the epitope recognized by mAb1TA close to the C-terminus of hTAF$_{II}$100 (see peptide 2 in Figure 1B) may be important in Pol II-directed transcription through interactions with general Pol II transcription factors.

Functional interaction between hTAF$_{II}$100 and the small subunit of TFIIF (TFIIFβ or RAP30)

To investigate whether hTAF$_{II}$100 interacts with one of the general Pol II transcription factors, HA-TAF$_{II}$100 was either co-expressed in SF9 cells individually with TFIIB, TFIIEα, TFIIEβ, or with the subunits of TFIIF (RAP74 and RAP30). Alternatively, SF9 cell extracts containing

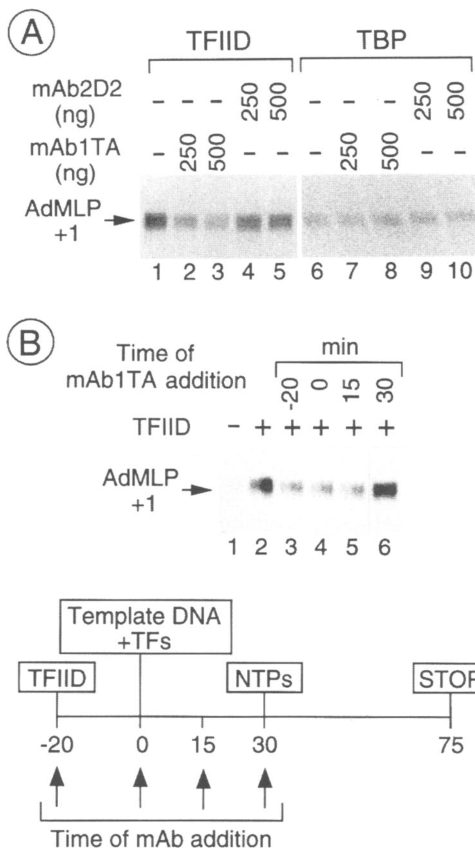


Fig. 5. The anti-h$TAF_{II}100$ antibody 1TA specifically inhibits TFIID-dependent Pol II transcription by interfering in the functional interaction between h$TAF_{II}100$ and TFIIFβ (RAP30). (**A**) TFIID (lanes 1–5) or TBP (lanes 6–10) were pre-incubated either in the absence or in the presence of different amounts of α-$TAF_{II}100$ mAbs (1TA and 2D2; as indicated) for 20 min at 25°C, then the AdMLP-containing template and the other general transcription factors (GTFs) were added. The position of the correctly initiated transcript from the AdMLP (+1), as determined by quantitative S1 nuclease analysis, is indicated. (**B**) MAb1TA exerts its inhibitory effect during pre-initiation complex formation. MAb1TA (500 ng) was either pre-incubated with TFIID for 20 min at 25°C before adding the template DNA and the other GTFs (–20 min, lane 3) or added simultaneously with TFIID, template DNA and the other GTFs (0 min, lane 4) or 15 (lane 5) and 30 min (lane 6) after the assembly of all the other components. In all cases, transcription was started at 30 min by the addition of NTPs. Correctly initiated transcripts (AdMLP +1) were determined by quantitative S1 nuclease analysis. The schematic assembly of the components of the transcription reactions is depicted under the figure.

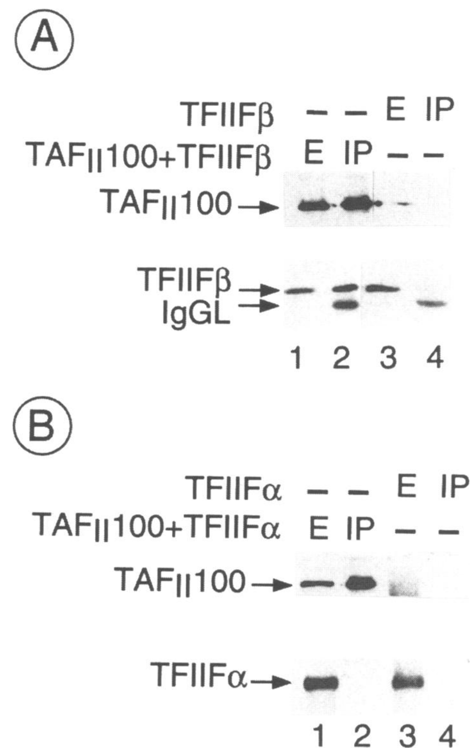


Fig. 6. h$TAF_{II}100$ interacts with TFIIFβ (RAP30). HA-$TAF_{II}100$ was either co-expressed with TFIIFβ (RAP30) (**A**) or TFIIFα (RAP74) (**B**) in SF9 cells. TFIIFβ (RAP30) and TFIIFα (RAP74) were also expressed individually as negative controls. Whole cell extracts were made (E) and $TAF_{II}100$ was immunoprecipitated (IP) using the α-HA tag antibody 12CA5. $TAF_{II}100$-bound proteins were then analysed by Western blot with mAb12CA5 and mAbs raised against RAP30 (A) or RAP74 (B) subunit of TFIIF. The position of immunoglobulin light chain (IgGL) is indicated.

HA-$TAF_{II}100$ were incubated together with either bacterially expressed TFIIA subunits, purified TFIIH or Pol II-containing fractions. From these extracts, HA-$TAF_{II}100$ was immunoprecipitated with the anti-HA mAb (12CA5), the resin-bound proteins were washed with IP buffer containing 500 mM KCl, and bound proteins analysed by Western blots using antibodies raised against the distinct transcription factors. Under these stringent conditions, TFIIFβ (RAP30) bound selectively to h$TAF_{II}100$ (Figure 6A, compare lanes 2 and 4). All the other basal transcription factors, including TFIIFα (or RAP74), did not interact with h$TAF_{II}100$ (Figure 6B, lane 2, and data not shown). When HA-$TAF_{II}100$ was co-expressed with both TFIIFα and β (RAP74 and RAP30) in SF9 cells, TFIIFα (RAP74) was also retained on HA-$TAF_{II}100$, conceivably through its interaction with TFIIFβ (RAP30) (data not shown).

Next, we analysed whether the protein–protein interaction between h$TAF_{II}100$ and TFIIF was functionally relevant by order-of-addition experiments using the *in vitro* transcription assay. To this end, two different sets of transcription reactions were assembled. In the first set, the supercoiled DNA template was pre-incubated with all basic factors except TFIIF to allow the formation of partial pre-initiation complexes, referred to as DA, DB, DAB or DBPol II complexes (Buratowski *et al.*, 1989; Maldonado *et al.*, 1990; Parvin *et al.*, 1994; Serizawa *et al.*, 1994). The mAb1TA was then added for 20 min, and the reaction mix completed with the TFIIF-containing fraction (Figure 7A, lanes 1 and 2). In the second set, the DNA template was pre-incubated with all the basic factors (including TFIIF) for 20 min before adding the antibody (lane 4). The reaction mixtures were then incubated further for 20 min and transcription started by NTP addition. MAb1TA inhibited transcription only when TFIIF was missing from the pre-incubation (Figure 7A, lane 2), but not when it was present (lane 4). This indicates that when partial PICs are formed, in the absence of TFIIF, the mAb1TA can still bind to h$TAF_{II}100$ and suggests that the antibody interferes in the h$TAF_{II}100$–TFIIF contact. This interaction seems to be important for basal Pol II transcription in the presence of TFIID *in vitro*.

To determine whether the h$TAF_{II}100$–TFIIFβ (RAP30) direct interaction is indeed prevented by mAb1TA, and

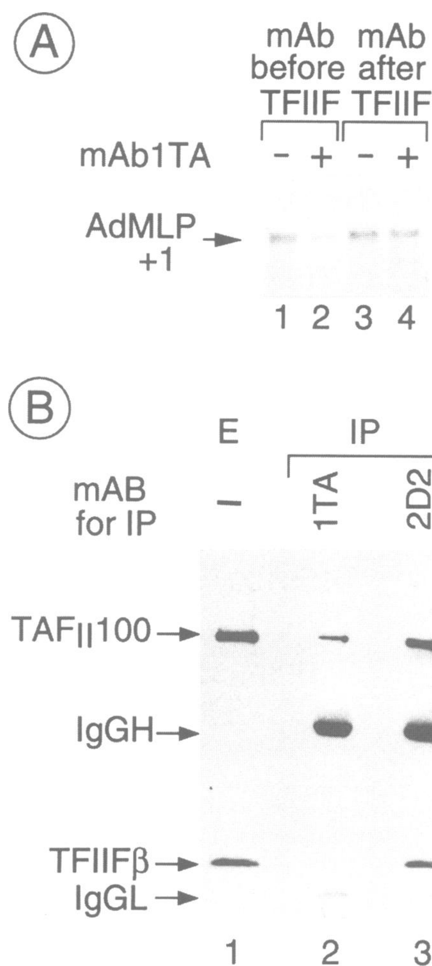


Fig. 7. (**A**) The $hTAF_{II}100$–TFIIF interaction is functionally relevant. Two sets of transcription reactions were set up with template DNA and all GTFs except TFIIF. One reaction was incubated with or without 500 ng of mAb1TA (mAb before TFIIF; lanes 1 and 2), whereas the other was completed with TFIIF (mAb after TFIIF; lanes 3 and 4). After 20 min, mAb1TA was added to one of the 'mAb after TFIIF' reactions (lane 4) and the 'mAb before TFIIF' reactions were completed by the addition of TFIIF. All the reactions were then incubated further at 25°C for another 20 min before the addition of NTPs. (**B**) MAb1TA interferes in the $hTAF_{II}100$–TFIIFβ (RAP30) interaction. HA-$TAF_{II}100$ was immunoprecipitated (IP) from the same SF9 cell extract (E; lane 1) that was used in Figure 6A with either mAb1TA (lane 2) or mAb2D2 (lane 3) and bound proteins analysed by Western blot with mAb12CA5 and mAb raised against the small subunit of TFIIF. Note that the presence of TFIIFβ (RAP30) in the mAb1TA IP (lane 2) was also undetectable on 5–10 times longer exposures than the one shown here. The positions of immunoglobulin heavy (IgGH) and light chains (IgGL) are indicated.

thus may be the reason for the transcriptional inhibition, we tried to co-immunoprecipitate $hTAF_{II}100$ and TFIIFβ (RAP30) with mAb1TA. HA-$TAF_{II}100$ and TFIIFβ (RAP30) were co-expressed in SF9 cells, WCEs made and $hTAF_{II}100$ was immunoprecipitated with either mAb1TA or mAb2D2 as a negative control, since mAb2D2 has almost no effect on transcription (Figure 5A, lanes 4–5). $hTAF_{II}100$-bound proteins were then analysed by Western blot with anti-HA and anti-RAP30 antibodies (Figure 7B). As expected, mAb1TA does not co-immunoprecipitate TFIIFβ (RAP30) with $hTAF_{II}100$ (Figure 7B, lane 2), whereas mAb2D2 does (lane 3). Taken together, these results indicate that mAb1TA blocks a direct protein–protein interaction between $TAF_{II}100$ and TFIIFβ (RAP30) that is required for efficient *in vitro* transcription.

Discussion

$hTAF_{II}100$ contains two functionally distinct domains

The present study reports the cloning of the cDNA encoding the 100 kDa component of human TFIID. $hTAF_{II}100$ is an evolutionary conserved subunit of TFIID as it has significant sequence similarity to both *Drosophila* and yeast TFIID subunits, $dTAF_{II}80$ and $yTAF_{II}90$ (Figure 1B; Dynlacht *et al.*, 1993; Kokubo *et al.*, 1993b; Reese *et al.*, 1994). The alignment of the amino acid sequences shows that these three proteins are more similar to each other in the C-terminal, WD repeat-containing halves than they are in the N-terminal halves. Proteins containing WD repeats regulate diverse cellular functions, such as cell division, cell fate determination, transmembrane signalling, mRNA modification and vesicle function and gene transcription (Neer *et al.*, 1994). To date, WD repeats have only been found in eukaryotic proteins and there has been no common function associated with them. However, the high homology amongst the WD repeats of $hTAF_{II}100$ and its *Drosophila* and yeast homologues (51 and 48% identity, respectively) and the well conserved positional distribution of the WD repeats may indicate a true functional homology (Neer *et al.*, 1994) amongst these $TAF_{II}s$ and suggests that they participate in evolutionary conserved interactions. Our results indicate that the WD repeats of $hTAF_{II}100$ are not absolutely necessary for interactions with other hTFIID subunits and suggest that these repeats may participate in interactions between the TFIID complex and other partner proteins, such as other members of the basal transcription machinery. Similarly, the WD repeats of $dTAF_{II}80$ are not required for protein–protein interactions *in vitro* with the TFIID subunits $dTAF_{II}110$ and dTBP (Kokubo *et al.*, 1993b). It is conceivable that each individual repeat interacts with a different partner and, as each repeat has a distinct but related amino acid sequence, the partner proteins need not have common structures.

In the N-terminal half of $hTAF_{II}100$, $dTAF_{II}80$ and $yTAF_{II}90$ there is a second region (between amino acids 210 and 374 in $hTAF_{II}100$ and the corresponding regions in the two other $TAF_{II}s$) that is also evolutionary conserved (66% similarity between $hTAF_{II}100$ and $dTAF_{II}80$, and 56% similarity between $hTAF_{II}100$ and $yTAF_{II}90$). C-terminal deletions in this region seriously impaired the ability of $hTAF_{II}100$ mutants to enter into the TFIID complex in HeLa cells (Figure 3C). The region between amino acids 282 and 353, that was absolutely required for the incorporation of $hTAF_{II}100$ mutants into the TFIID complex, contains a conserved putative α-helix (amino acids 313–324). This α-helix was identified by an improved secondary structure prediction program that takes into account the sequence alignments of the $TAF_{II}100$ homologues in yeast and *Drosophila* (Rost and Sander, 1993; Rost *et al.*, 1994). Thus, this minimal region, together with the adjacent amino acids (353–392), deletion of which weakened but did not abolish binding to TFIID, seems to be important for TAF–TAF interactions.

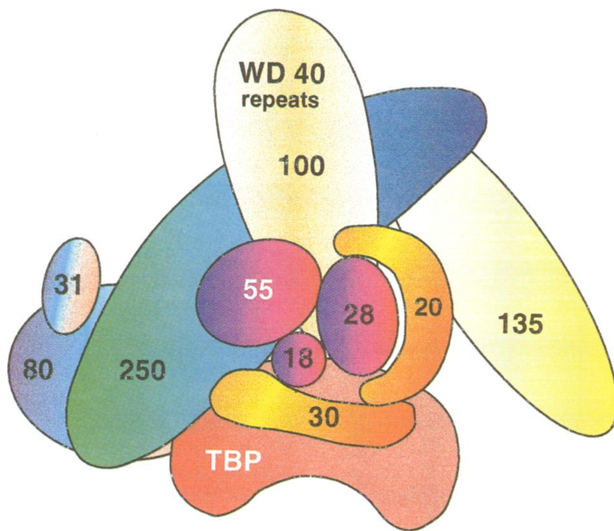


Fig. 8. Schematic representation of the $hTAF_{II}100$ interactions within the TFIID complex. The model includes most of the previously described human TAF_{II}–TAF_{II} and $hTAF_{II}$–TBP interactions (Ruppert *et al.*, 1993; Jacq *et al.*, 1994; Histake *et al.*, 1995; Klemm *et al.*, 1995; Mengus *et al.*, 1995). The numbers represent the apparent molecular masses of the TAF_{II}s in kDa.

The two anti-$hTAF_{II}100$ mAbs, which were raised against epitopes in the N-terminal (mAb2D2) and in the C-terminal (mAb1TA) regions of $hTAF_{II}100$ (see Figure 1A and B), have different effects on transcription. The fact that mAb1TA inhibits *in vitro* transcription is in good agreement with the suggestion that the C-terminal half of $hTAF_{II}100$ is responsible for making contacts between the TFIID complex and other transcription factors (see below). The other antibody, mAb2D2, does not inhibit transcription, indicating that its epitope is not involved directly in transcription. This epitope, in the non-conserved N-terminal region of $hTAF_{II}100$ (Figure 1B), is accessible in the native TFIID complex since mAb2D2 can immunoprecipitate the TFIID complex. This short region seems not to be involved in either TAF_{II}–TBP or TAF_{II}–TAF_{II} interactions (or other interactions that are important for transcription), suggesting that the domain responsible for interactions within the TFIID complex may begin C-terminal from the mAb2D2 epitope.

Taken together, all these results suggest that $hTAF_{II}100$, but also $dTAF_{II}80$ and $yTAF_{II}90$, can be divided into at least two functional domains, one located in the N-terminal half which is responsible for interactions within the TFIID complex and a second located in the C-terminal half containing the WD repeats, that may be involved in contacts between the TFIID complex and other partner factors (see also below).

$hTAF_{II}100$ interactions within the TFIID complex

$hTAF_{II}100$ interacts with several TAF_{II}s (see Figure 8 and Table I). These interactions seem to be direct protein–protein interactions for several reasons. First, the overexpressed human TAF_{II}s in SF9 cell extracts account for 5% or more of the total protein. Thus, as the human TAF_{II}s exceed by at least 1000-fold the endogenous insect cell TAF_{II} concentrations, it is unlikely that the insect cell TAF_{II}s can serve as bridging factors for these interactions. Furthermore, when human TAF_{II}s are overexpressed in HeLa cells, endogeneous bridging TAF_{II}s have not been detected by Western blots (data not shown). Second, we observed the same TAF_{II}–TAF_{II} interactions independently when the TAF_{II}s have either been pre-purified and incubated together before immunoprecipitation (data not shown) or co-expressed together and immunoprecipitated (Figure 4). The strong interaction of $hTAF_{II}100$ with $hTAF_{II}250$, $hTAF_{II}55$ and $hTAF_{II}28$ suggests that these $hTAF_{II}$s may be the main contact points of $hTAF_{II}100$ in the TFIID complex (see Figure 8). Both overexpressed $hTAF_{II}55$ and $hTAF_{II}28$ interact with the CΔ418 and CΔ353 $hTAF_{II}100$ mutants, but not with the CΔ282 mutant, in HeLa cell co-transfection experiments (data not shown), further suggesting that the N-terminal half of $hTAF_{II}100$ is responsible for the TAF_{II}–TAF_{II} interactions within the TFIID complex.

Previously, Dynlacht *et al.* (1993) found no interactions between the *Drosophila* homologue of $hTAF_{II}100$, $dTAF_{II}80$, and dTBP or $dTAF_{II}110$. In contrast, Kokubo *et al.* (1993b) described that both TBP and $dTAF_{II}110$ can bind to $dTAF_{II}80$. The contradictory TAF_{II}–TAF_{II} interactions described for $dTAF_{II}80$ are probably due to the different assay conditions used (Dynlacht *et al.*, 1993; Kokubo *et al.*, 1993b). In our hands, $hTAF_{II}100$ binds reproducibly to hTBP, but with a weaker affinity when compared with the binding of $hTAF_{II}100$ to other TAF_{II}s (see Figure 4). Moreover, $hTAF_{II}100$, TBP and $hTAF_{II}250$ form a stable partial TFIID complex (data not shown). In contrast, $dTAF_{II}80$ could only be incorporated in a higher order partial TFIID which includes TBP, $dTAF_{II}250$, $dTAF_{II}110$ and $dTAF_{II}60$ (Dynlacht *et al.*, 1993). In this respect, the interactions amongst the human TAF_{II}s and TBP are different from those described amongst the equivalent *Drosophila* proteins. We failed to detect an interaction between $hTAF_{II}100$ and $hTAF_{II}135$, the human homologue of $dTAF_{II}110$. One possible explanation for the lack of interaction may be that the $hTAF_{II}135$ protein is N-terminally truncated, since the isolated cDNA encodes only the C-terminal 733 amino acids of $hTAF_{II}135$. Thus, if there is an interaction between the full-length $hTAF_{II}135$ and $hTAF_{II}100$ then the N-terminal end of $hTAF_{II}135$ may be involved in this binding. The interactions between $dTAF_{II}80$ and the *Drosophila* homologues of $hTAF_{II}28$ ($dTAF_{II}30\beta$) or $hTAF_{II}55$ have not yet been tested. In the future, it will be interesting to investigate how these interactions compare with those described here for $hTAF_{II}100$ (see Table I and Figure 8). Note that, at present, the *Drosophila* homologue of $hTAF_{II}55$ is not known.

The possible role of $hTAF_{II}100$ in the TFIID complex

The direct interaction between $hTAF_{II}100$ and TFIIFβ (RAP30) was abolished by mAb1TA, the same antibody which inhibits basal Pol II transcription. Thus, our results suggest that this interaction is required for efficient basal transcription and that the C-terminal region of $hTAF_{II}100$, containing the mAb1TA epitope, is required to contact TFIIFβ (RAP30). The interaction between $hTAF_{II}100$ and TFIIFβ (RAP30) could be important for the recruitment of RNA polymerase II to the template, since a variety of evidence suggests that TFIIF (RAP74/30) enters into the pre-initiation complex together with Pol II (reviewed in Serizawa *et al.*, 1994). The inhibition by mAb1TA is not very strong (2- to 3-fold), possibly reflecting the fact that

other TAF_{II}s in the TFIID complex may functionally contact other members of the transcription machinery (Goodrich *et al.*, 1993; Histake *et al.*, 1995; Klemm *et al.*, 1995). Thus, the anti-$hTAF_{II}100$ antibody may destabilize only one of these contacts. A partial TFIID complex, containing only TBP and $hTAF_{II}250$, gives a reproducibly lower basal transcription activity than TBP alone (V.Dubrovskaya and L.Tora unpublished results; Kokubo *et al.*, 1993a). However, when the amount of TBP is equilibrated, purified TFIID complexes allow the same or higher basal transcription activities as TBP alone. This suggests that TAF_{II}s, such as $hTAF_{II}100$, can overcome the negative effect of $hTAF_{II}250$ in the native TFIID complexes possibly by stabilizing the formation of a productive PIC. In addition, TAF_{II} basic transcription factor contacts seem to confer a slight positive effect on transcription when saturating levels of TFIID or TBP are compared (data not shown), suggesting that these contacts not only overcome the negative effect of $hTAF_{II}250$ but that they positively participate in basal Pol II transcription.

At the present time, no direct interactions have been detected between $hTAF_{II}100$ and the acidic activation domain of VP16 or between $hTAF_{II}100$ and the two different activation domains of the human oestrogen receptor (data not shown). Similarly, no activators have been shown to interact with either $dTAF_{II}80$ or $yTAF_{II}90$. Further experiments will be necessary to determine whether $hTAF_{II}100$, or its homologues, are co-activators for specific activation domains. It is also conceivable that $hTAF_{II}100$ is not contacted by transcriptional activators but is a link between the TFIID complex and other components of the basal transcription machinery. A further possibility is that the $hTAF_{II}100$–RAP30 interaction is enhanced following interaction between transcriptional activators and other TAF_{II}s.

Materials and methods

Purification of the TFIID complexes and peptide sequencing of hTAF$_{II}$100

Nuclear extracts from 10^{12} HeLa cells were prepared and purified on heparin Ultrogel, phosphocellulose P11 and phenyl-5PW columns as described in Brou *et al.* (1993a). TFIID was affinity purified from the PC1.0-derived phenyl-5PW fractions using the purified anti-hTBP antibody 3G3. Bound TFIID was eluted by using an excess of the corresponding epitope peptide. The eluted PC1.0 TFIID was dialysed against buffer S [5 mM NaCl, 0.5 mM Tris–HCl pH 7.9, 0.005 mM dithiothreitol (DTT), 0.01% SDS] for 6 h, lyophilized and resolved by SDS–PAGE. The proteins were transferred to a PVDF membrane (Millipore, Immobilon P, 0.45 μm), stained briefly with Coomassie Brilliant Blue, excised and digested with trypsin. The eluted peptides were fractionated by reverse-phase HPLC and microsequenced as described by Brou *et al.* (1993b).

Cloning of the cDNA for the 100 kDa TBP-associated protein

The peptide sequence AFEDLETDDFTTATGHINLP was used to generate two degenerate primers (5′-GCA/C/T TTT GAG GAC/T CTG/T GAG AC-3′, 5′-CC T/CAA ATT G/ATT T/G/AAT G/ATG T/G/C/AGG-3′). These primers were used in PCR using a human HeLa cDNA library template to amplify the 60mer corresponding to the peptide sequence. The PCR products were verified by hybridization with a ^{32}P-labelled non-overlapping third degenerative primer (5′-GAT GAC/T TTC/T ACA/C/T ACA GCA/C/T AC-3′). The correct 60 bp long PCR product was then reamplified with *Eco*RI sites, inserted in a Bluescript SK+ vector (BSK+, Stratagene), sequenced and used as a probe for screening the HeLa cell random primed cDNA library made in the λZAP II system according to Stratagene. After the third round of screening, five positive clones were sequenced and two were found to encode the entire $hTAF_{II}100$ protein.

Construction of eukaryotic hTAF$_{II}$100 expression vectors and HeLa cell transfections

To construct the eukaryotic expression vector $pXJTAF_{II}100$, the *Xho*I–*Not*I $hTAF_{II}100$ cDNA fragment from BSK+ was inserted into the corresponding sites of pXJ42 (Xiao *et al.*, 1991). To express the HA epitope-tagged $hTAF_{II}100$ protein, the oligonucleotide 5′-CCCACCAT-GGCTACCCCTACGACGTGCCCGACTACGCCATCGAAC-3′ and its complementary strand were inserted into the *Apa*I–*Xho*I sites of the $pXJTAF_{II}100$ expression vector, to create pXJHA-$hTAF_{II}100$, thus adding the HA epitope-containing peptide (MGYPYDVPDYAIELEST) to the N-terminus of $hTAF_{II}100$. Expression vectors for the HA-ΔN418 and the HA-ΔC418 mutants were constructed by deleting from pXJHA-$TAF_{II}100$ either the *Xho*I–*Bam*HI fragment encoding the first 418 amino acids of $TAF_{II}100$ or the *Bam*HI fragment encoding amino acids 418–799 of $TAF_{II}100$, respectively. cDNA fragments encoding the other HA-$TAF_{II}100$ mutants shown in Figure 3C were generated by PCR amplification, digested with the appropriate restriction enzyme and cloned in the corresponding sites of the pXJ42 vector. All constructions were verified by sequencing.

HeLa cell transfections and WCE preparations were as described in Tasset *et al.* (1990). A total of 20 μg of DNA was used in each transfection (in 9 cm Petri dishes), which included 5 μg of the corresponding expression vector (as indicated in Figure 3), and carrier DNA (BSK+, Stratagene).

Construction of baculovirus expression vectors for TAF$_{II}$s, TFIIF and protein expression

The construction of the pVL1392-$HATAF_{II}250$ has been described (Jacq *et al.*, 1994). The cDNA encoding HA-$TAF_{II}100$ was excised from pXJHA-$TAF_{II}100$ by *Xba*I–*Spe*I and inserted into the corresponding sites of pVL1393 baculovirus expression vector (O'Reilly *et al.*, 1992). To express $TAF_{II}100$ with a histidine tag, the *Xho*I–*Not*I $TAF_{II}100$ cDNA fragment was inserted in-frame into the pAcSG HisNT-A vector (PharMingene). The cDNA encoding the C-terminal 733 amino acids of $hTAF_{II}135$ (G.Mengus and I.Davidson, unpublished result) was inserted into the *Eco*RI site of the pAcSG HisNT-A vector (PharMingene). The cDNA encoding HA-TAF30 was excised from pXJ-HATAF30 vector (Jacq *et al.*, 1994) and inserted into the pVL1392 vector (O'Reilly *et al.*, 1992). cDNAs encoding $hTAF_{II}18$, $hTAF_{II}20$, $hTAF_{II}28$ and $hTAF_{II}55$ (Mengus *et al.*, 1995; Lavigne *et al.*, 1996) were PCR amplified with the appropriate restriction sites, digested and inserted in-frame into the pAcSG HisNT-A vector (PharMingene). cDNAs encoding RAP74 and the RAP30 (Sopta *et al.*, 1989) were PCR amplified with *Xba*I sites and inserted in the corresponding sites of pAcAB3 (Belyaev and Roy, 1993) and pVL1392 (O'Reilly *et al.*, 1992), respectively. SF9 cell infection, plaque purification and WCE preparation were performed as described (O'Reilly *et al.*, 1992; Brou *et al.*, 1993c).

Immunization and monoclonal antibody production

Immunization and mAb production were essentially as described by Brou *et al.* (1993a). Mice were injected intraperitoneally three times at 2 week intervals, with 100 μg of the synthetic peptide (see peptides 1 and 2 in Figure 1B) coupled to ovalbumin. Spleen cells were fused with Sp2/0.Ag 14 myeloma cells and culture supernatants at day 10 were tested on recombinant and/or endogenous $hTAF_{II}100$ proteins by Western blot analysis, by immunostaining of transfected Cos-1 cells and by ELISA. The ovalbumin-coupled peptide 1-injected mice generated mAb2D2 and the ovalbumin-coupled peptide 2-injected mice generated mAb1TA. Both mAbs were found to be IgG_1,κ molecules.

The anti-TBP mAb (3G3), the anti-$TAF_{II}30$ mAb (2F4), the anti-$TAF_{II}18$ mAb (16TA), the anti-$TAF_{II}20$ mAb (22TA), the anti-$TAF_{II}28$ mAb (15TA), the anti-$TAF_{II}55$ mAb (19TA) and the anti-$TAF_{II}135$ mAb (20TA) are described in Brou *et al.* (1993a), Jacq *et al.* (1994), Mengus *et al.* (1995) and Lavigne *et al.* (1996). The anti-TFIIFα and anti-TFIIFβ mAbs were raised (as described above) against the respective full-length *E.coli* overexpressed recombinant TFIIF subunits. The anti-HA mAb (12CA5) was purchased from Boehringer Mannheim.

Co-immunoprecipitation and Western blot analysis

Routinely, 200 μl of the transfected HeLa cell extracts or 100 μl of infected SF9 cell extracts were immunoprecipitated with 20–50 μl of protein G–Sepharose (Pharmacia) and 2–5 μg of the different mAbs as indicated in the figures. The beads were then washed three times with 1 ml of IP buffer [25 mM Tris–HCl pH 7.9, 10% (v/v) glycerol, 0.1%

NP-40, 0.5 mM DTT, 5 mM $MgCl_2$] containing 500 mM KCl, and three times with 1 ml of IP buffer containing 100 mM KCl. After washing, bound proteins were either eluted with an excess of the corresponding epitope peptide and analysed by SDS–PAGE or directly 2–10 µl of beads were boiled in SDS sample buffer and proteins run on SDS–PAGE. Protein samples were then transferred to a nitrocellulose filter and probed with the appropriate antibodies. Western blot analyses were performed by standard methods using an ECL kit (Amersham).

In vitro transcription

The *E.coli* overexpression and the purification of recombinant human TBP, His-TFIIA α/β and His-TFIIAγ, TFIIB, His-TFIIEα and β was described previously (Moncollin *et al.*, 1992; Brou *et al.*, 1993a; Chalut *et al.*, 1994; Sun *et al.*, 1994). TFIIF and Pol II were purified as follows: the heparin–Ultrogel 0.6 M KCl-derived phosphocellulose 0.5 M KCl (PC0.5) fraction (Brou *et al.*, 1993a) was loaded on a DEAE–5PW HPLC column in 50 mM KCl-containing buffer A [25 mM Tris–HCl pH 7.9, 0.5 mM DTT, 0.1 mM EDTA, 10% glycerol (v/v)] and eluted stepwise with the same buffer containing 0.15, 0.25 and 1 M KCl. Pol II was eluting between 0.25 and 1 M KCl. To purify TFIIF, the PC0.5 fraction (Brou *et al.*, 1993a) was loaded on a Phenyl-5PW HPLC column in buffer A containing 0.9 M ammonium sulfate, and proteins were eluted with a linear 0.9–0 M ammonium sulfate gradient. TFIIF eluted in fractions containing ~0.7 M ammonium sulfate. The TFIIF- and Pol II-containing fractions were not contaminated by any other transcription factors, as verified by Western blot analysis (data not shown). All protein fractions were dialysed against buffer B [25 mM Tris–HCl pH 7.9, 50 mM KCl, 0.5 mM DTT, 0.1 mM EDTA, 20% glycerol (v/v)]. *In vitro* transcriptions were performed essentially as described by Brou *et al.* (1993a). Each 25 µl reaction contained 25 ng of supercoiled plasmid (pAL7; containing the AdMLP; Brou *et al.*, 1993c), 20–50 ng of the purified recombinant proteins, 2–3 µl of the purified fractions along with aliquots of the purified mAbs (as indicated) and an appropriate amount of buffer B. After the pre-incubation steps (see legend to Figure 5), transcription was initiated by addition of nucleoside triphosphates to 0.5 mM and $MgCl_2$ to 5 mM. Transcriptions were then incubated further at 25°C for 45 min. RNA transcripts from the AdMLP were detected by quantitative S1 nuclease mapping (Tora *et al.*, 1989).

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Accession number

The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X95525.

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