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.

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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 (Danielian et al. 1992; Baretino 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 hormone 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).

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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. RAR β 2-CAT contains the RAR β 2 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 RAR β 2 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] Δ 55-121, 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-RAR α (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 RAR α 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 RAR β and RAR γ AF-2s (Fig. 3B, lanes

Figure 3. (A) Expression of hTAF_{II}135 potentiates transactivation by the RAR α , 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 μ g of the 17m5-TATA-CAT reporter, 250 ng of the G4-RAR, ER, or VDR expression vectors, and 1 μ g of the G4-RXR expression vector with 0, 3, or 7 μ g 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 ($\pm$ 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 μ g 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-dnRAR α (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, RAR α 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 RAR α 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 RAR α 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 μ g 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 μ g 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 RAR β 2 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 RAR β 2 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 RAR β 2 promoter in the presence of cotransfected wild-type RAR α (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 potenti-

ate 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, RXR α , RXR β , and RXR γ 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 μ g of the hTAF_{II}135(630–1083) expression vectors. (D) hTAF_{II}135(630–1083) potentiates activation of the RAR β 2 promoter by endogenous NRs in Cos cells. Transfections contained 1 μ g of the RAR β 2–CAT reporter. (E) hTAF_{II}135(630–1083) enhances activation by coexpressed wild-type mouse (m)RAR α . Transfections contained 1 μ g of the RAR β 2–CAT reporter and 0 or 250 ng of mRAR α 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 pro-

motor 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 α 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 mu-

tated 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 μ g of the 17m5-TGTA-Luc reporter plasmid, 1 μ g of the TBP *spm3* expression vector, 250 ng of the G4-NR expression vectors, 0, 2, and 5 μ g 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 described in A. Transfections contained 250 ng of the G4-VDR and 1 μ g 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 RAR β 2 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 RAR β 2 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 μ M 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 μ m), 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)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, [³²P]-5'-end labeled, and used to screen a HeLa cell cDNA library in λ ZAPII (Stratagene). The filters were washed in 6 $\times$ 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 ³²P-labelled probe from nucleotides 1050–1456. This probe was used to screen 10⁶ phage from a human placental genomic DNA library. Filters were washed at 60°C in 0.2 $\times$ SSC and three positive clones were purified. A 4.7-kb BglIII–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.

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 *Hind*III sites between positions 1224–1236 without altering the amino acid sequence (5'-ACCCAGAGCCTGTCCCGGACGCCC-3' changed to 5'-ACCCAAAGCTTGTCTAGAACCC-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 *Hind*III 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 *Hind*III site at a position identical to that introduced in the cDNA. PCR was performed 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 *Hind*III sites of the pXJ40 expression vector. The resulting pXJ40 clone was then digested with *Hind*III and *Bgl*II and the *Hind*III–*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' primer 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 μ g of the luciferase reporter pRSV-Luc as internal standard for CAT assays or 2 μ g 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 α cis-RA, and 3,5,3'-triiodo-L-thyronine (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 phosphorimager analysis was performed on a Fujix BAS 2000 apparatus. In all cases, similar results ($\pm$ 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 μ l 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 μ g/ml of leupeptin, pepstatin, aprotinin, antipain, and chymostatin. Extracts were mixed with $\sim$ 1 μ g of the monoclonal antibodies and 50 μ l 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.

References

- Apone, L.M., C.A. Virbasius, J.C. Reese, and M.R. Green. 1996. Yeast TAF_{II}90 is required for cell-cycle progression through G₂/M but not for general transcription activation. *Genes & Dev.* **10**: 2368–2380.
- Barettino, D., M.D.M.V. Ruiz, and H. Stunnenberg. 1994. Characterisation of the ligand-dependent transactivation domain of the thyroid hormone receptor. *EMBO J.* **13**: 3039–3049.
- Beato, M., P. Herrlich, and G. Schutz. 1995. Steroid hormone receptors: Many actors in search of a plot. *Cell* **83**: 851–857.
- Bertolotti, A., Y. Lutz, D.J. Heard, P. Chambon, and L. Tora. 1996. hTAF_{II}68, a novel RNA/ssDNA-binding protein with homology to the proto-oncoproteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. *EMBO J.* **15**: 5022–5031.
- Bourguet, W., M. Ruff, P. Chambon, H. Gronemeyer, and D. Moras. 1995. Crystal structure of the ligand-binding domain of the human nuclear receptor RXR α . *Nature* **375**: 377–382.
- Brou, C., S. Chaudhary, I. Davidson, Y. Lutz, J. Wu, J.M. Egly, L. Tora, and P. Chambon. 1993. Distinct TFIID complexes mediate the effect of different transcriptional activators. *EMBO J.* **12**: 489–499.
- Burley, S.K. and R.G. Roeder. 1996. Biochemistry and structural biology of transcription factor TFIID. *Annu. Rev. Biochem.* **65**: 769–799.
- Caron, C., G. Mengus, V. Dubrovskaya, I. Davidson, and P. Jalinot. 1997. Human TAF_{II}28 interacts with the human T-cell leukemia virus type 1 Tax transactivator and promotes its transcriptional activity. *Proc. Natl. Acad. Sci.* **94**: 3662–3667.
- Cavaillès, V., S. Dauvois, F. L'Horsset, G. Lopez, S. Hoare, P.J. Kushner, and M.G. Parker. 1995. Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. *EMBO J.* **14**: 3741–3751.
- Chakravarti, D., V.J. LaMorte, M.C. Nelson, T. Nakajima, I.G. Schulman, H. Juguilon, M. Montminy, and R.M. Evans. 1996. Role CBP/P300 in receptor signaling. *Nature* **383**: 99–103.
- Chambon, P. 1994. The retinoid signalling pathway: Molecular and genetic analyses. *Semin. Cell. Biol.* **5**: 115–125.
- . 1996. A decade of molecular biology of retinoic acid receptors. *FASEB J.* **10**: 940–955.
- Chatterjee, S. and K. Struhl. 1995. Connecting a promoter bound protein to TBP bypasses the need for a transcriptional activation domain. *Nature* **374**: 820–822.
- Chaudhary, S., C. Brou, M.E. Valentin, N. Burton, L. Tora, P. Chambon, and I. Davidson. 1994. A cell-specific factor represses stimulation of transcription *in vitro* by transcriptional enhancer factor-1. *Mol. Cell. Biol.* **14**: 5290–5299.
- Chen, J.D. and R.M. Evans. 1995. A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* **377**: 454–457.
- Chen, J.L., L.D. Attardi, C.P. Verrijzer, K. Yokomori, and R. Tjian. 1994. Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. *Cell* **79**: 93–105.
- Chi, T. and M. Carey. 1996. Assembly of the isomerized TFIIA–TFIID–TATA ternary complex is necessary and sufficient for gene activation. *Genes & Dev.* **10**: 2540–2550.
- Chiang, C.-M., H. Ge, Z. Wang, A. Hoffman, and R.G. Roeder. 1993. Unique TATA-binding protein-containing complexes and cofactors involved in transcription by RNA polymerases II and III. *EMBO J.* **12**: 2749–2762.
- Danielan, P.S., R. White, J.A. Lees, and M.G. Parker. 1992. Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. *EMBO J.* **11**: 1025–1033.
- Dikstein, R., S. Zhou, and R. Tjian. 1996. Human TAF_{II}105 is a cell type-specific TFIID subunit related to hTAF_{II}130. *Cell* **87**: 137–146.
- Dubrowskaya, V., A.C. Lavigne, I. Davidson, J. Acker, A. Staub, and L. Tora. 1996. Distinct domains of hTAF_{II}100 are required for functional interaction with transcription factor TFIIF β (RAP30) and incorporation into the TFIID complex. *EMBO J.* **15**: 3702–3712.
- Durand, B., M. Saunders, C. Gaudon, B. Roy, R. Losson, and P. Chambon. 1994. Activation function 2 (AF-2) of the retinoic acid and 9-cis-retinoic acid receptors: Presence of a conserved autonomous constitutive activating domain and influence of the nature of the response element on AF-2 activity. *EMBO J.* **13**: 5370–5382.
- Dynlacht, B.D., T. Hoey, and R. Tjian. 1991. Isolation of coactivators associated with the TATA binding protein that mediate transcriptional activation. *Cell* **55**: 563–576.
- Eckner, R. 1996. p300 and CBP as transcriptional regulators and targets of oncogenic events. *Biol. Chem.* **377**: 685–688.
- Fondell, J.D., H. Ge, and R.G. Roeder. 1996. Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. *Proc. Natl. Acad. Sci.* **93**: 8329–8333.
- Geisberg, J.V., J.L. Chen, and R.P. Ricciardi. 1995. Subregions of the adenovirus E1A transactivation domain target multiple components of the TFIID complex. *Mol. Cell. Biol.* **15**: 6283–6290.
- Gill, G., E. Pascal, Z.H. Tseng, and R. Tjian. 1994. A glutamine-rich hydrophobic patch in transcription factor Sp1 contacts the dTAF_{II}110 component of the *Drosophila* TFIID complex and mediates transcriptional activation. *Proc. Natl. Acad. Sci.* **91**: 192–196.
- Goodrich, J.A. and R. Tjian. 1994. TBP–TAF complexes: Selectivity factors for eukaryotic transcription. *Curr. Opin. Cell Biol.* **6**: 403–409.
- Goodrich, J.A., T. Hoey, C.J. Thut, A. Admon, and R. Tjian. 1993. *Drosophila* TAF_{II}40 interacts with both a VP16 activation domain and the basal transcription factor TFIIB. *Cell* **75**: 519–530.
- Gruda, M.C., J.M. Zabolotny, J.H. Xiao, I. Davidson, and J.C. Alwine. 1993. Transcriptional activation by SV40 large T antigen: Interactions with multiple components of the transcription complex. *Mol. Cell. Biol.* **13**: 961–969.

- Hanstein, B., R. Eckner, J. DiRenzo, S. Halachmi, H. Liu, B. Searcy, R. Kurokawa, and M. Brown. 1996. p300 is a component of an estrogen receptor coactivator complex. *Proc. Natl. Acad. Sci.* **93**: 11540–11545.
- Hernandez, N. 1993. TBP, a universal eukaryotic transcription factor? *Genes & Dev.* **7**: 1291–1308.
- Hoey, T., B.D. Dynlacht, M.G. Peterson, B.F. Pugh, and R. Tjian. 1990. Isolation and characterisation of the *Drosophila* gene encoding the TATA box binding protein, TFIID. *Cell* **61**: 1179–1186.
- Hoey, T., R.O.J. Weinzierl, G. Gill, J.L. Chen, B.D. Dynlacht, and R. Tjian. 1993. Molecular cloning and functional analysis of *Drosophila* TAF_{II}110 reveal properties expected of co-activators. *Cell* **72**: 247–260.
- Hoffmann, A. and R.G. Roeder. 1996. Cloning and characterisation of human TAF_{20/15}: Multiple interactions suggest a central role in TFIID complex formation. *J. Biol. Chem.* **271**: 18194–18202.
- Hong, H., K. Kohli, A. Trivedi, D.L. Johnson, and M.R. Stallcup. 1996. GRIP1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors. *Proc. Natl. Acad. Sci.* **93**: 4948–4952.
- Hörlein, A.J., A.M. Näär, T. Heinzel, J. Torchia, B. Gloss, R. Kurokawa, A. Ryan, Y. Kamei, M. Söderström, C.K. Glass, and M.G. Rosenfeld. 1995. Ligand independent repression by the thyroid hormone receptor mediated by a nuclear receptor corepressor. *Nature* **377**: 397–404.
- Hwang, J.J., P. Chambon, and I. Davidson. 1993. Characterisation of the transcription activation function and the DNA binding domain of transcriptional enhancer factor-1. *EMBO J.* **12**: 2337–2348.
- Ingles, C.J., M. Shales, W.D. Cress, S.J. Triezenberg, and J. Greenblatt. 1991. Reduced binding of TFIID to transcriptionally compromised mutants of VP16. *Nature* **351**: 588–590.
- Jacq, X., C. Brou, Y. Lutz, I. Davidson, P. Chambon, and L. Tora. 1994. Human TAF_{II}30 is present in a distinct TFIID complex and is required for transcriptional activation by the estrogen receptor. *Cell* **79**: 107–117.
- Kamei, Y., L. Xu, T. Heinzel, J. Torchia, R. Kurokawa, B. Gloss, S.C. Lin, R.A. Heyman, D.W. Rose, C.K. Glass, and M.G. Rosenfeld. 1996. A CPB integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* **85**: 403–414.
- Kastner, P., M. Mark, and P. Chambon. 1995. Nonsteroid nuclear receptors: What are genetic studies telling us about their role in real life? *Cell* **83**: 859–869.
- Keaveney, M., A. Berkenstam, M. Felgenbutz, G. Vriend, and H. Stunnenberg. 1993. Residues in the TATA-binding protein required to mediate a transcriptional response to retinoic acid in EC cells. *Nature* **365**: 562–566.
- Klages, N. and M. Strubin. 1995. Stimulation of RNA polymerase II transcription initiation by recruitment of TBP in vivo. *Nature* **374**: 822–823.
- Klebanow, E.R., D. Poon, S. Zhou, and P.A. Weil. 1996. Isolation and characterisation of TAF₂₅, an essential yeast gene that encodes an RNA polymerase II-specific TATA-binding protein-associated factor. *J. Biol. Chem.* **271**: 13706–13715.
- Laudet, V. and H. Gronemeyer. 1995. Nuclear receptors. *Protein Profile* **2**: 1173–1236.
- Lavigne, A.C., G. Mengus, M. May, V. Dubrovskaya, L. Tora, P. Chambon, and I. Davidson. 1996. Multiple interactions between hTAF_{II}55 and other TFIID subunits: Requirements for the formation of stable ternary complexes between hTAF_{II}55 and the TATA binding protein. *J. Biol. Chem.* **271**: 19774–19780.
- LeDouarin, B., C. Zechel, J.M. Garnier, Y. Lutz, L. Tora, B. Pierat, D. Heery, H. Gronemeyer, P. Chambon, and R. Losson. 1995. The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-raf in the oncogenic protein T18. *EMBO J.* **14**: 2020–2033.
- LeDouarin, B., A.L. Nielsen, J.M. Garnier, H. Ichinose, F. Jeanmougin, R. Losson, and P. Chambon. 1996. A possible involvement of TIF1 α and TIF1 β in the epigenetic control of transcription by nuclear receptors. *EMBO J.* **15**: 6701–6715.
- Lee, J.W., F. Ryan, J.C. Swaffield, S.A. Johnston, and D.D. Moore. 1995. Interaction of thyroid-hormone receptor with a conserved transcriptional mediator. *Nature* **374**: 91–94.
- Lescure, A., Y. Lutz, D. Eberhardt, X. Jacq, A. Krol, I. Grummt, I. Davidson, P. Chambon, and L. Tora. 1994. The N-terminal domain of the human TATA-binding protein plays a role in transcription from the TATA-containing RNA polymerase II and III promoters. *EMBO J.* **13**: 1166–1175.
- Lin, Y.S., I. Ha, E. Maldonado, D. Reinberg, and M.R. Green. 1991. Binding of general transcription factor TFIIB to an acidic activating region. *Nature* **353**: 569–571.
- Mangelsdorf, D.J. and R.M. Evans. 1995. The RXR heterodimers and orphan receptors. *Cell* **83**: 841–850.
- Mangelsdorf, D.J., C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, and R.M. Evans. 1995. The nuclear receptor superfamily: The second decade. *Cell* **83**: 835–839.
- May, M., G. Mengus, A.C. Lavigne, P. Chambon, and I. Davidson. 1996. Human TAF_{II}28 promotes transcriptional stimulation by the activation function 2 of the retinoid X receptors. *EMBO J.* **15**: 3093–3104.
- Mazzarelli, J., B.J. Atkins, J.V. Geisberg, and R.P. Ricciardi. 1995. The viral oncoproteins Ad5 E1A, HPV 16 E7 and SV40 TAg bind a common region of the TBP-associated factor-110. *Oncogene* **11**: 1859–1864.
- Mengus, G., M. May, X. Jacq, A. Staub, L. Tora, P. Chambon, and I. Davidson. 1995. Cloning and characterisation of hTAF_{II}18, hTAF_{II}20, and hTAF_{II}28: Three subunits of the human transcription factor TFIID. *EMBO J.* **14**: 1520–1531.
- Moqtaderi, Z., Y. Bai, D. Poon, P.A. Weil, and K. Struhl. 1996a. TBP-associated factors are not generally required for transcriptional activation in yeast. *Nature* **383**: 188–191.
- Moqtaderi, Z., J. DePaulo, K. Struhl, and S. Buratowski. 1996b. Yeast homologues of higher eukaryotic TFIID subunits. *Proc. Natl. Acad. Sci.* **93**: 14654–14658.
- Nagpal, S., M. Saunders, P. Kastner, B. Durand, H. Nakshatri, and P. Chambon. 1992. Promoter context- and response element-dependent specificity of the transcriptional activation and modulating functions of the retinoic acid receptors. *Cell* **70**: 1007–1019.
- Nagpal, S., S. Friant, H. Nakshatri, and P. Chambon. 1993. RARs and RXRs: Evidence for two autonomous transactivation functions (AF-1 and AF-2) and heterodimerisation in vivo. *EMBO J.* **12**: 2349–2360.
- Onate, S.A., S.Y. Tsai, M.J. Tsai, and B.W. O'Malley. 1995. Sequence and characterisation of a coactivator for the steroid hormone receptor superfamily. *Science* **270**: 1354–1357.
- Parker, M.G. 1993. Steroid and related receptors. *Curr. Opin. Cell Biol.* **5**: 499–504.
- Poon, D., Y. Bai, A.M. Cambell, S. Björklund, Y.L. Kim, S. Zhou, R.D. Kornberg, and P.A. Weil. 1995. Identification and characterisation of a TFIID-like multiprotein complex from *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **92**: 8224–8228.
- Pugh, B.F. and R. Tjian. 1990. Mechanism of transcriptional activation by Sp1: Evidence for coactivators. *Cell* **61**: 1187–1197.

- . 1991. Transcription from a TATA-less promoter requires a multisubunit TFIID complex. *Genes & Dev.* **5**: 1935–1945.
- Reese, J.C., L.M. Apone, S.S. Walker, L.A. Griffen, and M.R. Green. 1994. Yeast TAF_{II}s in a multisubunit complex required for activated transcription. *Nature* **37**: 523–527.
- Renaud, J.P., N. Rochel, M. Ruff, V. Vivat, P. Chambon, H. Gronemeyer, and D. Moras. 1995. Crystal structure of the ligand binding domain of the human nuclear receptor RAR γ complexed with *all-trans* retinoic acid. *Nature* **378**: 681–689.
- Sauer, F., S.K. Hansen, and R. Tjian. 1995. Multiple TAF_{II}s directing synergistic activation of transcription. *Science* **270**: 1783–1778.
- Seipel, K., O. Georgiev, and W. Schaffner. 1992. Different activation domains stimulate transcription from remote ("enhancer") and proximal ("promoter") positions. *EMBO J.* **11**: 4961–4968.
- Sekiguchi, T., Y. Nohiro, Y. Nakamura, and T. Nishimoto. 1991. The human CCG1 gene essential for progression of the G1 phase, encodes a 210-kD nuclear DNA binding protein. *Mol. Cell Biol.* **11**: 3317–3325.
- Smith, C.L., S.A. Onate, M.J. Tsai, and B.W. O'Malley. 1996. CREB binding protein acts synergistically with steroid receptor coactivator-1 to enhance steroid receptor-dependent transcription. *Proc. Natl. Acad. Sci.* **93**: 8884–8888.
- Stargell, L.A. and K. Struhl. 1996a. Mechanisms of transcriptional activation in vivo: Two steps forward. *Trends Genet.* **12**: 311–315.
- . 1996b. A new class of activation-defective TATA-binding protein mutants: Evidence for two steps of transcriptional activation in vivo. *Mol. Cell Biol.* **16**: 4456–4464.
- Strubin, M. and K. Struhl. 1992. Yeast and human TFIID with altered DNA binding specificity for TATA elements. *Cell* **68**: 721–730.
- Struhl, K. 1995. Yeast transcriptional regulatory mechanisms. *Ann. Rev. Genet.* **29**: 651–674.
- Swaffield, J.C., K. Melcher, and S.A. Johnson. 1995. A highly conserved ATPase protein as a mediator between acidic activation domains and the TATA binding protein. *Nature* **374**: 88–91.
- Tanese, N., B.F. Pugh, and R. Tjian. 1991. Coactivators for a proline rich activator purified from the multisubunit human TFIID complex. *Genes & Dev.* **5**: 2212–2224.
- Tanese, N., D. Saluja, M.F. Vassallo, J.L. Chen, and A. Admon. 1996. Molecular cloning and analysis of two subunits of the human TFIID complex: hTAF_{II}130 and hTAF_{II}100. *Proc. Natl. Acad. Sci.* **93**: 13611–13616.
- Timmers, H.T.M., R. Meyers, and P.A. Sharp. 1992. Composition of transcription factor B-TFIID. *Proc. Natl. Acad. Sci.* **89**: 8140–8144.
- Tjian, R. and T. Maniatis. 1994. Transcriptional activation: A complex puzzle with few easy pieces. *Cell* **77**: 5–8.
- Tora, L., J.H. White, C. Brou, D. Tasset, N. Webster, E. Scheer, and P. Chambon. 1989. The human estrogen receptor has two independent non-acidic transcriptional activation functions. *Cell* **59**: 477–487.
- Tsai, M.J. and B.W. O'Malley. 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu. Rev. Biochem.* **63**: 451–486.
- Voegel, J.J., M.J.S. Heine, C. Zechel, P. Chambon, and H. Gronemeyer. 1996. TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO J.* **15**: 3667–3675.
- Vom Baur, E., C. Zechel, D. Heery, M. Heine, J.M. Garnier, V. Vivat, B. LeDouarin, H. Gronemeyer, P. Chambon, and R. Losson. 1995. Differential ligand-dependent interactions between the AF-2 activating domain of nuclear receptors and the putative transcriptional intermediary factors mSUG1 and TIF1. *EMBO J.* **15**: 110–124.
- Wagner, R.L., J.W. Apriletti, M.E. McGrath, B.L. West, J.D. Baxter, and R.J. Fletterick. 1995. A structural role for hormone in the thyroid hormone receptor. *Nature* **378**: 690–697.
- Walker, S.S., J.P. Reese, L.M. Apone, and M.R. Green. 1996. Transcription activation in cells lacking TAF_{II}s. *Nature* **383**: 185–188.
- Wurtz, J.M., W. Bourguet, J.P. Renaud, V. Vivat, P. Chambon, D. Moras, and H. Gronemeyer. 1996. A canonical structure for the ligand binding domain of nuclear receptors. *Nature Struct. Biol.* **3**: 87–94.
- Yao, T.P., G. Ku, N. Zhou, R. Scully, and D.M. Livingston. 1996. The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. *Proc. Natl. Acad. Sci.* **93**: 10626–10631.
- Zhou, Q., P.M. Lieberman, T.G. Boyer, and A.J. Berk. 1992. Holo-TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. *Genes & Dev.* **6**: 1964–1974.

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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