Human TAF<sub>II</sub>135 potentiates transcriptional activation by the AF-2s of the retinoic acid, vitamin D3, and thyroid hormone receptors in mammalian cells

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We report for the first time the cloning of a complete cDNA encoding the human TFIID subunit hTAF<sub>II</sub>135 (hTAF<sub>II</sub>130). Full-length hTAF<sub>II</sub>135 comprises 1083 amino acids and contains two conserved domains present also in dTAF<sub>II</sub>110 and hTAF<sub>II</sub>105. We show that expression of hTAF<sub>II</sub>135 in mammalian cells strongly and selectively potentiates transcriptional stimulation by the activation function-2 (AF-2) of the retinoic acid, thyroid hormone, and vitamin D3 receptors (RAR, TR, and VDR), but does not affect the AF-2s of the estrogen (ER) or retinoid X (RXR) receptors. The coactivator activity requires an hTAF<sub>II</sub>135 region that is located between the conserved domains but is itself not conserved in dTAF<sub>II</sub>110 and hTAF<sub>II</sub>105. Expression of hTAF<sub>II</sub>135 also stimulates RAR AF-2 activity when a promoter with a low-affinity TATA element (TGTA) is used, indicating that hTAF<sub>II</sub>135 overexpression compensates for the low-affinity of TBP for this promoter and may facilitate the recruitment of TFIID by the RAR AF-2.

[Key Words: TFIID; coactivators; TATA-binding protein; nuclear receptors; recruitment]

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The RNA polymerase II (Pol II) transcription factor TFIID comprises the TATA-binding protein (TBP) and TBP-associated factors (TAF<sub>II</sub>s) (Dynlacht et al. 1991; Pugh and Tjian 1991; Tanese et al. 1991; Timmers et al. 1992; Zhou et al. 1992; Brou et al. 1993; Chiang et al. 1993; for reviews, see Hernandez 1993; Goodrich and Tjian 1994; Zhou et al. 1992; Brou et al. 1993; Chiang et al. 1993; for reviews, see Hernandez 1993; Goodrich and Tjian 1994; Burley and Roeder 1996). The cDNAs encoding many TAF<sub>II</sub>s have been isolated revealing a striking sequence conservation from yeast to human (Jacq et al. 1994; Mengus et al. 1995; Bertolotti et al. 1996; Dubrovskaia et al. 1996; Hoffmann and Roeder 1996; Kekkonen et al. 1996; Lavigne et al. 1996; Moqtaderi et al. 1996a; for additional references, see reviews by Struhl 1995; Burley and Roeder 1996). TFIID plays a key role in regulated Pol II transcription in vitro, as TFIID, but not TBP, can support activator-dependent transcription (Hoey et al. 1990; Pugh and Tjian 1990; Zhou et al. 1992; Brou et al. 1993; for reviews, see Goodrich and Tjian 1994; Tjian and Maniatis 1994; Stargell and Struhl 1996a). Thus, in animal systems, TAF<sub>II</sub>s appear to be essential for the response to transcriptional activators in vitro.

Additional support for this TAF<sub>II</sub> function has come from studies showing direct and selective interactions between TAF<sub>II</sub>s and transcriptional activator proteins (Goodrich et al. 1993; Hoey et al. 1993; Chen et al. 1994; Gill et al. 1994; and references therein). For example, the estrogen receptor (ER) interacts directly with hTAF<sub>II</sub>30 and ligand-independent activation in vitro by the ER requires TFIID complexes containing hTAF<sub>II</sub>30 (Jacq et al. 1994). Interactions between multiple activators and TAF<sub>II</sub>s have been reported to result in transcriptional synergy in vitro (Sauer et al. 1995). Together, these studies show that TAF<sub>II</sub>s act as specific coactivators in vitro by engaging in direct and selective interactions with transactivators.

This model of TAF<sub>II</sub> function has been challenged by genetic studies in yeast where it has been shown that the presence of several yeast TAF<sub>II</sub>s is not required for activated transcription (Apone et al. 1996; Moqtaderi et al. 1996a; Walker et al. 1996). Nevertheless, TAF<sub>II</sub>s do play an essential role in yeast because mutant strains are not viable, being blocked in the cell cycle, reminiscent of what is observed in mammalian cells where TAF<sub>II</sub>250...
has been mutated (Sekiguchi et al. 1991; Reese et al. 1994; Poon et al. 1995; Apone et al. 1996; Klebanow et al. 1996). Therefore, there is a discrepancy between the fact that TAF<sub>n</sub>8s are dispensible for activator function in yeast, but are absolutely required for this function in animal cell extracts in vitro.

Evidence that hTAF<sub>n</sub>28 may function as coactivators in mammalian cells has come from the investigation of the role of hTAF<sub>n</sub>28 in transcriptional activation by nuclear receptors [NRs]. NRs generally comprise two activation functions [AFs], AF-1 located in the amino-terminal A/B region and AF-2 located in the ligand-binding domain [LBD] in the carboxy-terminal E region (for review, see Parker 1993, Chambon 1994, 1996; Tsai and O'Malley 1993, Chambon 1994, 1996; Beato 1995, Kastner et al. 1995, Lauder and Gronemeyer 1995, Mangelsdorf et al. 1995, Mangelsdorf and Evans 1995). The AF-2 is ligand inducible and requires the AF-2 activating domain core [AF-2 AD core], a conserved amphipathic α-helix at the carboxyl end of the LBD [Danielian et al. 1992; Barettoni et al. 1994; Durand et al. 1994; for review, see Chambon 1996]. Ligand binding induces a conformational change bringing the AF-2 AD core [α-helix H12] into contact with α-helix H4 of the LBD [Bourguet et al. 1995; Renaud et al. 1995; Wagner et al. 1995; Wurtz et al. 1996] allowing the NRs to interact with putative transcriptional intermediary factors [TIFs] required for AF-2 activity [Cavaillès et al. 1995; Le Douarin et al. 1995, 1996; Lee et al. 1995; Onate et al. 1995; Swaffield et al. 1995; Vom Baur et al. 1995; Chakravati et al. 1996; Fondell et al. 1996; Hanstein et al. 1996; Hong et al. 1996; Kamei et al. 1996; Smith et al. 1996; Voegel et al. 1996; Yao et al. 1996; and references therein].

We have shown that TAF<sub>n</sub>28 is depleted in Cos cell TFIID. Ectopic expression of recombinant hTAF<sub>n</sub>28, a fraction of which associates stably with Cos cell TFIID, strongly enhances activation by the retinoic acid receptor [RAR], the thyroid hormone receptor [TR], and VDR AF-2s, whereas no effect was seen on activation by the ER, the RXXR, or with unrelated activators. Using a promoter with a TGTA element instead of a TATA element, we show that coexpression of hTAF<sub>n</sub>135 and the cognate TBP spm3 mutant results in activation by the RAR AF-2. However, expression of hTAF<sub>n</sub>135, in the absence of coexpressed TBP spm3, also allowed activation by the RAR, TR, and VDR AF-2s, but did not affect the activity of the VP16 or TEF-1 AFs. This observation indicates that hTAF<sub>n</sub>135 most probably acts to facilitate the recruitment of the endogenous TFIID by the RAR, TR, and VDR AF-2s.

**Results**

**Molecular cloning of hTAF<sub>n</sub>135**

HeLa cell TFIID was isolated by chromatography, immunopurified with anti-TBP monoclonal antibodies [Mengus et al. 1995, Lavigne et al. 1996], and the 135-kD subunit was excised and the sequences of four tryptic peptide fragments [underlined in Fig. 1A] were obtained [see Materials and Methods]. Degenerate oligonucleotides derived from two of the peptide sequences [see Materials and Methods] were used to screen a HeLa cell cDNA library. Alignment of the sequences of four isolated cDNAs indicated the existence of a 732-amino-acid open reading frame [ORF] corresponding to the central and carboxy-terminal regions of hTAF<sub>n</sub>135 [see Fig. 1A]. Screening of several cDNA libraries failed to yield cDNAs extending further upstream, because of the high GC content of this region blocking reverse transcription.

To isolate a full-length hTAF<sub>n</sub>135 ORF, the first 400 nucleotides of the partial cDNA were used to screen a human genomic DNA library. A 4.7-kb genomic fragment was subcloned and sequenced [see Materials and Methods]. This fragment contained the first 101 amino acids of the cDNA followed by an intron beginning at nucleotide 1356 [see Fig. 1A]. Analysis of the genomic sequence upstream of the cDNA revealed a highly GC rich region, potentially encoding a further 351 amino acids initiated by an ATG codon. An in-frame stop codon was found upstream of this ATG [data not shown].

To determine whether the predicted sequence encodes hTAF<sub>n</sub>135, the total ORF of 1083 amino acids was expressed in transfected Cos cells [see Materials and Methods]. The recombinant protein was detected using an antibody to TBP (mAb 20TA), raised against bacterially produced histidine-tagged hTAF<sub>n</sub>135 [185-1083] [see Fig. 2; Materials and Methods]. mAb 20TA recognizes endogenous hTAF<sub>n</sub>135 in HeLa cell nuclear extracts [NE] and in the HeLa TFIID immunopurified [IP] using the anti-TBP mAb 3G3 [Fig. 1C, lanes 1, 4, 7, 8]. Recombinant hTAF<sub>n</sub>135 had an electrophoretic mobility identical to that of the endogenous HeLa cell TAF<sub>n</sub>135 [Fig. 1C, lanes 7-10], and could be precipitated from the transfected cell extracts by mAb 20TA [Fig. 1C, lane 10].
TAF\(_{135}\) enhances RAR AF-2 activity

**Figure 1.** (A) Nucleotide and amino acid sequence of hTAF\(_{135}\). The numbers indicate the nucleotides and the numbers in parenthesis the amino acids. The regions derived from the cDNA and genomic clones are indicated along with the position of an intron whose position was determined from sequencing of the genomic clone. A putative intron located between K228 and P261 is indicated by open brackets. The positions of the amino termini of the deletion mutants are indicated by the arrows. Peptides derived from endogenous HeLa cell hTAF\(_{135}\) are underlined. The open boxes show the peptides P1 and P2 used to generate antibodies and the filled box indicates the region required for coactivator activity. (B) Alignment of the amino acid sequences of hTAF\(_{135}\), hTAF\(_{105}\), and dTAF110. For clarity only the carboxy-terminal region where there are significant homologies is shown. The numbers indicate the position of the nucleotides in the genomic clone. A putative intron located between K228 and P261 is indicated by open brackets. The positions of the amino termini of the deletion mutants are indicated by the arrows. Peptides derived from endogenous HeLa cell hTAF\(_{135}\) are underlined. The open boxes show the peptides P1 and P2 used to generate antibodies and the filled box indicates the region required for coactivator activity.

**C**

- **Cell Extracts**
  - IP-TFID
  - hTAF\(_{135}\)
  - hTAF\(_{105}\)
  - TBP

- **HeLa N.E. Transfected Extracts**
  - hTAF\(_{135}\)
  - hTAF\(_{105}\)
  - TBP

- **Analysis of TAF\(_{135}\) and TAF\(_{105}\) Enhancing Activity**
  - mAbs 19TA + 20TA + 3G3 mAb 20TA Abanti-P1 + mAbs 20TA + is indicated by open brackets. The positions of TAFII\(_{135}\), TAFn\(_{105}\), the TBPs from human (HeLa), monkey (Cos), and mouse (MPC11), and the heavy chain of the antibody [lgG2H] used in the immunopurifications are indicated. (Middle) The extracts shown above each lane were immunoprecipitated with the mAbs shown above the panel and the blot was revealed with the anti-hTAF\(_{135}\) mAb 20TA shown below the panel. (Right) Lanes 11–13 and 14–16 contain aliquots of the same material. (N.E.) nuclear extract, (IP TFID) TFID immunopurified with the anti-TBP mAb 3G3, (S.N.) immunoprecipitation supernatant.
To confirm that the region deduced from the genomic sequence was present in hTAFII135, we generated antibodies against two peptides in this region (P1 and P2, open boxes in Fig. 1A). These antibodies recognized endogenous HeLa cell TAFII135 in nuclear extracts and in immunopurified TFIIID (Fig. 1C, cf. lanes 11-13 revealed with anti-P1 antibody with lanes 14-16 revealed with mAb20TA; data not shown for anti-P2 antibody). Together these results show that the deduced ORF shown in Figure 1A is in the correct reading frame to encode recombinant hTAFII135 with an electrophoretic mobility and antigenicity identical to that of the endogenous HeLa cell protein.

Comparison of the amino acid sequence of hTAFII135 with that of other TAFIIIs clearly indicated that the carboxy-terminal domain of hTAFII135 is highly homologous to that of dTAFII110 and hTAFII105 (Fig. 1B; Dikstein et al. 1996; Tanese et al. 1996). A second region in the central portion of these proteins is also conserved, particularly between hTAFII135 and hTAFII105, [amino acids 589-680 in Fig. 1B; see Tanese et al. 1996]. Our amino-terminal amino acid sequence extends the partial sequence reported by Tanese et al. (1996), which begins at G118. Unexpectedly, however, the amino acids amino-terminal to L398 differ from those reported by Dikstein et al. (1996).

**hTAFII135 potentiates transcriptional activation by the AF-2 of RARs in several cell lines**

Expression of hTAFII28 in Cos cells potentiated selectively and strongly transcriptional activation by the AF-2s located in the LBD (region E) of the RXR, ER, and VDR (May et al. 1996). Similarly we tested the ability of the newly cloned hTAFII135 to modulate transcriptional activation by NRs and other activators. Full-length hTAFII135 was coexpressed along with chimeras containing the AF-2s of different activators fused to the DNA-binding domain of the yeast activator Gal4 (G4), and the G4-responsive reporter gene 17m5–TATA–CAT [the reporters and activators are schematized in Fig. 2; see May et al. 1996].

The AF-2 in G4–RARα(DEF) activates transcription in transfected Cos cells in a ligand-dependent manner (Fig. 3A, lane 3) (Dikstein et al. 1996; Tanese et al. 1996). A second region in the central portion of these proteins is also conserved, particularly between hTAFII135 and hTAFII105, [amino acids 589-680 in Fig. 1B; see Tanese et al. 1996]. Our amino-terminal amino acid sequence extends the partial sequence reported by Tanese et al. (1996), which begins at G118. Unexpectedly, however, the amino acids amino-terminal to L398 differ from those reported by Dikstein et al. (1996).
TAF\textsubscript{II}135 enhances RAR AF-2 activity

8–14]. With both full-length TAF\textsubscript{II}135 and TAF\textsubscript{II}135(372–1083) activation was strictly ligand dependent (Fig. 3B, lanes 7, 11; data not shown). In contrast, no activation was seen either in the presence or absence of hTAF\textsubscript{II}135 using the dominant-negative mutant G4-dnRAR\textsubscript{II}(DEF) in which the AF-2 AD core has been deleted (Fig. 4C). Thus, hTAF\textsubscript{II}135 expression strongly potentiates the activity of the RAR AF-2.

The region of hTAF\textsubscript{II}135 required for this enhancement was localized using a series of deletion mutants. Coexpression of hTAF\textsubscript{II}135(372–1083) and (713–1083) resulted in a strong increase in RAR AF-2 activity, whereas hTAF\textsubscript{II}135(372–718) had no significant effect (Fig. 4A, lanes 3–8). Similarly, expression of hTAF\textsubscript{II}135(630–1083) strongly potentiated RAR AF-2 activity, whereas no significant effect was seen with hTAF\textsubscript{II}135(805–1083) (Fig. 4B, lanes 3–6). All deletion mutants were expressed at comparable levels, higher than that of full-length hTAF\textsubscript{II}135 (Fig. 4D). Thus, a domain residing within, or overlapping with, amino acids 713–805 of hTAF\textsubscript{II}135 (boxed in Fig. 1A) is required to potentiate the activity of the RAR AF-2.

We evaluated the possible cell specificity of the hTAF\textsubscript{II}135 enhancement effect. As in Cos cells, RAR\textsubscript{II} AF-2-activated transcription in CV1 cells was stimulated more than 20-fold by coexpression of hTAF\textsubscript{II}135(372–1083) (Fig. 5A), whereas it was enhanced only sixfold in HeLa cells where the optimum concentration of hTAF\textsubscript{II}135 expression vector was lower than in Cos and CV1 cells (Fig. 5B). In MPC11 lymphoid cells the RAR\textsubscript{II} AF-2 strongly activated transcription and coexpression of hTAF\textsubscript{II}135(630–1083) led to only a fourfold increase in its activity (Fig. 5C). These results show that hTAF\textsubscript{II}135 can potentiate activation by the RAR\textsubscript{II} AF-2 in several cell lines, albeit to different degrees.

The observation that ectopically expressed hTAF\textsubscript{II}135 enhances activation by the RAR AF-2 indicates that it is functionally limiting in the different cell types. The differential enhancement seen in these cell lines may reflect differences in the relative abundance of TAF\textsubscript{II}135. To test this possibility, the amount of TAF\textsubscript{II}135 present in HeLa, Cos, and MPC11 cell extracts was determined by immunoblotting. Each extract contained similar amounts of TBP and TAF\textsubscript{II}55 (Fig. 1C, lanes 1–3, bottom) as well as TAF\textsubscript{II}80 and TAF\textsubscript{II}18 (see May et al. 1996; data not shown). However, relative to both TBP and TAF\textsubscript{II}55, significantly less TAF\textsubscript{II}135 was seen in Cos and CV1 cell extracts, compared to HeLa and MPC11 cell extracts (Fig. 1C, lanes 1–3; data not shown). Confirming this result, TAF\textsubscript{II}135 was present, but less abundant in immunopurified Cos cell TFIIID than in HeLa and MPC11 cell TFIIIDs (Fig. 1C, lanes 4–6). Thus, the degree to
which ectopic expression of TAFII135 stimulates RAR AF-2 activity correlates inversely with the relative abundance of this protein in each cell type.

The experiments described so far were all performed using G4-AF-2 chimeras on a minimal promoter. To test whether hTAFII135 could also affect activation by wild-type NRs, the natural RARβ2 promoter was used. This promoter contains a RA response element (RARE) and is stimulated in the presence of RA. Transcription of a CAT reporter under the control of the RARβ2 promoter is stimulated by the endogenous RAR–RXR heterodimers upon addition of all-trans [T-RA] in transfected Cos cells (Nagpal et al. 1992, Fig. 5D, lanes 1,2). The coexpression of hTAFII135(630–1083) resulted in a fivefold increase in the ligand-dependent activity of this reporter (Fig. 5D, lanes 2–5). Expression of TAFII135 also enhanced ligand-dependent activation from the RARβ2 promoter in the presence of cotransfected wild-type RARα (Fig. 5E, lanes 3–6). Thus, coexpression of hTAFII135 enhances activation by endogenous and ectopically expressed wild-type RAR.

The coactivator activity of hTAFII135 is restricted to a subset of NR AF-2s

We then investigated whether hTAFII135 could potentiate activation by the AF-2s of other NRs. Expression of hTAFII135(1–1083) or (630–1083) resulted in >10-fold increase in the activity of the VDR and TR AF-2s (Figs. 3A, lanes 6–8, 13–15, 6A, lanes 7–10, and 6B, lanes 3–6). No significant increase in VDR and TR AF-2 activity was seen in the absence of ligand or with hTAFII135(805–1083) (Figs. 6A, lane 10, and B, lane 5; data not shown). In contrast, expression of hTAFII135(1–1083) or [630–1083] had no significant effect on the activity of the ER, RXRα, RXRβ, and RXRγ AF-2s (Fig. 3A, lanes 9–11, 16–18; data not shown), nor on activation by several activators that do not belong to the nuclear receptor superfamily (for example, see G4-TEF-1, Fig. 6B; see below), nor does it increase the amount of G4-NR chimeras expressed transiently (data not shown). Thus, expression of hTAFII135 does not result in a general nonspecific increase in transactivation, but its effect is restricted to only a subset of the NR AF-2s.

The NR specificity of hTAFII135 differs from that of hTAFII28, as its most potent effect was observed with the RAR and TR AF-2s, but not with the ER and RXR AF-2s, whereas the converse is true for hTAFII28 (May et al. 1996). However, as both hTAFII135 and hTAFII28 increased the activity of the VDR AF-2, we tested the effect of coexpression of both hTAFII135 and hTAFII28 on VDR AF-2 activity. Expression of hTAFII28 enhanced VDR AF-2 activity
Expression of hTAF$_{II135}$ enhances RAR AF-2 activity on a promoter containing a low affinity TATA element

To investigate the molecular mechanism by which hTAF$_{II135}$ enhances RAR AF-2 activity we used a promoter in which the TATA element has been mutated to TGTA. This TGTA element is not recognized efficiently by endogenous TBP, but is recognized by the altered specificity mutant TBP spm3 (Strubin and Struhl 1992). A luciferase reporter gene under the control of a promoter containing this mutated TGTA element and five G4-binding sites (17m5-TGTA-Luc; Keaveney et al. 1993) was transfected into Cos cells. This reporter was inactive even in the presence of coexpressed TBP spm3 (Fig. 8A, lane 1). The RAR$_{a}$ AF-2 alone did not activate transcription from this promoter, whereas coexpression of TBP spm3 resulted in a 10-fold stimulation of transcription (Fig. 8A, lanes 2,5). Coexpression of hTAF$_{II135}(630-1083)$ along with TBP spm3 resulted in a >25-fold increase in ligand-dependent RAR AF-2-activated transcription compared to that observed with TBP spm3 and the RAR AF-2 alone (Fig. 8A, lanes 5,7–11). Similar results were obtained with full-length hTAF$_{II135}$ (data not shown), whereas in control experiments, hTAF$_{II135}$ alone or in combination with TBP spm3 had no effect on transcription (Fig. 8A, lanes 3,4). Thus, ex-
pression of hTAF\textsubscript{135} enhances RAR AF-2 activity in the presence of cotransfected TBP spm3.

However, coexpression of hTAF\textsubscript{135}(630-1083) enhanced RAR AF-2 activity even in the absence of TBP spm3 [Fig. 8A, lane 6]. This stimulation was analogous to that seen with TBP spm3 and the RAR AF-2, but was 20-fold less than that seen with the combination of all three components showing that in the presence of coexpressed TAF\textsubscript{135}, the RAR AF-2 can activate transcription through the endogenous TFII D. Thus, hTAF\textsubscript{135} overexpression facilitates the formation of an initiation complex by the RAR AF-2 on the low affinity TGTA-containing promoter.

As observed with the RAR AF-2, the VDR and TR AF-2s alone did not activate transcription from the mutated TGTA promoter [Fig. 8B, lane 2, and Fig. 8C, lane 1], but coexpression of TBP spm3 or hTAF\textsubscript{135}(630-1083) resulted in significant and comparable levels of activation [Fig. 8B, lanes 3-4 and Fig. 8C, lanes 2-3]. However, in contrast to the RAR AF-2, coexpression of both hTAF\textsubscript{135} and TBP spm3 resulted in only a further threefold increase in VDR AF-2 activity [Fig. 8B, lanes 5,6], whereas no further increase was observed with the TR AF-2 [Fig. 8C, lanes 4,5]. Analogous results were obtained with full-length TAF\textsubscript{135} (data not shown). All effects were dependent on the presence of the corresponding ligands [Fig. 8B,C] and in control experiments, expression of TBP and hTAF\textsubscript{135} had no significant effect on promoter activity in the absence of the NR chimeras. Thus, as observed with the RAR AF-2, TAF\textsubscript{135}
TAF\(_{\text{II}135}\) enhances RAR AF-2 activity

**Figure 8.** (A) Expression of TBP spm\(_3\) and hTAF\(_{\text{II}135}\) enhances activation by the RAR AF-2. Transfections contained 2 \(\mu\)g of the 17m5-TGTA-Luc reporter plasmid, 1 \(\mu\)g of the TBP spm\(_3\) expression vector, 250 ng of the G4-NR expression vectors, 0, 2, and 5 \(\mu\)g of the TAF\(_{\text{II}135}(630-1083)\) expression vector and the cognate ligands as indicated. After correction for the \(\beta\)-galactosidase internal control, luciferase values were determined on a Berthold apparatus. (B) The layout is as described in A. Transfections contained 250 ng of the G4-VDR and 1 \(\mu\)g of the G4-RXR expression vectors. (C-D) The layout is as described above. Where indicated, transfections contained 250 ng of the G4-Spl and G4-AP-2 expression vectors and 100 ng of the G4-VP16 and G4-TEF-1 expression vectors.

Expression facilitates the formation of initiation complexes by the VDR and TR AF-2s on the low affinity TGTA-containing promoter.

Coexpression of hTAF\(_{\text{II}135}\) and TBP spm\(_3\) did not result in activation by the ER or RXR AF-2s [Fig. 8, A, lanes 12–16, and B, lanes 10–14, respectively], nor did hTAF\(_{\text{II}135}\) potentiate activation by the unrelated G4-AP-2 and G4-Spl activators [Fig. 8C, lanes 7–16; note that the G4-Spl chimera used in these experiments does not contain the region shown to interact with dTAF\(_{\text{II}10}\) and hTAF\(_{\text{II}135}\); Gill et al. 1994; Tanese et al. 1996], although they were expressed at comparable levels to the G4-NR chimeras [data not shown]. These results confirm that hTAF\(_{\text{II}135}\) acts selectively to enhance activation by the RAR, VDR, and TR AF-2s.

The effect of TAF\(_{\text{II}135}\) expression on the activity of two further unrelated transcriptional activators was determined. Even in the absence of TBP spm\(_3\), the potent VP16 and TEF-1 AFs stimulated transcription [Fig. 8D, lanes 6,11] and coexpression of TBP spm\(_3\) increased their activity [Fig. 8D, lanes 8,13]. In contrast, coexpression of hTAF\(_{\text{II}135}\) either alone or together with TBP spm\(_3\) had little effect [Fig. 8D, lanes 7,9,10,12,14,15]. These observations show that the VP16 and TEF-1 AFs can use the endogenous TFIIID to activate transcription from the TGTA promoter, and that their activity is not affected significantly by overexpression of hTAF\(_{\text{II}135}\). Thus, unlike the RAR, VDR, and TR AF-2s, these strong AFs are characterized by their ability to recruit efficiently cellular transcription factors to the TGTA-containing promoter in the absence of coexpressed hTAF\(_{\text{II}135}\).

Results similar, but not identical, to those described above were obtained with the NR chimeras in HeLa cells. The RAR AF-2 alone activated transcription in HeLa cells [Fig. 9, lanes 1–4] and coexpression of either TBP spm\(_3\) or hTAF\(_{\text{II}135}(630-1083)\) enhanced this activation by fourfold [Fig. 9, lanes 5–6]. Thus, overexpression of hTAF\(_{\text{II}135}\) in HeLa cells also promotes activation by the RAR AF-2 through the endogenous transcription machinery. However, the combination of hTAF\(_{\text{II}135}\) and TBP spm\(_3\) enhanced RAR AF-2 activity only two- to threefold more than that observed with either of the two
alone (lanes 7, 8) and therefore, to a lesser extent than in Cos cells.

No activation was seen in HeLa cells with the ER AF-2 alone, whereas coexpression of TBP spm3 resulted in ligand-dependent activation (Fig. 9, lanes 12, 13, 17–19). However, in contrast to the RAR AF-2, no significant ER AF-2 activity was seen in the presence of hTAFII135 and the activation seen in the presence of TBP spm3 was not increased significantly by the coexpression of hTAFII135 (lanes 14–16). Similarly, hTAFII135 had no effect on the weak activation seen with G4-Spl and TBP spm3 (lanes 20–24).

Together these results in HeLa and Cos cells with the TGTA promoter confirm, using an independent promoter configuration and gene expression assay, the results obtained with the 17m5-TATA-CAT and RARe2 reporters showing that hTAFII135 can enhance selectively activation by the RAR, VDR, and TR AF-2s.

**Discussion**

*hTAFII135* is related to *hTAFII105* and *dTAFII110*, but has no yeast homolog

We have characterized previously interactions between the carboxy-terminal domain of *hTAFII135* and other components of the TFIIID complex. By protein affinity chromatography in vitro and immunoprecipitations of transfected Cos cell extracts, we have shown that *hTAFII135* interacts with *hTAFII250* and *hTAFII20*, but not with *hTAFII100* or TBP (Mengus et al. 1995; Dubrovskaya et al. 1996). Here we describe the molecular cloning of full-length *hTAFII135* and show that it enhances RAR, VDR, and TR AF-2 activity in mammalian cells.

From cDNA and genomic fragments we have assembled an ORF encoding full-length *hTAFII135* of molecular mass identical to that of the endogenous HeLa cell protein. Antibodies directed against two amino-terminal peptides deduced from the genomic sequence recognize endogenous HeLa cell TAFII135 showing that this sequence is the bona fide TAFII135 ORF. Thus, the ORF reported here encodes a protein with electrophoretic and antigenic properties identical to those of endogenous HeLa cell TAFII135.

The sequence of the amino-terminal region of hTAFII135 reported here extends the partial cDNA sequence reported by Tanese et al. (1996), but differs from that reported by Dikstein et al. (1996). Comparison of our sequence with that of Tanese et al. indicates a deletion in the sequence of Tanese et al. between amino acids K228 and P261 (open brackets in Fig. 1A). Thus, the deleted sequence either corresponds to an intron, although there is no in-frame stop codon within this putative intron, nor is it flanked by consensus splice donor or acceptor sites, or results from reverse transcription or sequencing artifacts attributable to the high GC content (>90%) in this region.

TAFII135 clearly belongs to a family of proteins, including hTAFII105 and dTAFII110, containing a conserved carboxy-terminal domain that mediates interactions with other TFIIID subunits and the large subunit of TFIIA (Hoey et al. 1993; Mengus et al. 1995; Dikstein et al. 1996), as well as the interactions of hTAFII135 and dTAFII110 with the oncoproteins E1A, SV40 large T-antigen, and HPV E7 (Geisberg et al. 1995; Mazzarelli et al. 1995). This carboxy-terminal domain is clearly the hallmark of this family of proteins, conserved from *Drosophila* to humans. However, searches of the protein databases failed to reveal any homologous yeast proteins (G. Mengus et al., unpubl.). Thus, although yeast contains homologs for almost all other mammalian TAFII8 (Reese et al. 1994; Poon et al. 1995; Klebanow et al. 1996; Moqtaderi et al. 1996a,b; Walker et al. 1996), there appears to be no yeast homologs of the TAFII135, TAFII105, TAFII110 family.
AF-2, we were unable to detect RAR-hTAF135 interactions, et al., unpubl.), showing that vertebrate TAFHs may of other functional differences noted among these recep-
tors. Thus, TAF135 may be a specific coactivator for
other metazoans or yeast.

TAF135 specifically enhances activation by the class II NRs, RAR, TR, and VDR, which preferentially form heterodimers with RXRs. These NRs also share the abil-
ity to repress transcription in the absence of their cognate ligands and, at least for the RAR and TR, interact with corepressors such as a nuclear receptor corepressor [N-CoR] and SMRT [silencing mediator of retinoid and thyroid hormone receptors] [Chen and Evans 1995; Hor-
lein et al. 1995]. On the other hand, the ER, whose ac-
tivity is enhanced preferentially by hTAF135 [May et al. 1996], functions as a homodimer and does not act as a ligand-independent repressor. The differential ability of the NRs to respond to TAF135, therefore, is reminiscent of other functional differences noted among these recep-
tors. Thus, TAF135 may be a specific coactivator for certain class II NRs, whereas hTAF135 may enhance the activity of the class I NRs preferentially. However, both of these TAF135s enhance VDR AF-2 activity and their coexpression results in an additive effect.

Enhancement of NR AF-2 activity required the integ-
rity of a hTAF135 domain located within or overlapping with amino acids residues 713-805. This region is located between the two highly conserved domains of the TAF135, TAF105, TAF110 proteins, but is itself poorly conserved. Accordingly, expression of dTAF110 had no significant effect on RAR AF-2 activity (G. Mengus, et al., unpubl.), showing that vertebrate TAF135s may have evolved to perform specific functions not found in other metazoans or yeast.

Possible molecular mechanisms for TAF135 function

Although hTAF135 acts as a coactivator for the RAR AF-2, we were unable to detect RAR-hTAF135 interactions either in transfected Cos cells or in yeast two hybrid assays, whereas control experiments revealed strong hTAF135-hTAF20 interactions [our unpublished data]. Nevertheless, we show that TAF135 is function-
ally limiting for the activity of certain NR AF-2s in several mammalian cell lines and that the degree to which TAF135 expression enhances AF-2 activity correlates inversely with TAF135 levels in these cells. A stronger enhancement is observed in Cos cells that contain the lowest amounts of TAF135, whereas the effect is weaker in HeLa and MPC11 cells where TAF135 is relatively more abundant. As ectopically expressed hTAF135 associates with endogenous TFIIID [Tanese et al. 1996], it is likely that the enhancement of NR AF-2 activity results from an increase in the amount of TFIIID complexes containing TAF135, a situation analogous to that previously observed with TAF28 in Cos cells [May et al. 1996]. These observations support our previous proposal that substochiometric TAF135s present in only a subset of TFIIID complexes may mediate the effect of certain transactivators [Brou et al. 1993; jacq et al. 1994]. Interestingly, the related TAF105 is also present in substochiometric amounts in TFIIID from B cells [Dikstein et al. 1996].

To address the molecular mechanism of hTAF135 function, we used a promoter in which the TATA element has been replaced by a TGTA element to which the endogenous TFIIID binds with low affinity. With this reporter, coexpression of the cognate TBP pwm3 mutant and TAF135 resulted in strong activation by the RAR AF-2. However, coexpression of hTAF135 in the absence of TBP pwm3 also enhanced RAR AF-2 activity showing that TAF135 allows RAR AF-2-dependent formation of initiation complexes containing endogenous TFIIID, thereby compensating for its low affinity for the TGTA element. When coexpressed with TAF135, the RAR AF-2 behaves like strong AFs, exemplified here by VP16 or TEF-1, which activate transcription from the TGTA-containing promoter in the absence of cotrans-
formed TBP pwm3, irrespective of TAF135 overexpres-
sion. Considering the importance of the recruitment of TFIIID in transcriptional activation [Klages and Strubin 1995; Chatterjee and Struhl 1995; Chi and Carey 1996; Stargell and Struhl 1996b], the reported interactions be-
tween these strong AFs and TBP or other components of the preinitiation complex [Ingles et al. 1991; Lin et al. 1991; Gruda et al. 1993; for review, see Tjian and Mani-
atis 1994], may suffice to allow efficient recruitment of endogenous TFIIID to the TGTA-containing promoter. In contrast, for the RAR, VDR, and TR AF-2s, overexpression of TAF135 would be required for TFIIID to be recruted efficiently.

Several possible molecular mechanisms may be envis-
age to explain how TAF135 could enhance recruit-
ment of TFIIID by the NR AF-2. In view of the observed specificity of TAF135 for certain NRs and the lack of evidence for direct interactions with these NRs, it is tempting to speculate that TAF135 may interact with putative RAR-, VDR-, and TR-specific TAFs, thereby enhanced the recruitment of TFIIID. Further experiments will be required to identify interaction partners of TAF135 and lead to a better understanding of the molecular basis of its coactivator activity.
Materials and methods

Purification of HeLa cell TFIIID and cloning of hTAF135

Nuclear extract and TFIIID preparation was as described (Brou et al. 1993; Chaudhary et al. 1994). Immunopurified TFIIID was dialyzed against buffer containing 5 mM NaCl, 0.5 mM 8-Mercaptoethanol, and stained briefly with Coomassie blue. hTAF135 was excised and digested in situ on the membrane with trypsin. The eluted peptides were then separated by reverse phase high-pressure liquid chromatography (HPLC) and microsequenced. Degenerate oligonucleotides from peptides IMITTPQQIQLNPL, and AATVTSALQP, were synthesized, [α2P]-5'-end labeled, and used to screen a HeLa cell cDNA library. Three positive clones were purified. A 4.7-kb fragment, which hybridized to an oligonucleotide derived from both peptides were purified and in vivo excision was performed. The resulting clone was further modified to introduce a restriction site and a consensus Kozak sequence, and an ATG, and the 3' primer an ATG, and the 3' primer a BamHI restriction site; the proteins expressed from the pAT6 vector are B10-tagged, with B10 being the ER epitope for the mAb B10. The TBP spm3, G4 chimera expression vectors, and reporter plasmids were as described previously (Tora et al. 1989, Seipel et al. 1992, NagpaI et al. 1992, 1993; Hwang et al. 1993, Keaveney et al. 1993, Durand et al. 1994; Mengus et al. 1995; May et al. 1996 and references therein).

Construction of recombinant plasmids

To construct the expression vector for full-length hTAF135, the cDNA was first modified by site-directed mutagenesis to introduce XbaI and HindIII sites between positions 1224–1236 without altering the amino acid sequence. 5'-ACCAGAGCCTGTCCCGGACGCC-3' and 5'-ACCCAGAGCTGTCAGAACC-3', restriction sites shown in boldface type. The resulting clone was further modified to introduce a BamHI restriction site at a position identical to that introduced by PCR into the original genomic fragment. PCR amplification was used to generate a 32P-labeled probe from nucleotides 1050–1456. This probe was used to screen 106 phage from a human placental cDNA library. Filters were washed in 6x SSC at 45°C and 50°C and subjected to autoradiography. Four clones hybridizing to oligonucleotides derived from both peptides were purified and in vivo excision was performed. The cDNA inserts were sequenced completely with internal primers using an Applied Biosystems automated DNA sequencing machine. The resulting data were analyzed, and database searching was performed using the Genetics Computer Group (GCG) software (University of Wisconsin) sequence analysis programs. To isolate full-length hTAF135, PCR amplification was used to generate a 32P-labeled probe from nucleotides 1050–1456. This probe was used to screen 109 phage from a human placental genomic DNA library. Filters were washed at 60°C in 0.2x SSC and three positive clones were purified. A 4.7-kb BglII–HindIII fragment, which hybridized to an oligonucleotide corresponding to amino acids 372–378, was cloned and sequenced. Because of the high GC content, automated DNA sequencing was performed at 60°C in the presence of 10% DMSO.

Antibody preparation

mAbs against TBP [3G3, 2C11] and TAF135 [9TA] were as described previously (Brou et al. 1993, Lescure et al. 1994, Lavigne et al. 1996). mAb 20TA was raised against the pET-expressed carboxy-terminal domain of hTAF135 purified from E. coli by affinity chromatography on nickel NTA-agarose (Pharmacia) essentially as described (Brou et al. 1993; Lescure et al. 1994). Peptides P1 and P2 were coupled to ovalbumin and injected into rabbits as described (May et al. 1996).

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Transfections, CAT, luciferase assays, and immunoprecipitations

Cos-1, CV1, MPC11, and HeLa cells were transfected by the calcium phosphate precipitate technique. In addition to the expression vectors or reporters described in each figure, all transfections contained 1 μg of the luciferase reporter pRSV–Luc as internal standard for CAT assays or 2 μg of pXJ-LacZ as internal standard for luciferase assays, and pBSK–DNA as carrier. Transfections were performed in dextran-charcoal-treated medium and ligands were added [50 nM T-RA, 9cis-RA, and 3,5,3'-triiodothyronine (T3), 100 nM 1,25(OH)2D3, and 15 nM E2] at the same time as the DNA–calcium phosphate coprecipitate. Cells were harvested 48 hr after transfection and luciferase, β-galactosidase, and CAT assays were performed by standard procedures. Quantitative phoshorlager analysis was performed on a Fuji BAS 2000 apparatus. In all cases, similar results (+20%) were obtained in at least three independent transfections and the results of typical experiments are shown in the figures.

Immunoprecipitations were performed essentially as described (Mengus et al. 1995). After transfections, cell extracts were prepared by three cycles of freeze–thaw in 100 μl of buffer A [50 mM Tris-HCl [pH 7.9], 20% glycerol, 1 mM diithiothreitol, and 0.1% NP-40] containing 0.5 mM KCl and 2.5 μg/ml of leupeptin, pepstatin, aprotinin, antipain, and chymostatin. Extracts were mixed with –1 μg of the monoclonal antibodies and 50 μl of protein G-Sepharose and incubated at 4°C for 2 hr with rotation. The precipitated proteins were washed four times with 1 ml of buffer A containing 1.0 M KCl and once with buffer A containing 0.1 M KCl. The proteins were then detected on Western blots using an Amersham ECL kit.
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Note

The nucleotide sequence reported in this paper has been assigned accession no. Y11354 in the EMBL database.

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