Human $TAF_{II}135$ 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 $hTAF_{II}135$ ($hTAF_{II}130$). Full-length $hTAF_{II}135$ comprises 1083 amino acids and contains two conserved domains present also in $dTAF_{II}110$ and $hTAF_{II}105$. We show that expression of $hTAF_{II}135$ 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 $hTAF_{II}135$ region that is located between the conserved domains but is itself not conserved in $dTAF_{II}110$ and $hTAF_{II}105$. Expression of $hTAF_{II}135$ also stimulates RAR AF-2 activity when a promoter with a low-affinity TATA element (TGTA) is used, indicating that $hTAF_{II}135$ 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_{II}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; Burley and Roeder 1996). The cDNAs encoding many TAF_{II}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; Dubrovskaya et al. 1996; Hoffmann and Roeder 1996; Klebanow 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_{II}s appear to be essential for the response to transcriptional activators in vitro.

Additional support for this TAF_{II} function has come from studies showing direct and selective interactions between TAF_{II}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 $hTAF_{II}30$ and ligand-independent activation in vitro by the ER requires TFIID complexes containing $hTAF_{II}30$ (Jacq et al. 1994). Interactions between multiple activators and TAF_{II}s have been reported to result in transcriptional synergy in vitro (Sauer et al. 1995). Together, these studies show that TAF_{II}s act as specific coactivators in vitro by engaging in direct and selective interactions with transactivators.

This model of TAF_{II} function has been challenged by genetic studies in yeast where it has been shown that the presence of several yeast TAF_{II}s is not required for activated transcription (Apone et al. 1996; Moqtaderi et al. 1996a; Walker et al. 1996). Nevertheless, TAF_{II}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 $TAF_{II}250$

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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_{II}s are dispensible for activator function in yeast, but are absolutely required for this function in animal cell extracts in vitro.

Evidence that $hTAF_{II}$s may function as coactivators in mammalian cells has come from the investigation of the role of $hTAF_{II}28$ 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 1994; Beato et al. 1995; Kastner et al. 1995; Laudet 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 (Danielan et al. 1992; Barettino 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 $TAF_{II}28$ is depleted in Cos cell TFIID. Ectopic expression of recombinant $hTAF_{II}28$, a fraction of which associates stably with Cos cell TFIID, strongly enhances activation by the retinoid X receptor (RXR), the vitamin D receptor (VDR), and ER AF-2s (May et al. 1996). This activity did not involve direct $hTAF_{II}28$–NR interactions, but was abrogated by mutations impairing $hTAF_{II}28$–TBP interactions, suggesting that $hTAF_{II}28$ acts as a bridging factor between the NR AF-2-associated TIFs and the basal transcription machinery through TBP (May et al. 1996). Expression of $hTAF_{II}28$ also enhances activation by the viral Tax transactivator, which interacts directly with $hTAF_{II}28$ and TBP to form a ternary complex. Expression of TBP and $TAF_{II}28$ increases activation by Tax in HeLa cells by up to 40-fold (Caron et al. 1997). Thus, although TAF_{II}s may not be required for activation in yeast, the above results provide strong evidence that $hTAF_{II}28$ can act as a specific coactivator in mammalian cells.

Previously, we have reported the cloning of the carboxy-terminal region of $hTAF_{II}135$ (Mengus et al. 1995). We now report the cloning of full-length $hTAF_{II}135$ ($hTAF_{II}130$ in Dikstein et al. 1996; Tanese et al. 1996). $hTAF_{II}135$ is homologous to $hTAF_{II}105$ and $dTAF_{II}110$ in the carboxy-terminal and central regions, but has no yeast homolog. Expression of $hTAF_{II}135$ in several mammalian cell lines strongly potentiates transactivation by the retinoic acid receptor (RAR), the thyroid homone receptor (TR), and VDR AF-2s, whereas no effect was seen on activation by the ER, the RXR, or with unrelated activators. Using a promoter with a TGTA element instead of a TATA element, we show that coexpression of $hTAF_{II}135$ and the cognate TBP spm3 mutant results in activation by the RAR AF-2. However, expression of $hTAF_{II}135$, 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 $hTAF_{II}135$ most probably acts to facilitate the recruitment of the endogenous TFIID by the RAR, TR, and VDR AF-2s.

Results

Molecular cloning of $hTAF_{II}135$

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 peptides (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 $hTAF_{II}135$ (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 $hTAF_{II}135$ 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 $hTAF_{II}135$, the total ORF of 1083 amino acids was expressed in transfected Cos cells (see Materials and Methods). The recombinant protein was detected using a monoclonal antibody (mAb 20TA), raised against bacterially produced histidine-tagged $hTAF_{II}135$(815–1083) (see Fig. 2; Materials and Methods). mAb 20TA recognizes endogenous $hTAF_{II}135$ 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 $hTAF_{II}135$ had an electrophoretic mobility identical to that of the endogenous HeLa cell $TAF_{II}135$ (Fig. 1C, lanes 7–10), and could be precipitated from the transfected cell extracts by mAb 20TA (Fig. 1C, lane 10).

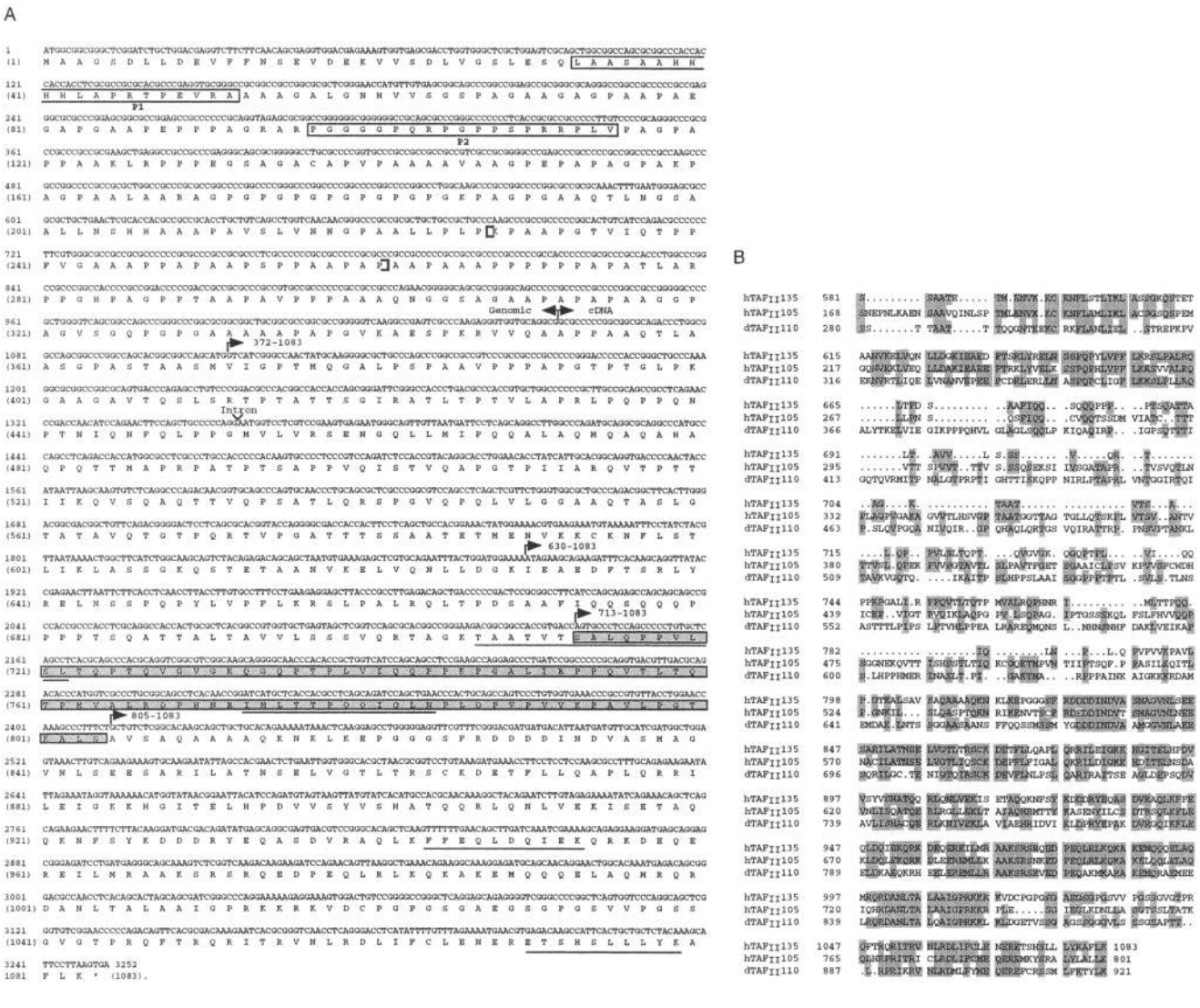

Figure 1. (*A*) Nucleotide and amino acid sequence of hTAF$_{II}$135. The numbers indicate the nucleotides and the numbers in parenthesis the amino acids. The regions derived from the cDNA and genomic clones are indicated along with the position of an intron whose position was determined from sequencing of the genomic clone. A putative intron located between K228 and P261 is indicated by open brackets. The positions of the amino termini of the deletion mutants are indicated by the arrows. Peptides derived from endogenous HeLa cell hTAF$_{II}$135 are underlined. The open boxes show the peptides P1 and P2 used to generate antibodies and the filled box indicates the region required for coactivator activity. (*B*) Alignment of the amino acid sequences of hTAF$_{II}$135, hTAF$_{II}$105, and dTAF$_{II}$110. For clarity only the carboxy-terminal region where there are significant homologies is shown. The numbers indicate the amino acid coordinates in each protein. Amino acids conserved in at least two of the three proteins are shaded. (*C*) Quantitative comparison of TAF$_{II}$135 levels in different cell types and comparison of the electrophoretic and antigenic properties of endogenous and recombinant hTAF$_{II}$135. (*Left*) Lanes *1–3* contain the cell extracts shown above each lane and in lanes *4–6* the TFIIDs immunopurified from these extracts with the anti-TPB mAb3G3. The blot was first revealed with mAb 20TA (*top*) to reveal TAF$_{II}$135 and then reincubated with mAbs 3G3 and 19TA (*bottom*) to reveal TBP and TAF$_{II}$55, respectively. The positions of TAF$_{II}$135, TAF$_{II}$55, the TBPs from human (HeLa), monkey (Cos), and mouse (MPC11), and the heavy chain of the antibody [IgG(H)] used in the immunopurifications are indicated. (*Middle*) The extracts shown above each lane were immunoprecipitated with the mAbs shown above the panel and the blot was revealed with the anti-hTAF$_{II}$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 TFIID) TFIID immunopurified with the anti-TBP mAb 3G3; (S.N.) immunoprecipitation supernatant.

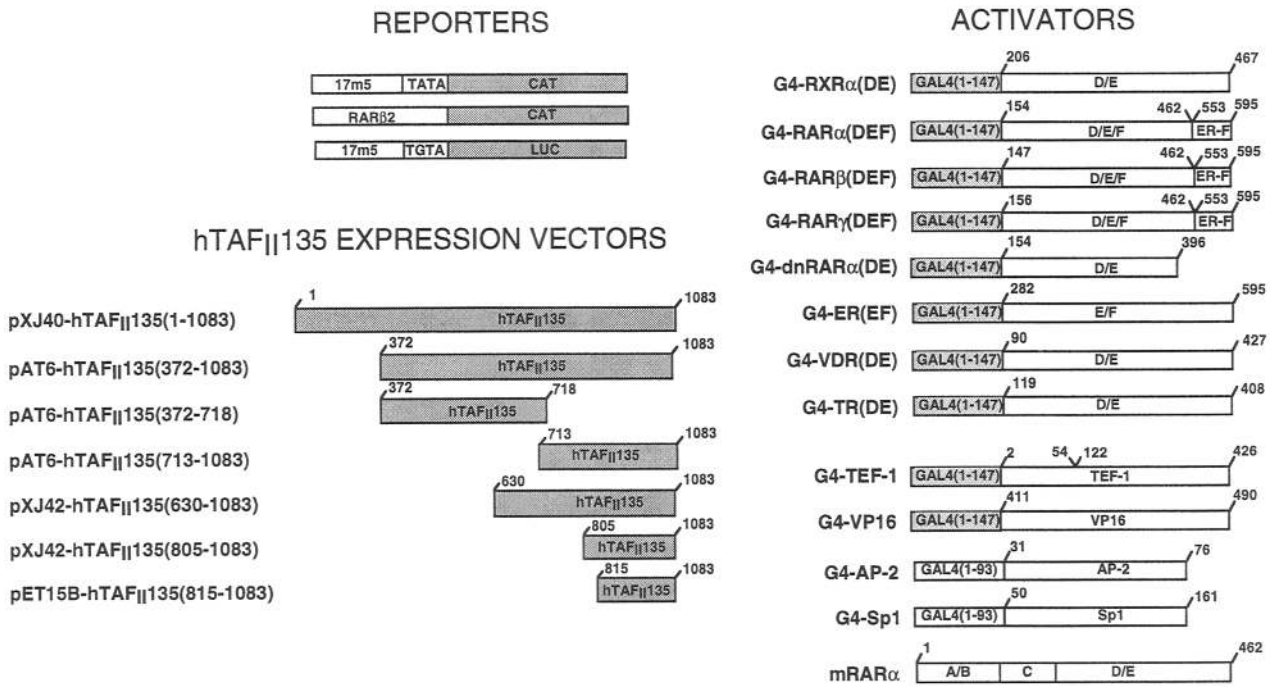

Figure 2. Schematic representation of the reporter gene and expression vectors. (Reporters) 17m5–TATA–CAT contains five Gal4-binding sites inserted 38 nucleotides upstream of the adenovirus major late promoter and the chloramphenicol-acetyltransferase (CAT) gene. RARβ2–CAT contains the RARβ2 promoter fused to the CAT gene as described previously (Nagpal et al. 1992). 17m5–TGTA–Luc contains five Gal4-binding sites upstream of the mutated RARβ2 TATA element and the luciferase gene as previously described (Keaveney et al. 1993). (hTAF$_{II}$135 expression vectors) The vectors expressing wild-type, B10-tagged (pAT6), and mutant derivatives of hTAF$_{II}$135 are schematized. The numbers are the amino acid coordinates in each case. (Activators) The vectors expressing all RAR, RXR, VDR, TR derivatives, G4–TEF-1(2-426)Δ55–121, G4–VP16, G4–AP-2, and G4–Sp1 are schematized. Gal4 is abbreviated to G4. In all cases the numbers indicate the amino acid coordinates in the native proteins.

To confirm that the region deduced from the genomic sequence was present in hTAF$_{II}$135, we generated antibodies against two peptides in this region (P1 and P2, open boxes in Fig. 1A). These antibodies recognized endogenous HeLa cell TAF$_{II}$135 in nuclear extracts and in immunopurified TFIID (Fig. 1C, cf. lanes 11–13 revealed with anti-P1 antibody with lanes 14–16 revealed with mAb20TA; data not shown for anti-P2 antibody). Together these results show that the deduced ORF shown in Figure 1A is in the correct reading frame to encode recombinant hTAF$_{II}$135 with an electrophoretic mobility and antigenicity identical to that of the endogenous HeLa cell protein.

Comparison of the amino acid sequence of hTAF$_{II}$135 with that of other TAF$_{II}$s clearly indicated that the carboxy-terminal domain of hTAF$_{II}$135 is highly homologous to that of dTAF$_{II}$110 and hTAF$_{II}$105 (Fig. 1B; Dikstein et al. 1996; Tanese et al. 1996). A second region in the central portion of these proteins is also conserved, particularly between hTAF$_{II}$135 and hTAF$_{II}$105, (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).

hTAF$_{II}$135 potentiates transcriptional activation by the AF-2 of RARs in several cell lines

Expression of hTAF$_{II}$28 in Cos cells potentiated selectively and strongly transcriptional activation by the AF-2s located in the LBD (region E) of the RXR, ER, and VDR (May et al. 1996). Similarly we tested the ability of the newly cloned hTAF$_{II}$135 to modulate transcriptional activation by NRs and other activators. Full-length hTAF$_{II}$135 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. 3, A, lane 3, and B, lanes 2 and 3). Strikingly, coexpression of full-length hTAF$_{II}$135(1–1083) led to a greater than 20-fold increase in transactivation by the RAR AF-2 (Fig. 3A, lanes 4,5), whereas expression of hTAF$_{II}$135 alone had no effect (Fig. 3A, lane 2). A similar strong increase in RARα AF-2 activity was seen with the hTAF$_{II}$135(372–1083) deletion mutant (Figs. 3B, lanes 5 and 6, and 4A, lanes 3 and 4). Analogous effects were also observed with the RARβ and RARγ AF-2s (Fig. 3B, lanes

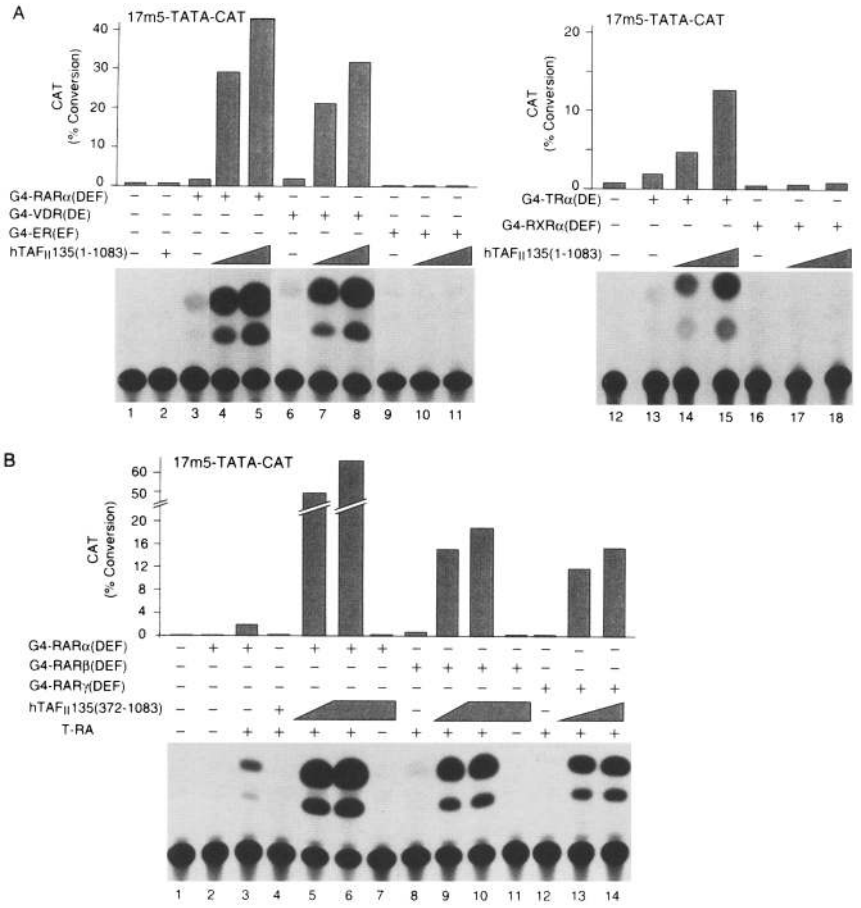

Figure 3. (*A*) Expression of hTAF$_{II}$135 potentiates transactivation by the RARα, VDR, and TR AF-2s in Cos cells. (*Bottom*) The autoradiography of CAT assays performed with extracts from cells transfected with the expression vectors shown above each lane. Transfections contained 1 μg of the 17m5–TATA–CAT reporter, 250 ng of the G4–RAR, ER, or VDR expression vectors, and 1 μg of the G4–RXR expression vector with 0, 3, or 7 μg of the hTAF$_{II}$135(1–1083) expression vector. All transfections contained the appropriate ligands. (*Top*) The quantitative phosphorImager analysis of the CAT assays represented in bottom. Values are expressed as percentage of the total chloramphenicol that was acetylated. In all figures similar results (± 20%) were obtained in at least three independent transfections and the results of a typical experiment are shown. (*B*) The layout is as in *A*. Transfections contained 0, 2, or 5 μg of the hTAF$_{II}$135(372–1083) deletion mutant expression vector and 250 ng of the G4–RAR expression vectors. The transfections in control lanes (i.e., lane *4*) contain always the highest concentration of the hTAF$_{II}$135 expression vector. The presence or absence of all-*trans*-retinoic acid (T-RA) in the transfections is indicated.

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

The region of hTAF$_{II}$135 required for this enhancement was localized using a series of deletion mutants. Coexpression of hTAF$_{II}$135(372–1083) and (713–1083) resulted in a strong increase in RAR AF-2 activity, whereas hTAF$_{II}$135(372–718) had no significant effect (Fig. 4A, lanes 3–8). Similarly, expression of hTAF$_{II}$135(630–1083) strongly potentiated RAR AF-2 activity, whereas no significant effect was seen with hTAF$_{II}$135(805–1083) (Fig. 4B, lanes 3–6). All deletion mutants were expressed at comparable levels, higher than that of full-length hTAF$_{II}$135 (Fig. 4D). Thus, a domain residing within, or overlapping with, amino acids 713–805 of hTAF$_{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$_{II}$135 enhancement effect. As in Cos cells, RARα AF-2-activated transcription in CV1 cells was stimulated more than 20-fold by coexpression of hTAF$_{II}$135(630–1083) (Fig. 5A), whereas it was enhanced only sixfold in HeLa cells where the optimum concentration of hTAF$_{II}$135 expression vector was lower than in Cos and CV1 cells (Fig. 5B). In MPC11 lymphoid cells the RARα AF-2 strongly activated transcription and coexpression of hTAF$_{II}$135(630–1083) led to only a fourfold increase in its activity (Fig. 5C). These results show that hTAF$_{II}$135 can potentiate activation by the RARα AF-2 in several cell lines, albeit to different degrees.

The observation that ectopically expressed hTAF$_{II}$135 enhances activation by the RAR AF-2 indicates that it is functionally limiting in the different cell types. The differential enhancement seen in these cell lines may reflect differences in the relative abundance of TAF$_{II}$135. To test this possibility, the amount of TAF$_{II}$135 present in HeLa, Cos, and MPC11 cell extracts was determined by immunoblotting. Each extract contained similar amounts of TBP and TAF$_{II}$55 (Fig. 1C, lanes 1–3, bottom) as well as TAF$_{II}$20 and TAF$_{II}$18 (see May et al. 1996; data not shown). However, relative to both TBP and TAF$_{II}$55, significantly less TAF$_{II}$135 was seen in Cos and CV1 cell extracts, compared to HeLa and MPC11 cell extracts (Fig. 1C, lanes 1–3; data not shown). Confirming this result, TAF$_{II}$135 was present, but less abundant in immunopurified Cos cell TFIID than in HeLa and MPC11 cell TFIIDs (Fig. 1C, lanes 4–6). Thus, the degree to

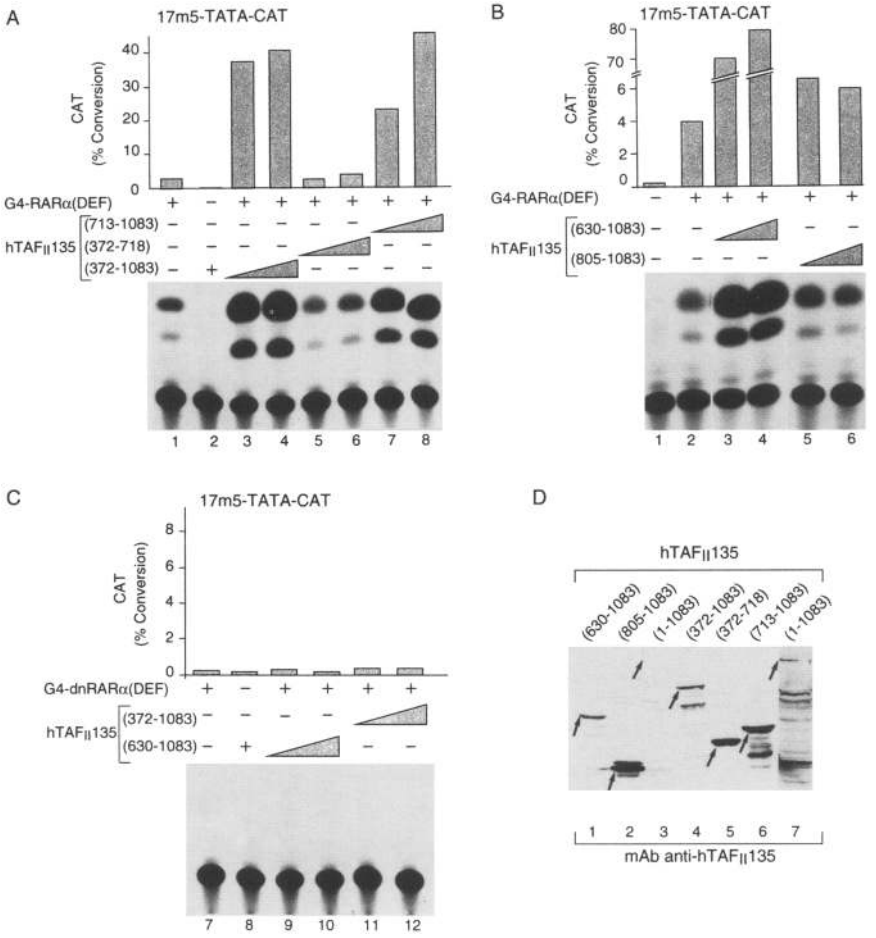

Figure 4. (*A–B*) Deletion mutants in hTAF$_{II}$135 define a region important for its coactivator activity. The layout is as described in Fig. 3A. Cos cell transfections contained 0, 2, or 5 μg of the indicated hTAF$_{II}$135 mutant expression vectors. (*C*) The coactivator effect of TAF$_{II}$135 requires the AF-2 AD core. The layout is as in *A–B*. (*D*) Expression of hTAF$_{II}$135 deletion mutants. Transfections were performed with 5 μg of the expression vectors shown above each lane and the Western blot was revealed with the mAbs 20TA directed against hTAF$_{II}$135 and B10 directed against B10-tagged hTAF$_{II}$135 constructs. The wild-type and mutant proteins are indicated by the arrows. Lane 7 shows a longer exposure of lane 3.

which ectopic expression of TAF$_{II}$135 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 hTAF$_{II}$135 could also affect activation by wild-type NRs, the natural RARβ2 promoter was used. This promoter contains a RA response element (RARE) and is stimulated in the presence of RA. Transcription of a CAT reporter under the control of the RARβ2 promoter is stimulated by the endogenous RAR–RXR heterodimers upon addition of all-*trans* (T-)RA in transfected Cos cells (Nagpal et al. 1992, Fig. 5D, lanes 1,2). The coexpression of hTAF$_{II}$135(630–1083) resulted in a fivefold increase in the ligand-dependent activity of this reporter (Fig. 5D, lanes 2–5). Expression of TAF$_{II}$135 also enhanced ligand-dependent activation from the RARβ2 promoter in the presence of cotransfected wild-type RARα (Fig. 5E, lanes 3–6). Thus, coexpression of hTAF$_{II}$135 enhances activation by endogenous and ectopically expressed wild-type RAR.

The coactivator activity of hTAF$_{II}$135 is restricted to a subset of NR AF-2s

We then investigated whether hTAF$_{II}$135 could potentiate activation by the AF-2s of other NRs. Expression of hTAF$_{II}$135(1–1083) or (630–1083) resulted in >10-fold increase in the activity of the VDR and TR AF-2s (Figs. 3A, lanes 6–8, 13–15, 6A, lanes 7–10, and 6B, lanes 3–6). No significant increase in VDR and TR AF-2 activity was seen in the absence of ligand or with hTAF$_{II}$135(805–1083) (Figs. 6A, lane 10, and B, lane 5; data not shown). In contrast, expression of hTAF$_{II}$135(1–1083) or (630–1083) had no significant effect on the activity of the ER, RXRα, RXRβ, and RXRγ AF-2s (Fig. 3A, lanes 9–11, 16–18; data not shown), nor on activation by several activators that do not belong to the nuclear receptor superfamily (for example, see G4–TEF-1, Fig. 6B; see below), nor does it increase the amount of G4–NR chimeras expressed transiently (data not shown). Thus, expression of hTAF$_{II}$135 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 hTAF$_{II}$135 differs from that of hTAF$_{II}$28, as its most potent effect was observed with the RAR and TR AF-2s, but not with the ER and RXR AF-2s, whereas the converse is true for hTAF$_{II}$28 (May et al. 1996). However, as both hTAF$_{II}$135 and hTAF$_{II}$28 increased the activity of the VDR AF-2, we tested the effect of coexpression of both hTAF$_{II}$s on VDR AF-2 activity.

Expression of hTAF$_{II}$28 enhanced VDR AF-2 activity

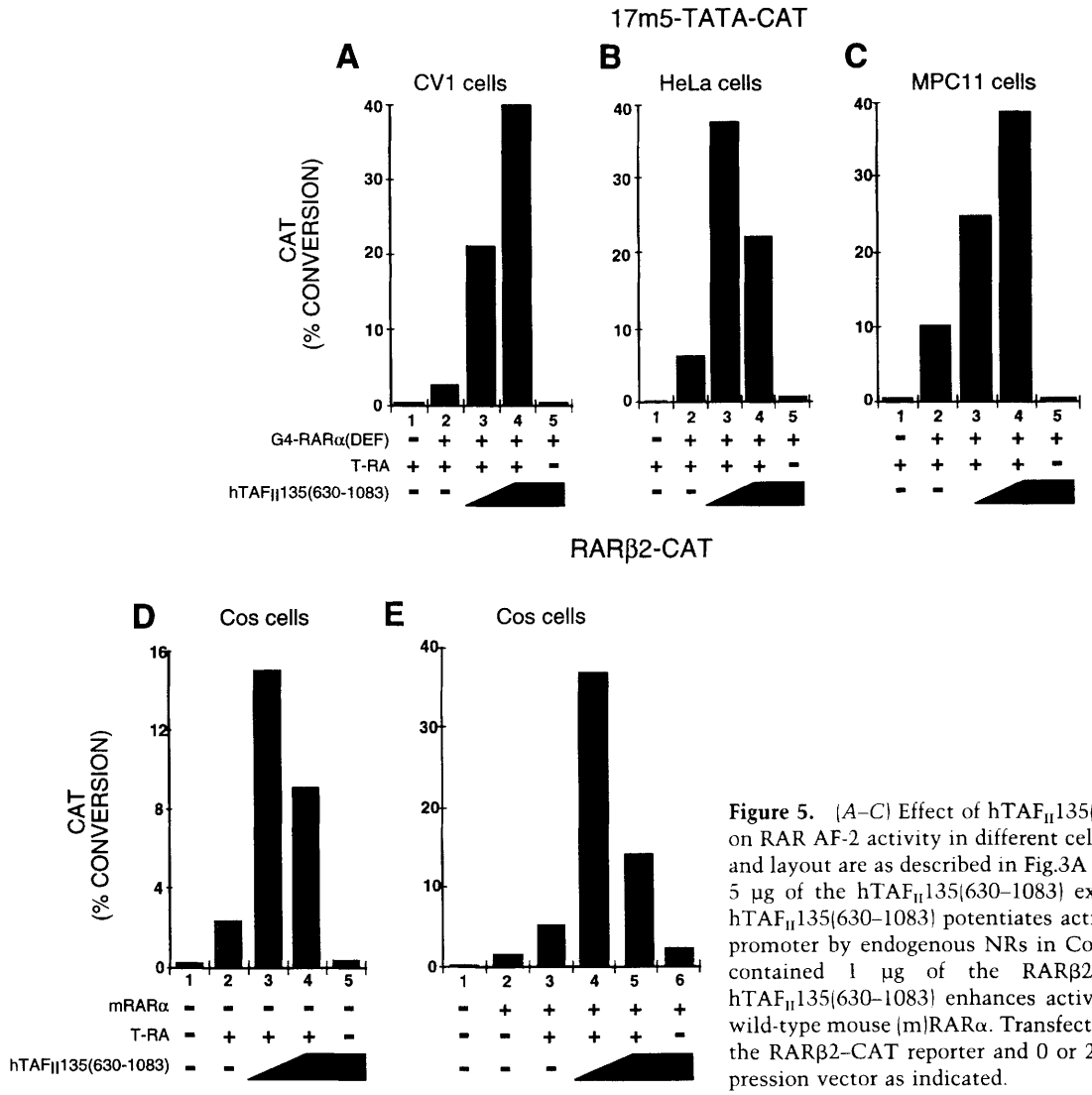

Figure 5. (*A–C*) Effect of hTAF$_{II}$135(630–1083) expression on RAR AF-2 activity in different cell types. Transfections and layout are as described in Fig.3A and contained 0, 2, or 5 μg of the hTAF$_{II}$135(630–1083) expression vectors. (*D*) hTAF$_{II}$135(630–1083) potentiates activation of the RARβ2 promoter by endogenous NRs in Cos cells. Transfections contained 1 μg of the RARβ2–CAT reporter. (*E*) hTAF$_{II}$135(630–1083) enhances activation by coexpressed wild-type mouse (m)RARα. Transfections contained 1 μg of the RARβ2–CAT reporter and 0 or 250 ng of mRARα expression vector as indicated.

fivefold, whereas expression of TAF$_{II}$135 had a 10-fold effect (Fig. 7A, lanes 2,4,6). Coexpression of hTAF$_{II}$135 with a suboptimal amount of hTAF$_{II}$28 resulted in a greater increase in VDR AF-2 activity than that seen with hTAF$_{II}$135 alone (e.g., cf. Fig. 7A, lanes 3, 5, and 7). In contrast, ER AF-2 activity was enhanced sixfold by hTAF$_{II}$28 expression, but was not affected significantly by hTAF$_{II}$135 (Fig. 7B, lanes 2–6). Furthermore, the expression of TAF$_{II}$135 did not increase ER AF-2 activity in the presence of TAF$_{II}$28 (Fig. 7B, lanes 3,7,8). Thus, although hTAF$_{II}$135 and hTAF$_{II}$28 act together to enhance VDR AF-2 activity, no such additive effect was seen with the ER AF-2.

Expression of hTAF$_{II}$135 enhances RAR AF-2 activity on a promoter containing a low affinity TATA element

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

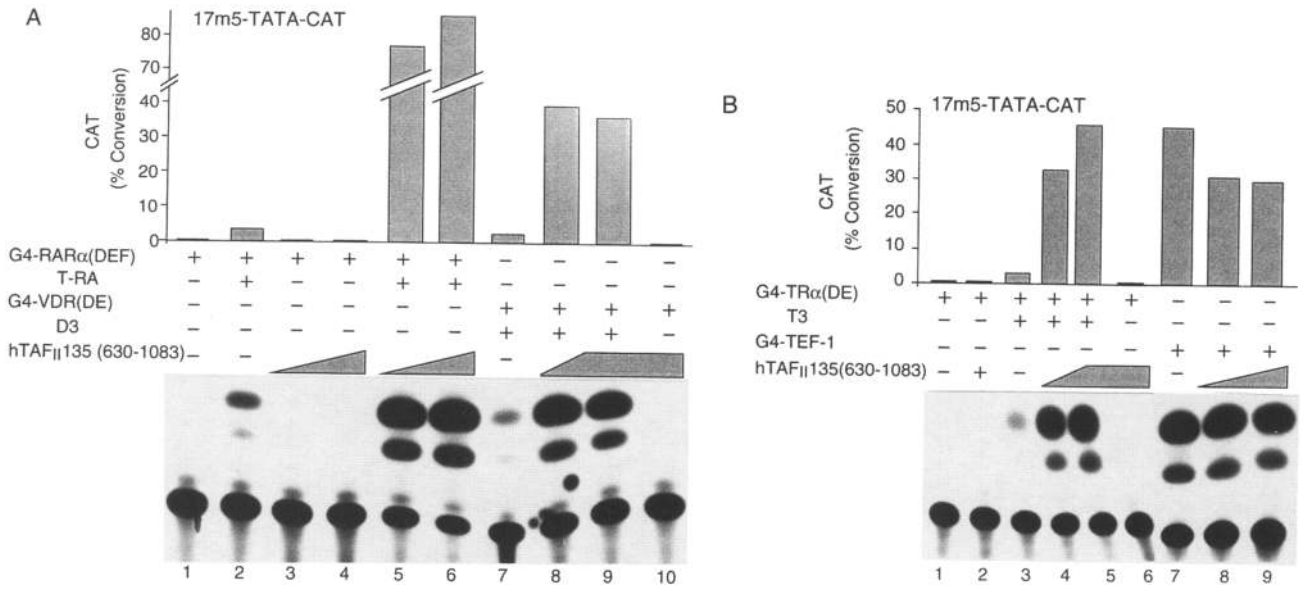

Figure 6. (*A–B*) The hTAF$_{II}$135(630–1083) mutant potentiates activation by the RAR, VDR, and TR AF-2s, but not by the TEF-1 AF. The layout is as in Fig. 3A. The presence or absence of the cognate ligands T-RA, 1,25(OH)$_2$ vitamin D3 (D3), and thyroid hormone (T3) in the transfections is indicated. Transfections 7–9 in *B* contained 100 ng of the G4–TEF-1 expression vector.

pression of hTAF$_{II}$135 enhances RAR AF-2 activity in the presence of cotransfected TBP spm3.

However, coexpression of hTAF$_{II}$135(630–1083) enhanced RAR AF-2 activity even in the absence of TBP spm3 (Fig. 8A, lane 6). This stimulation was analogous to that seen with TBP spm3 and the RAR AF-2, but was 20-fold less than that seen with the combination of all three components showing that in the presence of coexpressed TAF$_{II}$135, the RAR AF-2 can activate transcription through the endogenous TFIID. Thus, hTAF$_{II}$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$_{II}$135(630–1083) resulted in significant and comparable levels of activation (Fig. 8B, lanes 3–4 and Fig. 8C, lanes 2–3). However, in contrast to the RAR AF-2, coexpression of both hTAF$_{II}$135 and TBP spm3 resulted in only a further threefold increase in VDR AF-2 activity (Fig. 8B, lanes 5,6), whereas no further increase was observed with the TR AF-2 (Fig. 8C, lanes 4,5). Analogous results were obtained with full-length TAF$_{II}$135 (data not shown). All effects were dependent on the presence of the corresponding ligands (Fig. 8B,C) and in control experiments, expression of TBP and hTAF$_{II}$135 had no significant effect on promoter activity in the absence of the NR chimeras. Thus, as observed with the RAR AF-2, TAF$_{II}$135

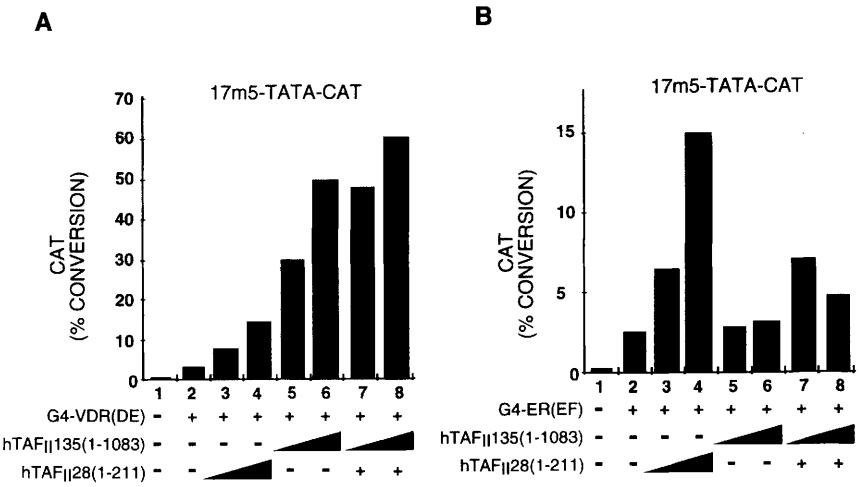

Figure 7. (*A–B*) Both hTAF$_{II}$28 and hTAF$_{II}$135 enhance VDR AF-2 activity, whereas ER AF-2 activity is enhanced selectively by hTAF$_{II}$28. Transfections contained 0, 2, or 4 μg of the hTAF$_{II}$28 expression vector and 0, 3, or 7 μg of the hTAF$_{II}$135 expression vector as indicated. Transfections in lanes 7 and 8 contained the suboptimal 2 μg of the hTAF$_{II}$28 expression vector.

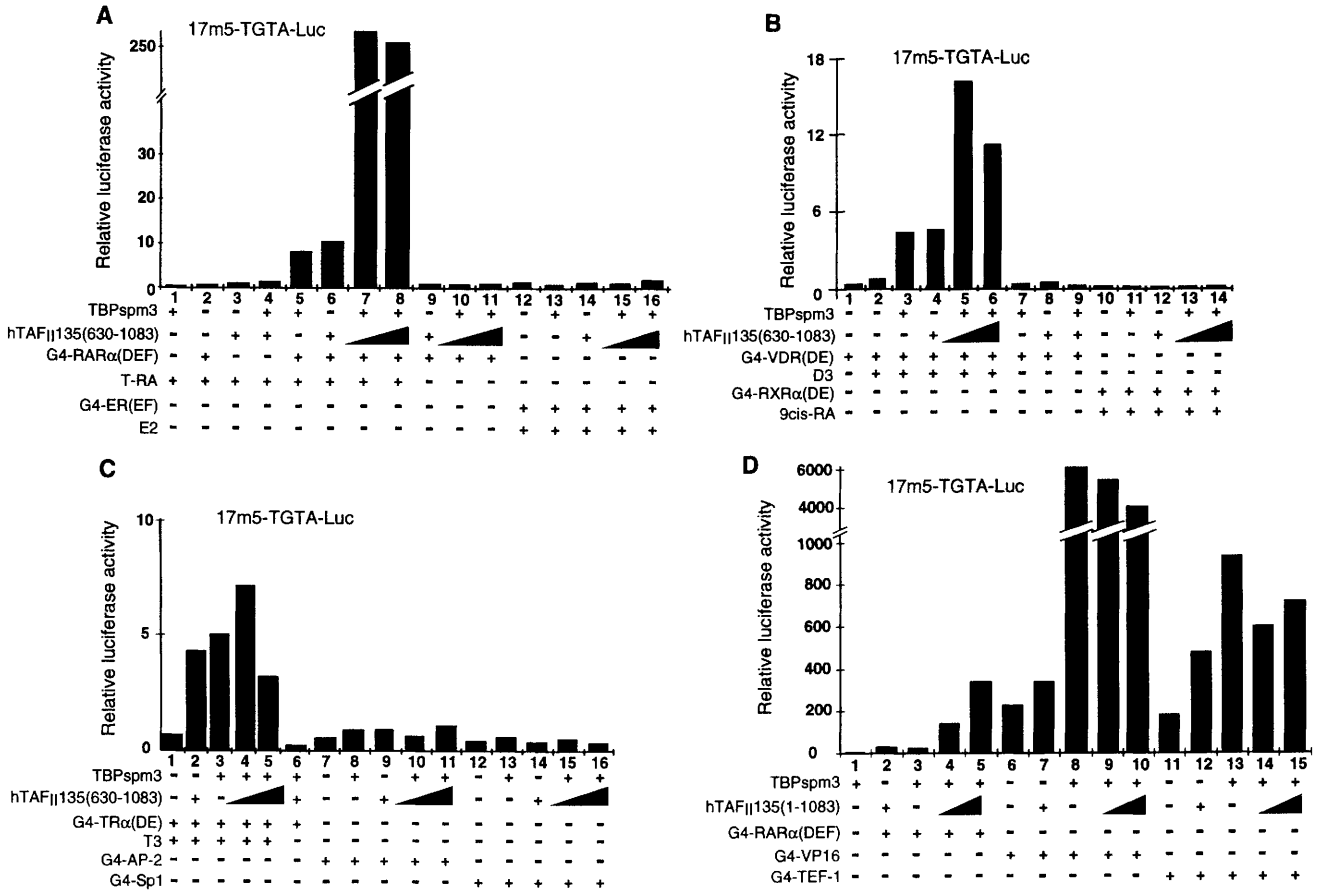

Figure 8. (*A*) Expression of TBP spm3 and hTAF$_{II}$135 enhances activation by the RAR AF-2. Transfections contained 2 μg of the 17m5–TGTA–Luc reporter plasmid, 1 μg of the TBP spm3 expression vector, 250 ng of the G4–NR expression vectors, 0, 2, and 5 μg of the TAF$_{II}$135(630–1083) expression vector and the cognate ligands as indicated. After correction for the β-galactosidase internal control, luciferase values were determined on a Berthold apparatus. (*B*) The layout is as as described in *A*. Transfections contained 250 ng of the G4–VDR and 1 μg of the G4–RXR expression vectors. (*C–D*) The layout is as described above. Where indicated, transfections contained 250 ng of the G4–Sp1 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$_{II}$135 and TBP spm3 did not result in activation by the ER or RXR AF-2s (Fig. 8, A, lanes 12–16, and B, lanes 10–14, respectively), nor did hTAF$_{II}$135 potentiate activation by the unrelated G4–AP-2 and G4–Sp1 activators (Fig. 8C, lanes 7–16; note that the G4–Sp1 chimera used in these experiments does not contain the region shown to interact with dTAF$_{II}$110 and hTAF$_{II}$135; Gill et al. 1994; Tanese et al. 1996), although they were expressed at comparable levels to the G4–NR chimeras (data not shown). These results confirm that hTAF$_{II}$135 acts selectively to enhance activation by the RAR, VDR, and TR AF-2s.

The effect of TAF$_{II}$135 expression on the activity of two further unrelated transcriptional activators was determined. Even in the absence of TBP spm3, the potent VP16 and TEF-1 AFs stimulated transcription (Fig. 8D, lanes 6,11) and coexpression of TBP spm3 increased their activity (Fig. 8D, lanes 8,13). In contrast, coexpression of hTAF$_{II}$135 either alone or together with TBP spm3 had little effect (Fig. 8D, lanes 7,9,10,12,14,15). These observations show that the VP16 and TEF-1 AFs can use the endogenous TFIID to activate transcription from the TGTA promoter, and that their activity is not affected significantly by overexpression of hTAF$_{II}$135. Thus, unlike the RAR, VDR, and TR AF-2s, these strong AFs are characterized by their ability to recruit efficiently cellular transcription factors to the TGTA-containing promoter in the absence of coexpressed hTAF$_{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 spm3 or hTAF$_{II}$135(630–1083) enhanced this activation by fourfold (Fig. 9, lanes 5–6). Thus, overexpression of hTAF$_{II}$135 in HeLa cells also promotes activation by the RAR AF-2 through the endogenous transcription machinery. However, the combination of hTAF$_{II}$135 and TBP spm3 enhanced RAR AF-2 activity only two- to threefold more than that observed with either of the two

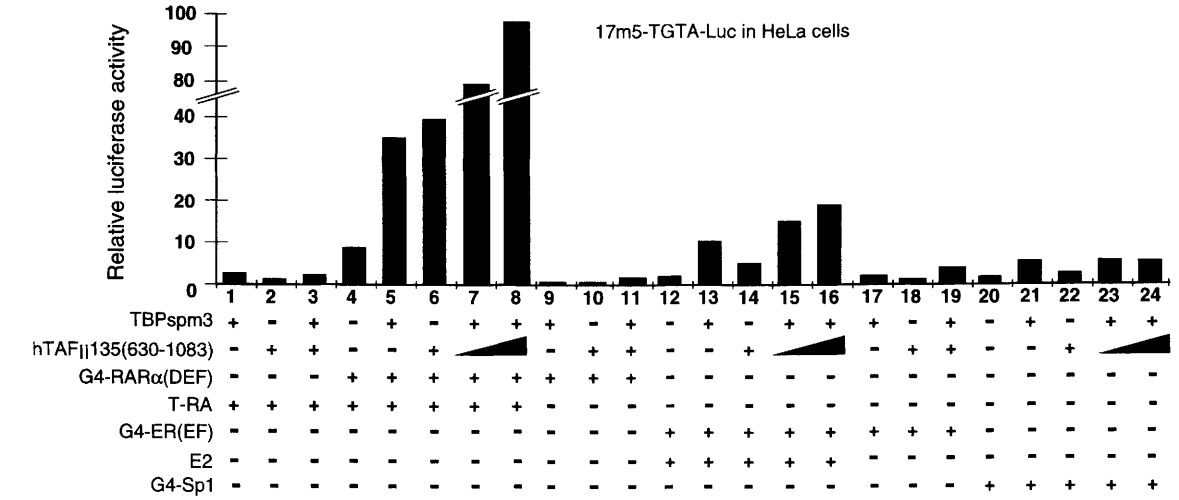

Figure 9. Expression of hTAF$_{II}$135(630–1083) potentiates RAR AF-2 activity in HeLa cells. Transfections were performed with the amounts of each plasmid indicated in Fig. 8A.

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 hTAF$_{II}$135 and the activation seen in the presence of TBP spm3 was not increased significantly by the coexpression of hTAF$_{II}$135 (lanes 14–16). Similarly, hTAF$_{II}$135 had no effect on the weak activation seen with G4–Sp1 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 RARβ2 reporters showing that hTAF$_{II}$135 can enhance selectively activation by the RAR, VDR, and TR AF-2s.

Discussion

hTAF$_{II}$135 is related to hTAF$_{II}$105 and dTAF$_{II}$110, but has no yeast homolog

We have characterized previously interactions between the carboxy-terminal domain of hTAF$_{II}$135 and other components of the TFIID complex. By protein affinity chromatography in vitro and immunoprecipitations of transfected Cos cell extracts, we have shown that hTAF$_{II}$135 interacts with hTAF$_{II}$250 and hTAF$_{II}$20, but not with hTAF$_{II}$100 or TBP (Mengus et al. 1995; Dubrovskaya et al. 1996). Here we describe the molecular cloning of full-length hTAF$_{II}$135 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 hTAF$_{II}$135 of molecular mass identical to that of the endogenous HeLa cell protein. Antibodies directed against two amino-terminal peptides deduced from the genomic sequence recognize endogenous HeLa cell TAF$_{II}$135 showing that this sequence is the bona fide TAF$_{II}$135 ORF. Thus, the ORF reported here encodes a protein with electrophoretic and antigenic properties identical to those of endogenous HeLa cell TAF$_{II}$135.

The sequence of the amino-terminal region of hTAF$_{II}$135 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.

TAF$_{II}$135 clearly belongs to a family of proteins, including hTAF$_{II}$105 and dTAF$_{II}$110, containing a conserved carboxy-terminal domain that mediates interactions with other TFIID subunits and the large subunit of TFIIA (Hoey et al. 1993; Mengus et al. 1995; Dikstein et al. 1996), as well as the interactions of hTAF$_{II}$135 and dTAF$_{II}$110 with the oncoproteins E1A, SV40 large T-antigen, and HPV E7 (Geisberg et al. 1995; Mazzarelli et al. 1995). This carboxy-terminal domain is clearly the hallmark of this family of proteins, conserved from *Drosophila* to humans. However, searches of the protein databases failed to reveal any homologous yeast proteins (G. Mengus et al., unpubl.). Thus, although yeast contains homologs for almost all other mammalian TAF$_{II}$s (Reese et al. 1994; Poon et al. 1995; Klebanow et al. 1996; Moqtaderi et al. 1996a,b; Walker et al. 1996), there appears to be no yeast homologs of the TAF$_{II}$135,TAF$_{II}$105, TAF$_{II}$110 family.

$TAF_{II}135$ is a specific coactivator for a subset of NRs in mammalian cells

We show that $hTAF_{II}135$ can enhance transactivation by several NR AF-2s in mammalian cells. $hTAF_{II}135$ enhanced the activity of the NR AF-2s present in G4 chimeras and enhanced activation of the RARβ2 promoter by endogenous and ectopically expressed wild-type RAR. This enhancement required the integrity of the RAR AF-2 AD core showing that $hTAF_{II}135$ did not act on an unknown AF-2-independent AF located in the receptor LBD. In contrast, expression of $hTAF_{II}135$ had no effect on the activity of the ER and RXR AF-2s, and it did not potentiate the effect of several unrelated activators (VP16, TEF-1). Interestingly, although no $TAF_{II}135$ homolog or NRs exist in yeast, ectopically introduced RAR AF-2 can function in this organism. Similarly, other proteins that are NR coactivators in mammalian cells, for example p300/CBP, also have no homolog in yeast (Eckner 1996), suggesting that the cofactors, which mediate RAR AF-2 activity in yeast, are distinct from those in mammalian cells.

$TAF_{II}135$ specifically enhances activation by the class II NRs, RAR, TR, and VDR, which preferentially form heterodimers with RXRs. These NRs also share the ability 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; Hörlein et al. 1995). On the other hand, the ER, whose activity is enhanced preferentially by $hTAF_{II}28$ (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 $TAF_{II}135$ therefore, is reminiscent of other functional differences noted among these receptors. Thus, $TAF_{II}135$ may be a specific coactivator for certain class II NRs, whereas $hTAF_{II}28$ may enhance the activity of the class I NRs preferentially. However, both of these TAF_{II}s enhance VDR AF-2 activity and their coexpression results in an additive effect.

Enhancement of NR AF-2 activity required the integrity of a $hTAF_{II}135$ domain located within or overlapping with amino acids residues 713–805. This region is located between the two highly conserved domains of the $TAF_{II}135$,$TAF_{II}105$,$TAF_{II}110$ proteins, but is itself poorly conserved. Accordingly, expression of $dTAF_{II}110$ had no significant effect on RAR AF-2 activity (G. Mengus, et al., unpubl.), showing that vertebrate TAF_{II}s may have evolved to perform specific functions not found in other metazoans or yeast.

Possible molecular mechanisms for $TAF_{II}135$ function

Although $hTAF_{II}135$ acts as a coactivator for the RAR AF-2, we were unable to detect RAR–$hTAF_{II}135$ interactions either in transfected Cos cells or in yeast two hybrid assays, whereas control experiments revealed strong $hTAF_{II}135$–$hTAF_{II}20$ interactions (our unpublished data). Nevertheless, we show that $TAF_{II}135$ is functionally limiting for the activity of certain NR AF-2s in several mammalian cell lines and that the degree to which $TAF_{II}135$ expression enhances AF-2 activity correlates inversely with $TAF_{II}135$ levels in these cells. A stronger enhancement is observed in Cos cells that contain the lowest amounts of $TAF_{II}135$, whereas the effect is weaker in HeLa and MPC11 cells where $TAF_{II}135$ is relatively more abundant. As ectopically expressed $hTAF_{II}135$ associates with endogenous TFIID (Tanese et al. 1996), it is likely that the enhancement of NR AF-2 activity results from an increase in the amount of TFIID complexes containing $TAF_{II}135$, a situation analogous to that previously observed with $TAF_{II}28$ in Cos cells (May et al. 1996). These observations support our previous proposal that substochiometric TAF_{II}s present in only a subset of TFIID complexes may mediate the effect of certain transactivators (Brou et al. 1993; Jacq et al. 1994). Interestingly, the related $TAF_{II}105$ is also present in substochiometric amounts in TFIID from B cells (Dikstein et al. 1996).

To address the molecular mechanism of $hTAF_{II}135$ function, we used a promoter in which the TATA element has been replaced by a TGTA element to which the endogenous TFIID binds with only low affinity. With this reporter, coexpression of the cognate TBP spm3 mutant and $TAF_{II}135$ resulted in strong activation by the RAR AF-2. However, coexpression of $hTAF_{II}135$ in the absence of TBP spm3 also enhanced RAR AF-2 activity showing that $TAF_{II}135$ allows RAR AF-2-dependent formation of initiation complexes containing endogenous TFIID, thereby compensating for its low affinity for the TGTA element. When coexpressed with $TAF_{II}135$, 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 cotransfected TBP spm3, irrespective of $TAF_{II}135$ overexpression. Considering the importance of the recruitment of TFIID in transcriptional activation (Klages and Strubin 1995; Chatterjee and Struhl 1995; Chi and Carey 1996; Stargell and Struhl 1996b), the reported interactions between 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 Maniatis 1994), may suffice to allow efficient recruitment of endogenous TFIID to the TGTA-containing promoter. In contrast, for the RAR, VDR, and TR AF-2s, overexpression of $TAF_{II}135$ would be required for TFIID to be recruited efficiently.

Several possible molecular mechanisms may be envisaged to explain how $TAF_{II}135$ could enhance recruitment of TFIID by the NR AF-2. In view of the observed specificity of $TAF_{II}135$ for certain NRs and the lack of evidence for direct interactions with these NRs, it is tempting to speculate that $TAF_{II}135$ may interact with putative RAR-, VDR-, and TR-specific TIFs, thereby enhancing the recruitment of TFIID. Further experiments will be required to identify interaction partners of $TAF_{II}135$ and lead to a better understanding of the molecular basis of its coactivator activity.

Materials and methods

Purification of HeLa cell TFIID and cloning of hTAF$_{II}$135

Nuclear extract and TFIID preparation was as described (Brou et al. 1993; Chaudhary et al. 1994). Immunopurified TFIID was dialyzed against buffer containing 5 mM NaCl, 0.5 mM Tris-HCl (pH 7.9), 5 μM DTT, and 0.01% SDS for 6–8 hr. The TFIID was then lyophilized and resolved by SDS-PAGE. Proteins were electroblotted overnight in 50 mM Tris, 50 mM boric acid onto a PVDF membrane (Millipore Immobilon P, 0.45 μM), and stained briefly with Coomassie blue. hTAF$_{II}$135 was excised and digested in situ on the membrane with trypsin. The eluted peptides were then separated by reverse phase high-pressure liquid chromatography (HPLC) and microsequenced. Degenerate oligonucleotides from peptides IMLTTPQQIQLNPL, AT(C/T)ATG(C/T)T(C/G) AC(A/C/T)AC(A/C/T)CC(A/C/T)CA(A/G)CA(A/G)AT(C/T)CA(A/G)(C/T)T(C/G)AA(C/T)CC, and AATVTSALQP, GC(A/C/T)GC(A/C/T)AC(A/C/T)GT(C/G/T)AC(A/C/T)TC(C/T)GC(A/C/T) CT(C/G/T)CA(A/G)CC: GC (A/C/T)GC(A/C/T)AC(A/C/T)GT(C/G/T)AC(A/C/T)TC(C/T)GC(A/C/T) TTGCA(A/G)CC: GC(A/C/T)GC(A/C/T)AC(A/C/T)GT(C/G/T)AC(A/C/T)AGCGC(A/C/T)TTGCA(A/G)CC: GC(A/C/T)GC(A/C/T)AC(A/C/T)GT(C/G/T)AC(A/C/T)AGCGC(A/C/T)CT(C/G/T) CA(A/G)CC were synthesized, [^{32}P]-5′-end labeled, and used to screen a HeLa cell cDNA library in λ ZAPII (Stratagene). The filters were washed in 6× SSC at 45°C or 50°C and subjected to autoradiography. Four clones hybridizing to oligonucleotides derived from both peptides were purified and in vivo excision was performed. The cDNA inserts were sequenced completely with internal primers using an Applied Biosystems automated DNA sequencer. The resulting data were analyzed, and database searching was performed using the Genetics Computer Group (GCG) software (University of Wisconsin) sequence analysis programs. To isolate full-length hTAF$_{II}$135, PCR amplification was used to generate a ^{32}P-labelled probe from nucleotides 1050–1456. This probe was used to screen 10^6 phage from a human placental genomic DNA library. Filters were washed at 60°C in 0.2× SSC and three positive clones were purified. A 4.7-kb *Bgl*II–*Hin*dIII 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.

Construction of recombinant plasmids

To construct the expression vector for full-length hTAF$_{II}$135, the cDNA was first modified by site-directed mutagenesis to introduce *Xba*I and *Hin*dIII sites between positions 1224–1236 without altering the amino acid sequence (5′-ACCCAGAGCCTGTCCCGGACGCCC-3′ changed to 5′-ACCCA**AAGCTT**G**TCTAGA**ACCC-3′, restriction sites shown in boldface type). The resulting clone was further modified to introduce a *Bam*HI restriction site after the stop codon. The modified plasmid was digested with *Hin*dIII and *Bam*HI and the cDNA fragment purified. The 5′-end of the hTAF$_{II}$135 coding sequence was generated by PCR from the genomic fragment using a 5′ primer containing an *Eco*RI site and a consensus Kozak sequence, and a 3′ primer with a *Hin*dIII site at a position identical to that introduced in the cDNA. PCR was perfomed with Vent DNA polymerase in thermopol buffer containing 10% DMSO, 5 mM KCl, 0.15 mM C7-deaza-dGTP, and 0.05 mM dGTP. The PCR fragment was cloned between the *Eco*RI and *Hin*dIII sites of the pXJ40 expression vector. The resulting pXJ40 clone was then digested with *Hin*dIII and *Bgl*II and the *Hin*dIII–*Bam*HI cDNA fragment was introduced to generate a full-length hTAF$_{II}$135 ORF. The other hTAF$_{II}$135 expression vectors were generated by PCR using appropriately located primers. For cloning in pXJ42 the 5′ primer contained a *Bam*HI site, a consensus Kozak sequence, and an ATG, and the 3′ primer an *Eco*RI restriction site; for the pET15 construct the 5′ primer contained an *Nde*I site and the 3′ priner a *Bam*HI restriction site; for cloning in pAT6, the primers contained *Nhe*I restriction sites; 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; Nagpal 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).

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, 9*cis*-RA, and 3,5,3′-triiodo-L-thyronine (T3), 100 nM 1,25(OH)$_2$D$_3$, 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 phosphorImager analysis was performed on a Fujix 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 dithiothreitol, and 0.1% NP-40] containing 0.5 M 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.

Antibody preparation

mAbs against TBP (3G3, 2C1) and TAF$_{II}$55 (19TA) were as described previously (Brou et al. 1993; Lescure et al. 1994; Lavigne et al. 1996). mAb 20TA was raised against the pET-expressed carboxy-terminal domain of hTAF$_{II}$135 purified from *Escherichia coli* by affinity chromatography on nickel NTA$^+$ 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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Note

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

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