MOLECULAR AND CELLULAR BIOLOGY, Oct. 1998, p. 5734–5743 Vol. 18, No. 10
0270-7306/98/$04.00+0

Distinct Subdomains of Human $TAF_{II}130$ Are Required for Interactions with Glutamine-Rich Transcriptional Activators

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Received 23 February 1998/Returned for modification 1 April 1998/Accepted 1 July 1998

TFIID is a multiprotein complex consisting of the TATA box binding protein and multiple tightly associated proteins (TAF_{II}s) that are required for transcription by selected activators. We previously reported cloning and partial characterization of human $TAF_{II}130$ ($hTAF_{II}130$). The central domain of $hTAF_{II}130$ contains four glutamine-rich regions, designated Q1 to Q4, that are involved in interactions with the transcriptional activator Sp1. Mutational analysis has revealed specific regions within the glutamine-rich (Q1 to Q4) central region of $hTAF_{II}130$ that are required for interaction with distinct activation domains. We tested amino- and carboxyl-terminal deletions of $hTAF_{II}130$ for interaction with Sp1 activation domains A and B (Sp1A and Sp1B) and the N-terminal activation domain of CREB (CREB-N) by using the yeast two-hybrid system. Our results indicate that Sp1B interacts almost exclusively with the Q1 region of $hTAF_{II}130$. In contrast, Sp1A makes multiple contacts with Q1 to Q4 of $hTAF_{II}130$, while CREB-N interacts primarily with the Q1-Q2 $hTAF_{II}130$ subdomain. Consistent with these interaction studies, overexpression of the Q1-to-Q4 region in HeLa cells inhibits Sp1- but not VP16-mediated transcriptional activation. These findings indicate that the Q1-to-Q4 region of $hTAF_{II}130$ is required for Sp1-mediated transcriptional enhancement in mammalian cells and that different activation domains target distinct subdomains of $hTAF_{II}130$.

The role of TAFs in transcriptional regulation has been intensely studied in vitro as well as in vivo over the past several years (reviewed in references 3, 5, 32, and 40). Results from the early in vitro studies have revealed that TAFs play an essential role in mediating transcriptional activation by a variety of activators, and as such, they are considered coactivators. TAFs have been shown to directly contact selected activators, and these interactions are required for activated transcription in vitro. In vivo studies conducted with yeast, however, have suggested that TAFs may not be required at all gene promoters to regulate transcription (29, 47). Further work has revealed that they may be essential for transcription of selected genes that govern the cell cycle progression in yeast (1, 48). Studies carried out with the *Drosophila* embryo have also demonstrated that specific TAF-activator interactions are required for activation of selected genes in vivo (41).

$hTAF_{II}130$ is a human homolog of *Drosophila* $TAF_{II}110$ ($dTAF_{II}110$), the first TAF demonstrated to possess coactivator activity (6, 20). Unlike other TAFs, $hTAF_{II}130$ and $dTAF_{II}110$ display limited sequence similarities (26, 45). $hTAF_{II}130$ is also unique among TAFs in that no apparent homolog exists in yeast. Furthermore, $hTAF_{II}130$ may be the product of a member of a gene family, since at least one additional related but distinct gene product, $hTAF_{II}105$, has been found in the TFIID complex purified from differentiated B cells (11).

Protein-protein interaction assays as well as in vitro transcription assays have provided evidence for direct interaction of activators with one or more TAFs in the TFIID complex (6, 17, 20, 21). Significantly, such studies have suggested that different activators may interact selectively with specific TAF proteins. For example, glutamine-rich activation domains of Sp1 and the cyclic AMP-responsive transcription factor CREB bind $hTAF_{II}130$ (45) and $dTAF_{II}110$ (14, 20), the activation domains of VP16 and p53 bind $hTAF_{II}32$ (23, 24) and $dTAF_{II}40$ (17, 23, 24, 46), the retinoblastoma susceptibility gene product binds $hTAF_{II}250$ (42), and the estrogen receptor interacts with $hTAF_{II}30$ that is present in a subset of TFIID complex (21). These interactions are thought to participate in the recruitment and/or stabilization of the preinitiation complex at the promoter, leading to increased levels of transcription. TAFs may also play a role in positioning TFIID onto promoter DNA, in conjunction with TFIIA. In the context of promoter-bound TFIID, site-specific photo-cross-linking of $hTAF_{II}130$ to the adenovirus major late promoter was observed (31). Furthermore, *Drosophila* $TAF_{II}60$ was shown to bind to the conserved downstream core promoter element (2), while a recent study indicated that yeast $TAF_{II}145$ functions to recognize selected core promoters (43). It is evident from these studies that TAFs serve multiple functions as a coactivator and a promoter selectivity factor. In addition, a regulatory function has been suggested for TAFs, as recent findings indicate that $TAF_{II}250$ contains protein kinase (10) and histone acetyltransferase (28) activities. As integral components of the preinitiation complex, TAFs also participate in protein-protein interactions with components of the general transcription machinery (3).

As a step towards understanding the function of $hTAF_{II}130$, we have identified the regions of several activators that interact with $hTAF_{II}130$. We then compared and contrasted these activator-TAF interactions by using individual activators and deletions of $hTAF_{II}130$. This analysis of TAF-activator interactions should provide an understanding of how multiple activators cooperate to activate transcription by targeting the same TAF protein in the general transcription machinery.

The human transcription factor Sp1 contains glutamine-rich

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activation domains, A and B (9). Using protein-protein interaction assays in yeast, we have determined the regions within hTAF$_{II}$130 required for interaction with the A and B activation domains of Sp1 as well as the N-terminal activation domain of CREB. The deletion analyses suggest that different activation domains interact with distinct subdomains of hTAF$_{II}$130. Furthermore, transient expression of the central portion of hTAF$_{II}$130 reveals domain-specific inhibition of Sp1-mediated transcription in HeLa cells. We also show that Sp1B mutants that fail to interact with hTAF$_{II}$130 in the yeast two-hybrid assay display reduced transcription in transient-transfection assays in cultured cells. These results suggest that hTAF$_{II}$130 is likely to serve as a target for multiple activators in mammalian cells.

MATERIALS AND METHODS

Construction of C-terminal and N-terminal deletion derivatives of hTAF$_{II}$130. All hTAF$_{II}$130 derivatives used in this study were cloned into the pEG202 vector downstream of the LexA DNA binding domain (DBD) (18) and in frame with the introduced hemagglutinin antigen (HA) tag. For construction of C-terminal deletion derivatives, pAS-hTAF$_{II}$130 (residues 270 to 947) was linearized at the 3′ end of the hTAF$_{II}$130 cDNA sequence and digested with nuclease *Bal* 31 at 30°C for different times as described previously (37). Each deletion pool was then digested with *Eco*RI (upstream of the HA tag in pAS [12]), and the DNA fragments were purified and ligated to pEG202 digested with *Eco*RI and *Bam*HI (blunt ended). For construction of N-terminal deletion derivatives, pAS-hTAF$_{II}$130N/C (residues 270 to 700) (45) was linearized with *Eco*RI at the 5′ end of the insert sequence and digested with nuclease *Bal* 31 at 25°C, followed by digestion with *Sal*I. The DNA fragments were gel purified and subcloned into *Nco*I (blunt ended) and *Sal*I sites in pEG202 downstream of the LexA DBD and the introduced HA tag sequence. All constructs were sequenced across the cloning junction to select for the deletions that were in frame with the LexA DBD.

Yeast two-hybrid methods. The pEG202-hTAF$_{II}$130 deletion derivatives and the pJG4-5 vector (18) constructs encoding the B42 transcriptional activation domain fused to Sp1A (residues 83 to 262), Sp1B (residues 263 to 542) (a gift of Grace Gill, Harvard Medical School), or CREB-N (residues 3 to 296) were cotransformed into yeast strain EGY48 as described previously (20). Mutants of the Sp1B and Sp1B-c (residues 421 to 542) activation domains, cloned into the pGAD vector (16) (gifts of G. Gill), were cotransformed into yeast strain W303 with pEG202-hTAF$_{II}$130N/C (residues 270 to 700) as described previously (45). The transformed yeast cells were grown on a selection medium containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) for qualitative detection of the β-galactosidase activity. For quantitative β-galactosidase assays, transformed yeast cells were grown in a liquid selection medium for 24 to 36 h before induction (overnight), and β-galactosidase activity was measured in triplicate as described previously (15). Each experiment was repeated a minimum of three times. The expression of the fusion proteins was confirmed by immunoblotting with anti-HA antibody.

Transient-transfection assays with cultured mammalian cells. The pSG424 vector (36) constructs carrying the Gal4 DBD (residues 1 to 147) fused to Sp1A/B (residues 83 to 621), Sp1B (residues 263 to 542), Sp1B-c (residues 421 to 542), and their mutant derivatives were generous gifts of G. Gill (16). COS cells were transfected with a Gal4-Sp1 fusion construct and a UASp59RLG reporter plasmid (a gift of David Ron, New York University Medical Center) containing two copies of the Gal4 binding site upstream of the minimal angiotensinogen promoter (35), using the DEAE-dextran method as described previously (37). Transient transfections in HeLa cells were performed by using Lipofectamine (Life Technologies, Inc.) according to the manufacturer's instructions with minor modifications. Quantities of the cytomegalovirus (CMV)-driven expression plasmid DNA containing subdomains of HA-ΔNhTAF$_{II}$130 (amino acids 1 to 947) (45) were optimized for comparable levels of protein expression (see Fig. 5) as determined by immunoblotting with anti-HA antibody and an ECL chemiluminescence detection kit (Amersham Life Science). Each transfection in HeLa cells included a fixed amount of CMVhTAF$_{II}$130 derivative and/or empty CMV vector as well as CMVlacZ (0.15 μg), 5xGal4-E1b-luciferase reporter (44) (0.5 to 0.75 μg), and one of the following activators in the pSG424 vector: Gal4-Sp1A/B (0.25 μg), Gal4-Sp1B (0.25 μg), or Gal4-VP16 (0.05 μg). The HA-tagged CMVhTAF$_{II}$130 derivatives utilized were as follows: wild-type hTAF$_{II}$130 (residues 1 to 947) (2 μg), hTAF$_{II}$130N/C (residues 270 to 700) (1.25 to 3 μg), derivative 4 (residues 270 to 700/Δ454-525) (3 μg), derivative 10 (residues 270 to 409) (0.25 μg), derivative 13 (residues 270 to 350) (2 μg), N334 (residues 1 to 334) (0.1 μg), N288 (residues 1 to 288) (0.09 μg), and N297 (residues 1 to 297) (0.075 μg). At 40 h posttransfection, cells were washed twice in phosphate-buffered saline and harvested in 1× Reporter Lysis Buffer (Promega). Luciferase activity was quantified in a reaction mixture containing 25 mM glycylglycine (pH 7.8), 15 mM $MgSO_4$, 1 mM ATP, 0.1 mg of bovine serum albumin per ml, and 1 mM dithiothreitol. A Lumat LB 9507 luminometer (EG&G Berthold) was used to measure activity with 1 mM D-luciferin (Analytical Luminescence Laboratory) as the substrate. All transfections were performed in duplicate a minimum of three times.

RESULTS

The first glutamine-rich domain (Q1) within the central region of hTAF$_{II}$130 is sufficient for interaction with activation domain B of Sp1. Using the yeast two-hybrid system, we previously found that the central region (residues 270 to 700) of hTAF$_{II}$130 (designated hTAF$_{II}$130N/C) was sufficient to interact with activation domain B of Sp1 (Sp1B) (45). To further define subregions of the hTAF$_{II}$130 central domain for interaction with Sp1B, we generated a series of N-terminal and C-terminal deletions of hTAF$_{II}$130. Deletion mutants of hTAF$_{II}$130 were subcloned into a yeast plasmid downstream of the LexA DBD/HA tag sequence and tested for their ability to interact with the Sp1B domain.

The central region of hTAF$_{II}$130 contains four glutamine-rich regions (designated Q1 to Q4) (see the legend to Fig. 1). Figure 1 shows the results of the interaction assay with Sp1B and the C-terminal deletion mutants of hTAF$_{II}$130. Surprisingly, the hTAF$_{II}$130 C-terminal deletion mutants lacking the Q2, Q3, and Q4 glutamine-rich regions had little effect on the interaction with Sp1B (derivatives 1 to 12). hTAF$_{II}$130 containing only the Q1 region (derivative 13) was found to be sufficient for interaction with Sp1B. Deletion of Q1 (derivative 15) reduced the interaction to 28%. The central region of hTAF$_{II}$130 contains a sequence (CI, residues 449 to 528) that has a high degree of similarity with dTAF$_{II}$110 (45). We tested a derivative lacking most of the CI sequence (derivative 4) and found that the conserved sequence CI was not required for interaction of hTAF$_{II}$130 with Sp1B. Although derivatives 6 to 8 were found to be weakly active in the absence of Sp1B, they still showed significant interactions with Sp1B, as the β-galactosidase activity measured was significantly enhanced over the basal levels in the presence of Sp1B (data not shown). The expression of all mutant hTAF$_{II}$130 proteins was confirmed by immunoblotting of the yeast cell lysates with anti-HA antibody (data not shown).

To test whether other glutamine-rich regions of hTAF$_{II}$130 (Q2, Q3, and Q4) could functionally substitute for Q1, we tested a series of N-terminal deletion mutants of hTAF$_{II}$130 in the yeast two-hybrid system. As shown in Fig. 2, deletion of a region containing Q1 (derivative 18) severely decreased (to 4.4%) the ability of hTAF$_{II}$130 to interact with Sp1B even in the presence of other glutamine-rich regions. These results suggest that a domain within amino acids 270 to 350 of hTAF$_{II}$130 (derivative 13) contains the sequences important for interaction with Sp1B and that the other domains (Q2, Q3, and Q4) cannot functionally substitute for Q1.

Different activators interact with distinct regions of hTAF$_{II}$130. The central portion of hTAF$_{II}$130, like dTAF$_{II}$110, also interacts with activation domain A of Sp1 (Sp1A) and the N-terminal activation domain of CREB (CREB-N) (14, 20, 45). We wanted to test whether these activators interacted with a common region or distinct regions within hTAF$_{II}$130. The hTAF$_{II}$130 deletion mutants described above were tested for their interaction with Sp1A and CREB-N by using the yeast two-hybrid system. As shown in Fig. 3, deletion of a region containing Q1 (derivative 18) of hTAF$_{II}$130 did not impair its interaction with Sp1A. This is in contrast to the result obtained with Sp1B (compare results with derivative 18 in Fig. 2 and 3), where deletion of Q1 virtually eliminated interaction. hTAF$_{II}$130 lacking Q1 and a portion of Q2 (derivative 19) retained 43% of the activity with Sp1A, whereas the same

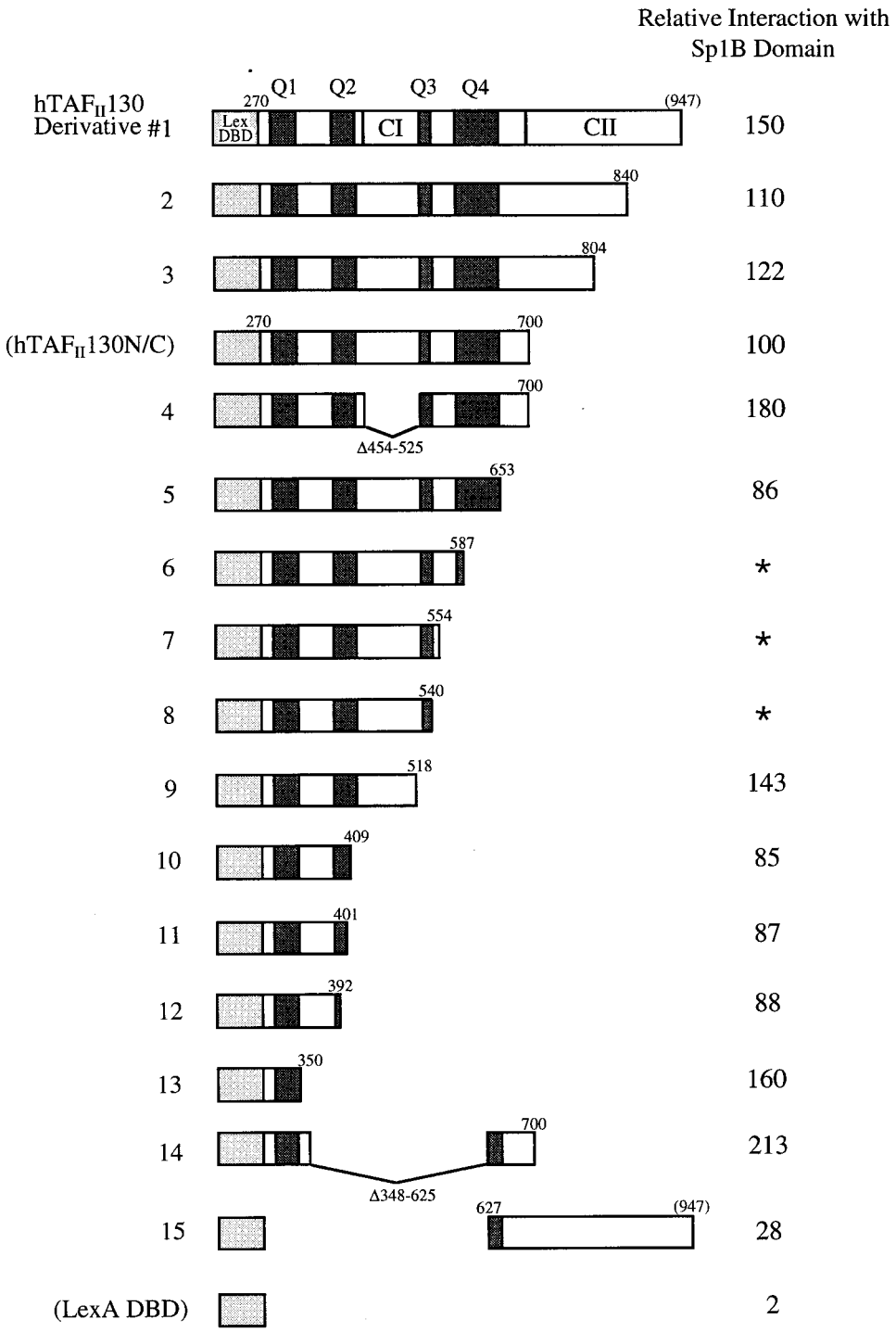


FIG. 1. Carboxyl-terminal deletion analysis of $hTAF_{II}130$ reveals the Q1 region to be important for interaction with Sp1B. Derivatives of $hTAF_{II}130$ fused to the LexA DBD in pEG202 are shown schematically. Yeast (EGY48) was transformed with pEG202-$hTAF_{II}130$ fusion constructs and an Sp1B (residues 263 to 542) fusion construct in pJG4-5 along with the reporter plasmid. The percent β-galactosidase activity relative to that of $hTAF_{II}130N/C$ in each transformant is represented at the right. Asterisks indicate $hTAF_{II}130$ derivatives that activate weakly in the absence of Sp1B. All assays were done in triplicate. Expression of $hTAF_{II}130$ deletion mutants and Sp1B was confirmed by immunoblotting (data not shown). Q1, residues 300 to 347, 25% glutamine content; Q2, residues 388 to 419, 25% glutamine content; Q3, residues 528 to 550, 30% glutamine content; Q4, residues 580 to 651, 19% glutamine content. The numbering of the amino acid residues is as in reference 45.

construct interacted poorly with Sp1B (2.1%) (derivative 19 in Fig. 2). Interestingly, derivative 10 (Fig. 3), containing Q1 and Q2, interacted with Sp1A (42%) as well as derivative 21 (Fig. 3), which contained Q3 and Q4 (42%). This finding suggests that unlike Sp1B, Sp1A makes multiple contacts with $hTAF_{II}130$. We also observed that Sp1A interacted more strongly with $hTAF_{II}130$ than did Sp1B (30 to 60% higher activity) (data not shown). Sp1A was also shown to interact

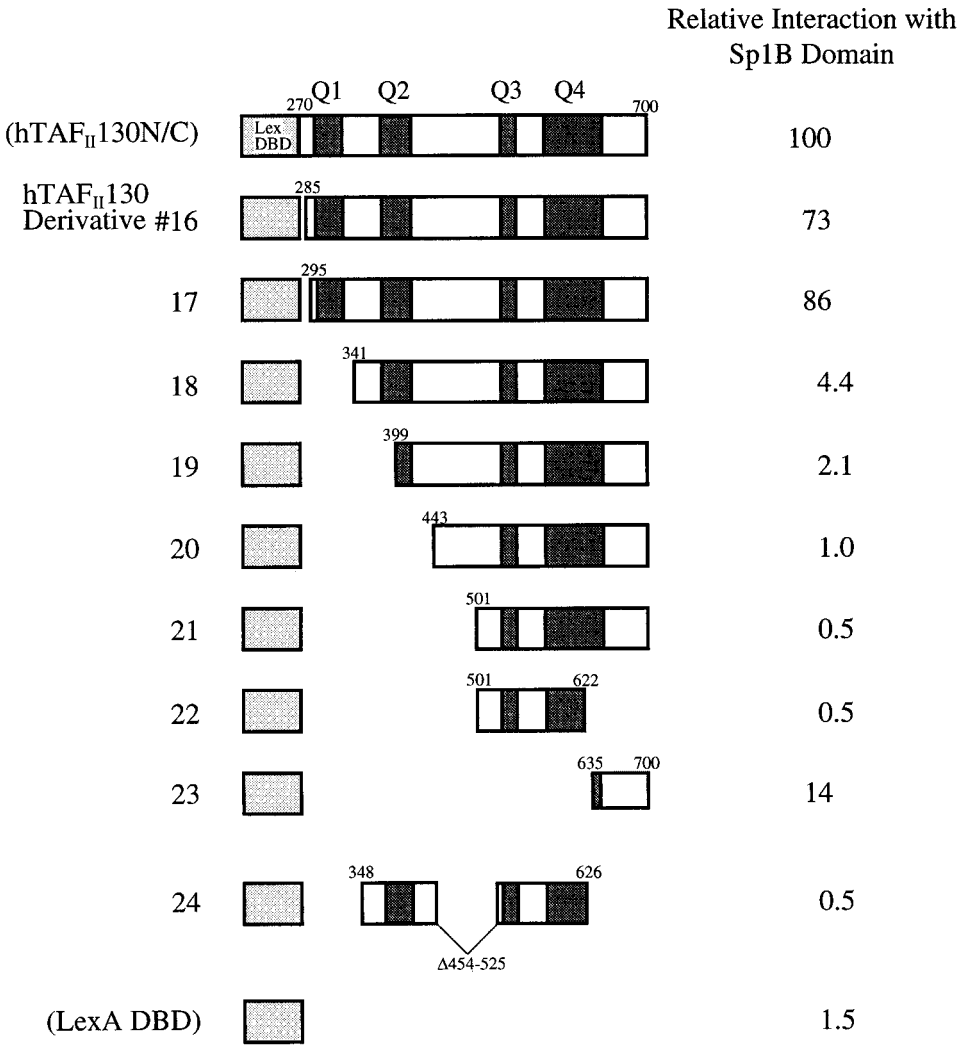


FIG. 2. Amino-terminal deletion analysis of hTAF$_{II}$130, showing that the Q1 region of hTAF$_{II}$130 is essential for interaction with Sp1B. The pEG202 plasmids expressing the N-terminal deletions of hTAF$_{II}$130N/C were cotransformed with pJG4-5-Sp1B into yeast (EGY48) as described in the legend to Fig. 1. The β-galactosidase activity relative to that of hTAF$_{II}$130N/C (set to 100%) is shown at the right.

with the N-terminal 308 amino acids of dTAF$_{II}$110, which exclude most of the highly conserved domain CI (20). Thus, dTAF$_{II}$110 and hTAF$_{II}$130 may have additional structural similarities, not apparent in the primary amino acid sequence, that permit their interactions with Sp1A.

The N-terminal glutamine-rich activation domain of CREB, on the other hand, preferentially interacted with a region encompassing Q1 and Q2 of hTAF$_{II}$130. Unlike the case for Sp1B, deletion of Q1 did not impair the interaction of hTAF$_{II}$130 with CREB-N (compare results with derivative 18 in Fig. 2 and 3); however, deletion of the sequences between Q1 and Q2 (derivative 19; Fig. 3) reduced the activity with CREB-N to 14%. Furthermore, derivatives 11 to 13, which contained Q1 and a partial Q2, interacted with CREB-N at reduced levels, suggesting additional interactions between Q2 and CREB-N. Interestingly, the C-terminal half of hTAF$_{II}$130, containing Q3 and Q4, did not interact efficiently with CREB-N, unlike with Sp1A (derivatives 20 and 21 in Fig. 3). Based on the hTAF$_{II}$130 N-terminal (derivatives 18 and 19) and C-terminal (derivatives 10 and 12) deletion constructs, a region involved in interaction with CREB-N appeared to encompass Q1 and Q2. This is in contrast to the interactions of Sp1A with hTAF$_{II}$130 (Q1 to Q4) and of Sp1B with hTAF$_{II}$130 (Q1). Thus, different activation domains appear to interact with distinct subdomains of hTAF$_{II}$130.

The ability of mutants of Sp1B to interact with hTAF$_{II}$130 correlates with their ability to activate transcription in mammalian cells. To demonstrate that Sp1B-hTAF$_{II}$130 interaction correlates with Sp1's ability to activate transcription, we tested previously characterized mutants of Sp1B (16) in the yeast two-hybrid system with hTAF$_{II}$130 (Fig. 4). Linker substitution mutations in the carboxyl-terminal half of the Sp1B domain (M37 and M38) resulted in a 65 to 91% decrease in the ability of Sp1B to interact with hTAF$_{II}$130 (Fig. 4A). Although the C-terminal subdomain of Sp1B (Sp1B-c) was sufficient to interact with hTAF$_{II}$130, substitution mutants B-c/M37 and B-c(W→A) interacted poorly with hTAF$_{II}$130 (Fig. 4B), supporting the above-described finding that the C-terminal half of Sp1B contains the sequences required for interaction with hTAF$_{II}$130. As with dTAF$_{II}$110 (16), the replacement of two glutamines and one asparagine with alanine residues did not affect the interaction of the mutant B-c (Q→A) with hTAF$_{II}$130.

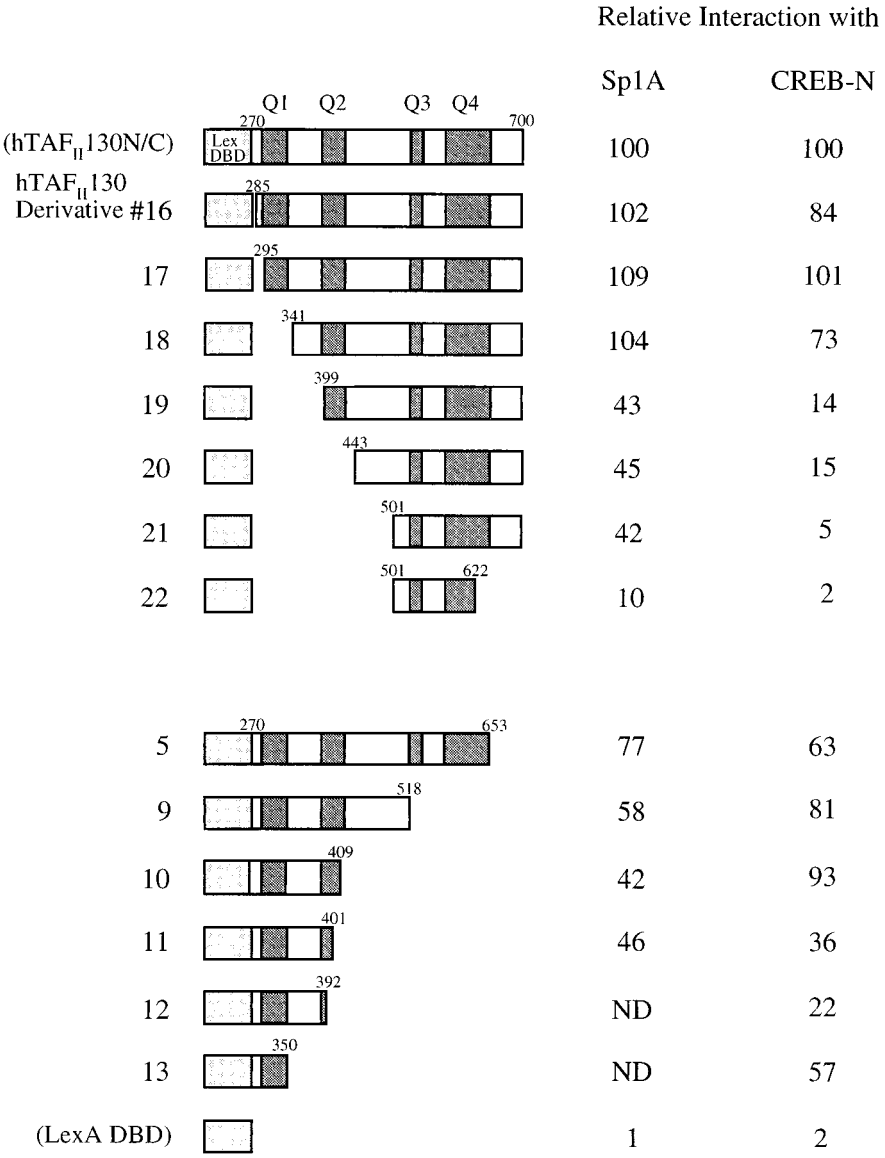
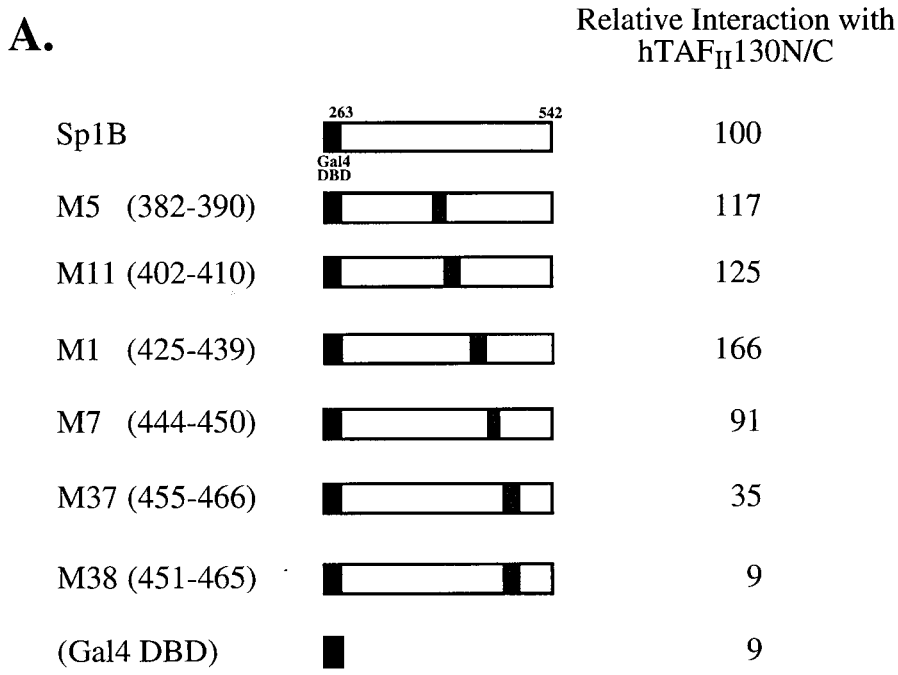


FIG. 3. Different activators interact with distinct regions within hTAF$_{II}$130. pEG202-hTAF$_{II}$130 derivatives were cotransformed into yeast with pJG4-5 plasmids expressing either Sp1A (residues 83 to 262) or CREB-N (residues 3 to 296) along with the reporter plasmid. All other conditions were as described in the legend to Fig. 1. The hTAF$_{II}$130 derivatives shown have the same numbers as in Fig. 1 and 2. The β-galactosidase activity of hTAF$_{II}$130N/C measured with pJG4-5–activator fusions was taken as 100%. ND, not determined.

To correlate the ability of the Sp1B derivatives to interact with hTAF$_{II}$130 with their ability to activate transcription in mammalian cells, we tested the expression of a luciferase reporter gene containing the Gal4 binding sites by cotransfection of plasmids expressing Gal4–Sp1B-c or its mutant derivatives into COS cells. Gal4–Sp1B-c efficiently activated the reporter gene (90 to 100% of the activation by Gal4-Sp1B [data not shown]), whereas Gal4-DBD showed 5 to 10% of the activity of Gal4–Sp1B-c (data not shown). The linker substitution mutation significantly compromised the activation of the reporter gene (Sp1B-c/M37) (24%), as did the W→A substitution mutation in Sp1B-c (6%) (Fig. 4B). By contrast, Sp1B-c bearing the Q→A mutation retained activity close to that of the wild type. Thus, Sp1B-c mutants that interacted poorly with hTAF$_{II}$130 in the yeast two-hybrid assay also failed to direct efficient transcription of the reporter gene in mammalian cells.

Transient expression of the hTAF$_{II}$130 central domain selectively interferes with Sp1-mediated activation of the reporter gene in HeLa cells. To further demonstrate the role of hTAF$_{II}$130 in mediating transcriptional activation by Sp1, we performed transient-transfection assays in HeLa cells with Sp1-responsive luciferase reporter constructs. HeLa cells were cotransfected with the expression plasmids carrying HA-tagged subdomains of hTAF$_{II}$130 as shown schematically in Fig. 5A. The amount of DNA transfected was adjusted so as to achieve comparable levels of protein expression, as shown in the representative anti-HA immunoblot (Fig. 5B). Cotransfection of a reporter construct bearing five Gal4 binding sites with a plasmid expressing the Gal4-Sp1A/B activator directed a high level of luciferase activity, which was decreased three- to fourfold in the presence of two hTAF$_{II}$130 subdomains, hTAF$_{II}$130N/C and derivative 4 (Fig. 5C). By contrast, constructs N334 and



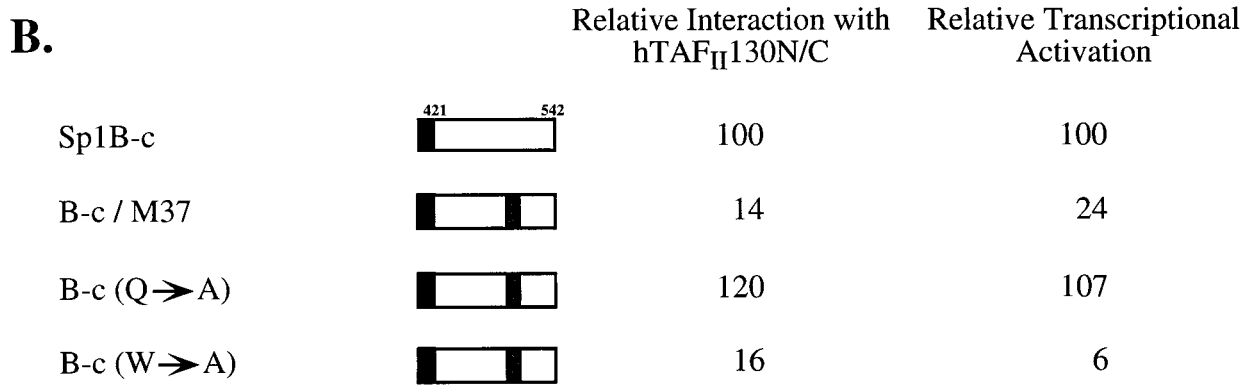


FIG. 4. The C-terminal subdomain of Sp1B interacts with hTAF$_{II}$130 and activates transcription in mammalian cells. (A) Linker substitution mutants of Sp1B (16) were tested for interaction with hTAF$_{II}$130 in the yeast two-hybrid assay. Yeast (W303) was cotransformed with pEG202-hTAF$_{II}$130N/C and Sp1B mutants in pGAD. The percent β-galactosidase activity was measured relative to that of the wild-type Sp1B. (B) Substitution mutants of Sp1B-c were tested for interaction with hTAF$_{II}$130 in the yeast two-hybrid assay. Sp1B-c mutants in pGAD were transformed into yeast along with pEG202-hTAF$_{II}$130N/C. The percent β-galactosidase activity was measured relative to that of the wild-type Sp1B-c. To determine the transcriptional activities of these Sp1B-c mutants, plasmids expressing the indicated Sp1B mutants fused to the Gal4 DBD were transfected into COS cells along with a Gal4-driven luciferase reporter gene and assayed for activation of transcription. The resulting luciferase activity was expressed relative to the activity of the wild-type Sp1B-c domain. All assays were done in triplicate.

N288, expressing the N-terminal subdomains of hTAF$_{II}$130, had no detectable effect on Gal4-Sp1A/B-mediated transcription. The finding that a deletion of the conserved region CI (derivative 4) did not affect the ability of the hTAF$_{II}$130 central domain to inhibit transcription is in agreement with the result from the yeast two-hybrid system in which the ΔCI construct remained capable of interacting with Sp1 (derivative 4 in Fig. 1). Additionally, construct N334, expressing a portion of Q1, did not inhibit activation by Sp1A/B, suggesting that additional Q regions are necessary for full inhibition of Sp1A/B.

We next tested the effects of transiently expressing the wild-type hTAF$_{II}$130 cDNA (amino acids 1 to 947) as well as derivatives carrying a subset of Q-rich regions (Fig. 6A) on the reporter gene activated by Gal4-Sp1B. Derivatives 10 and 13 contain the same hTAF$_{II}$130 sequences as those shown to interact with Sp1B in the yeast two-hybrid system (derivatives 10 and 13 in Fig. 1). We found that wild-type hTAF$_{II}$130 (amino acids 1 to 947), as well as derivatives 10 and 13, decreased Gal4-Sp1B-mediated reporter gene activity but that the N-terminal subdomain N297 did not (Fig. 6B). To demonstrate that the squelching effect was specific for Sp1B, the Gal4-driven reporter gene was cotransfected with a plasmid expressing Gal4-VP16. Figure 6C shows that coexpression of hTAF$_{II}$130 subdomains had little effect on the Gal4-VP16-mediated activation of transcription, suggesting that the hTAF$_{II}$130 central domain had a specific effect on Sp1-mediated transcrip-

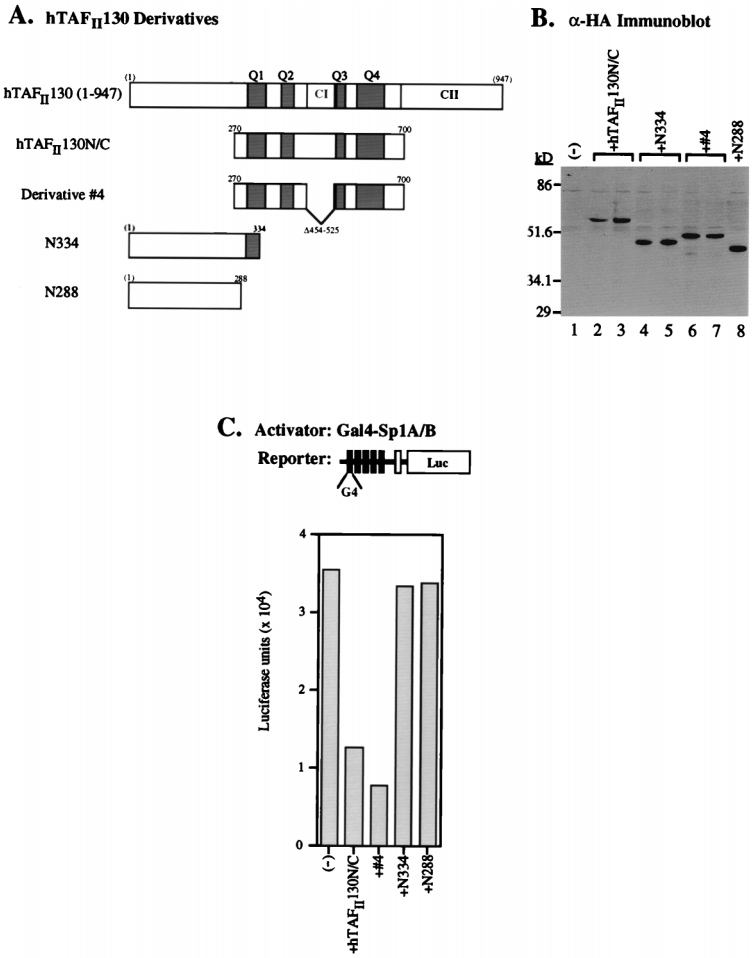


FIG. 5. Expression of the central domain of hTAF$_{II}$130 results in domain-specific inhibition of Gal4-Sp1-mediated transcriptional activation in HeLa cells. (A) Plasmid constructs that express subdomains of hTAF$_{II}$130 (tagged with HA) are depicted schematically. Derivative 4 contains the same hTAF$_{II}$130 sequence as in Fig. 1. Constructs N334 and N288 contain the N-terminal 334 and 288 amino acids of hTAF$_{II}$130, respectively. (B) A representative anti-HA (α-HA) Western immunoblot of cell lysates used in the luciferase assay, demonstrating comparable levels of protein expression of transfected HA-hTAF$_{II}$130 derivatives. Lysates of HeLa cells transfected with no hTAF$_{II}$130 derivative (lane 1), hTAF$_{II}$130N/C (lanes 2 and 3), N334 (lanes 4 and 5), derivative 4 (lanes 6 and 7), and N288 (lane 8) are shown. (C) Luciferase activity in the lysates of HeLa cells (as shown in panel B) transfected with the reporter construct, the indicated hTAF$_{II}$130 derivative, and Gal4-Sp1A/B (residues 83 to 621).

tion. In these experiments, hTAF$_{II}$130N/C, derivative 10, and N297 were expressed at comparable levels, whereas hTAF$_{II}$130 (amino acids 1 to 947) and derivative 13 were expressed at lower levels (data not shown). Similar results were obtained with different concentrations of activator proteins; thus, domain-specific transcriptional inhibition by hTAF$_{II}$130 was observed over a broad range of the reporter gene activity (data not shown). Taken together, the TAF-activator interaction studies carried out with yeast and cultured mammalian cells indicate that different activators bind distinct subdomains of hTAF$_{II}$130 and suggest a mechanism for coordinated action of multiple promoter-bound activators on the general transcription machinery.

DISCUSSION

Interactions between multiple activation domains and hTAF$_{II}$130. Recent studies on transcriptional activators that bind to enhancers to form a stereospecific enhanceosome complex have revealed multiple protein-protein contacts between DNA-bound activators, as well as between activators and their target proteins. It has been proposed that such extended networks of protein-protein interactions contribute to transcriptional synergy (reviewed in reference 4). For example, transcriptional activators bound to beta interferon enhancer appear to contact multiple regions of their target protein CBP (CREB-binding protein) or components of the basal transcriptional machinery to enhance transcription (22, 27). A previous study has already established that interactions between activators with multiple members of the general transcription machinery lead to synergistic transcription (7). It has also been shown with the *Drosophila hunchback* promoter that specific activator-TAF interactions are sufficient for simple as well as synergistic activation by multiple enhancer factors (38, 39). Thus, transcriptional synergy may be achieved by multiple protein-protein interactions between single or multiple domains of activators and single or multiple surfaces of their target proteins.

The results presented in this paper suggest that distinct subdomains of hTAF$_{II}$130 might also serve as targets for multiple transcriptional activation domains. Using N- and C-terminal deletion mutants of the hTAF$_{II}$130 central domain, we demonstrate that specific regions within this domain are required for interaction with the glutamine-rich activation domains A and B of Sp1 and CREB. The central domain of

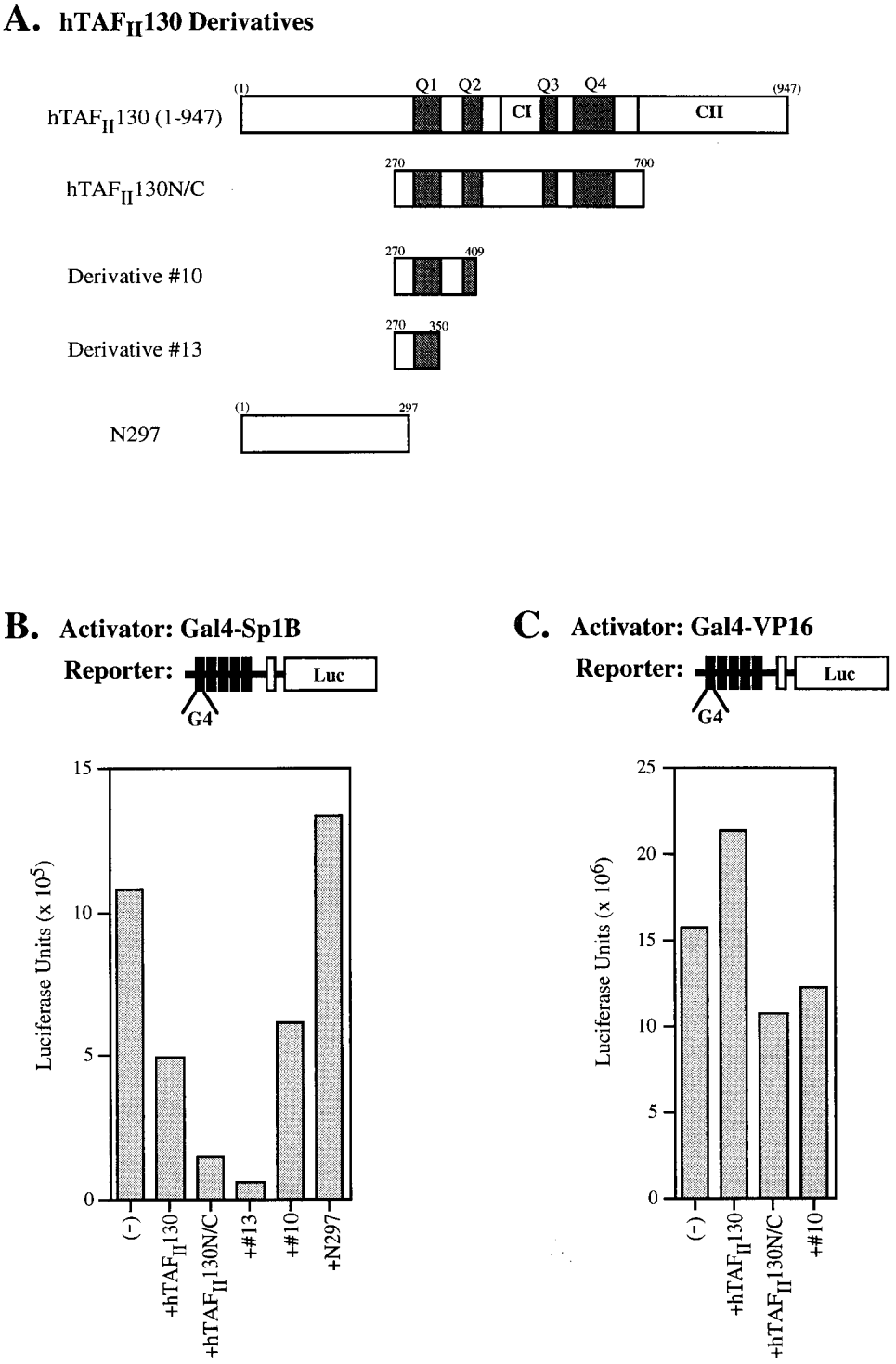


FIG. 6. Wild-type hTAF$_{II}$130 and subdomains of hTAF$_{II}$130 that interact with Sp1B domain inhibit transcriptional activation by Gal4-Sp1B (residues 263 to 542) but not by Gal4-VP16 in HeLa cells. (A) Schematic representation of hTAF$_{II}$130 derivatives used in the experiment. hTAF$_{II}$130 (1-947), wild-type hTAF$_{II}$130 carrying amino acids 1 to 947. Derivatives 10 and 13 contain the same hTAF$_{II}$130 sequences as those tested in the yeast two-hybrid assay (depicted in Fig. 1). (B and C) Luciferase activity in the lysates of HeLa cells transfected with the reporter construct, the indicated hTAF$_{II}$130 derivative, and Gal4-Sp1B (B) or Gal4-VP16 (C) was determined. Although not shown in panel C, N297 was also tested with Gal4-VP16 in a separate experiment, and like other hTAF$_{II}$130 derivatives, it was found to have no significant effect on the transcriptional activation by Gal4-VP16.

hTAF$_{II}$130 contains four glutamine-rich sequences (Q1 to Q4) flanking region CI, a sequence highly conserved between dTAF$_{II}$110 and hTAF$_{II}$130. CI is not required for interactions with these activation domains, suggesting that structural determinants within the nonconserved segment of hTAF$_{II}$130 mediate TAF-activator interactions. Interestingly, calculation of the percent glutamine content for the central domain (Q1 to Q4) of hTAF$_{II}$130 reveals 17% glutamine outside region CI

and 5% within region CI. For $dTAF_{II}110$, similar analysis reveals a 12.5% glutamine content outside region CI and 6% within region CI, whereas $hTAF_{II}105$ maintains a rather low (6%) glutamine content throughout the entire central domain, including region CI. Previous work with $dTAF_{II}110$ has shown that the amino-terminal 308 amino acid residues that exclude most of region CI are also sufficient to interact with Sp1A (20), in agreement with our finding that the conserved region is not required for TAF-Sp1 interactions. We are currently generating point mutations within the $hTAF_{II}130$ central domain to further dissect these TAF-activator interactions.

Although the majority of $hTAF_{II}130$ derivatives fused to the LexA DBD were transcriptionally inactive in yeast, C-terminal truncations that fell between residues 540 and 587 (Fig. 1) were found to be weakly active in the absence of Sp1. Interestingly, further removal of part of the highly conserved CI sequence from the truncated $hTAF_{II}130$ derivatives abolished self-activation (derivative 9 [Fig. 1]), suggesting that self-activation might have been caused by unmasking of the intact CI sequence. Although the function of CI is yet to be determined, the C-terminal conserved region CII in $dTAF_{II}110$ is required for interaction with $dTAF_{II}250$, $dTAF_{II}30\alpha$, and TFIIA-L (41, 45, 49), suggesting that CI might also serve a conserved function. Perhaps unmasking of the $hTAF_{II}130$ CI sequence in yeast may have permitted interaction(s) with a conserved domain of a yeast TAF or a general transcription factor, leading to tethering of the transcription machinery to the promoter and activation of the reporter gene (34).

Functional significance of the Sp1-$hTAF_{II}130$ interactions. We have also observed that Sp1A interacted more strongly with $hTAF_{II}130$ than did Sp1B in the yeast two-hybrid assay and in vitro binding assay (data not shown), similar to the observations made with $dTAF_{II}110$ (20). Perhaps multiple contacts made between Sp1A and subdomains of $hTAF_{II}130$ may explain why Sp1A functions as a more potent activator than Sp1B in transient-transfection studies (9). Since distinct regions of $hTAF_{II}130$ are targeted by Sp1A and B domains, these two domains in full-length Sp1 are likely to interact cooperatively with $hTAF_{II}130$ in vivo. Indeed, it has been shown that domains A and B, in addition to the carboxyl-terminal domain D, are all required for synergistic activation by Sp1 (33). Interestingly, the carboxyl-terminal domain of Sp1 that includes the zinc finger DBD and domain D has been shown to interact with $hTAF_{II}55$ (8). Thus, binding of Sp1 to different TAFs as well as different regions of the same TAF could result in cooperative interactions between Sp1 and TFIID and strong activation of transcription by the full-length Sp1 protein. It is worth noting that CREB also possesses two discrete activation domains, the kinase-inducible domain and the glutamine-rich activation domain Q2, both of which have been shown to be required for signal-dependent activation of transcription in vitro (30). The phosphorylation-dependent kinase-inducible domain has been shown to interact with RNA polymerase II via the coactivator CBP, and the Q2 activation domain appears to recruit TFIID via $hTAF_{II}130$. These experiments suggest that multiple interactions between an activator and the components of the transcriptional machinery are required for full activity of CREB.

It has been demonstrated that the carboxyl-terminal half of the Sp1B domain (Sp1B-c) is sufficient for interaction of Sp1B with $dTAF_{II}110$ and that mutants of Sp1B-c that failed to interact with $dTAF_{II}110$ also activated transcription at reduced levels in *Drosophila* Schneider cells (16). We have shown in the present study that the same mutants of Sp1B-c interacted poorly with $hTAF_{II}130$ and were compromised for their ability to activate transcription in mammalian cells. Thus, both in insect cells and in mammalian cells we find a correlation between the ability of Sp1 to interact with $hTAF_{II}130$ or $dTAF_{II}110$ and its ability to activate transcription. Moreover, despite the differences in the primary amino acid sequences between the $hTAF_{II}130$ central domain and the amino terminus of $dTAF_{II}110$, both proteins appear to interact with Sp1B in an analogous manner, suggesting a functional conservation between the two TAF proteins. It remains to be seen whether the interacting surfaces have similar structural characteristics.

Effects of transiently expressing $hTAF_{II}130$ in cultured cells. We have found that transient expression of the central domain of $hTAF_{II}130$ containing Q1 to Q4 ($hTAF_{II}130N/C$) as well as of subdomains of $hTAF_{II}130$ containing Q1 alone (derivative 13) or Q1 and Q2 (derivative 10) decreased transcriptional activation of the reporter gene by Gal4-Sp1B, consistent with the finding that Sp1B interacted strongly with Q1 in the yeast two-hybrid study. In the same transient-transfection assay, we also found that wild-type $hTAF_{II}130$ (amino acids 1 to 947) inhibited transcription by Gal4-Sp1B (Fig. 6B). It was previously reported that transient expression of full-length $dTAF_{II}110$ did not affect transcriptional activation by Sp1 in insect cells (13). It is possible that overexpression of $dTAF_{II}110$ was not sufficient to block activation by the full-length Sp1 used in that experiment, since full-length Sp1 has multiple potential targets within TFIID, including $hTAF_{II}55$, as discussed above. By contrast, in another study, transient expression of the full-length as well as the C-terminal portions of $hTAF_{II}130$ was reported to significantly enhance transcription of the reporter genes driven by the AF-2 activation domains of the retinoic acid, vitamin D_3, and thyroid hormone receptors (26). The authors of that study found $hTAF_{II}130$ to be limiting in vivo in some cell lines and thus speculated that overexpression might result in an increase in TFIID available for recruitment to promoters driven by AF-2. Interestingly, unlike the glutamine-rich activation domains described in this paper, AF-2 domains of selected nuclear receptors did not directly contact $hTAF_{II}130$. The authors proposed that $hTAF_{II}130$ might contact a common intermediary protein(s) that binds AF-2 domains in a subset of nuclear receptors. Finally, the conserved C-terminal 105 amino acids of $hTAF_{II}130$ have been reported to interact with the CR3 activation domain of E1A. In that study, the C-terminal fragment of $hTAF_{II}130$ was shown to specifically inhibit E1A-mediated transcriptional activation when transiently expressed in mammalian cells (25).

Although TAFs are present as integral components of the general transcription machinery, individual TAFs might be required by only a subset of activators in a eukaryotic cell. It is possible that short stretches of amino acid residues may be sufficient to provide specific points of contact between a given activator and a TAF. Thus, it is reasonable to envision 8 to 12 TAFs in the TFIID complex providing enough surface for interaction with a large number of activators present in a eukaryotic cell. Posttranslational modifications, differential splicing, and tissue-specific expression of TAFs may further add to the specificity of activator-TAF interactions. Indeed, the recent discovery of a new complex composed of TRF (TBP-related factor) and novel TAF subunits further increases the repertoire of TAFs required for coactivator function in different cell types (19). Binding of different activators to different TAFs or to distinct subdomains within the same TAF may allow TFIID to respond to multiple signals from activators bound upstream of the transcriptional initiation site, resulting in the coordinated expression of genes.

ACKNOWLEDGMENTS

We are grateful to Grace Gill of Harvard Medical School for her help and generous gifts of the yeast and mammalian plasmids carrying Sp1 derivatives and to Michael Garabedian of New York University Medical Center for many valuable discussions. We thank Kelly Vogel and Amy Kun for technical assistance, Eileen Rojo-Niersbach and Stavros Giannakopoulos for their help with the project, Muktar Mahajan for advice on the yeast two-hybrid assay, Sobha Pisharody for assistance with cell culture, and David Ron for the gift of the reporter plasmid. Critical reading of the manuscript by Michael Garabedian and Grace Gill was greatly appreciated.

This work was supported by a grant from the National Institutes of Health (R01-BM51314). D.S. was supported by a National Institutes of Health Training Grant (5T32 AI07180), and N.T. was supported in part by The Irma T. Hirschl Trust.

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