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Human $TAF_{II}28$ interacts with the human T cell leukemia virus type I Tax transactivator and promotes its transcriptional activity

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ABSTRACT **The Tax protein encoded by human T cell leukemia virus type I transactivates the viral promoter by forming a complex with several cellular factors bound to three repeats of a specific upstream regulatory sequence. We have shown that transactivation by Tax was correlated with its ability to interact with the C-terminal moiety of the TATA box-binding protein (TBP). In the present study, the ability of Tax to interact with several human TBP-associated factors (TAF_{II}s) was analyzed. We show that Tax interacts selectively with $hTAF_{II}28$ in transfected HeLa cells. A direct interaction between Tax and $hTAF_{II}28$ was also observed *in vitro* with purified proteins. In transient expression studies we show that overexpression of $hTAF_{II}28$ significantly increased transactivation by Tax, both in the absence and in the presence of overexpressed TBP. The ability of $hTAF_{II}28$ to potentiate transactivation correlated with the ability of Tax to interact with $hTAF_{II}28$ and also with the ability of $hTAF_{II}28$ to interact with TBP. Coexpression of TBP and $hTAF_{II}28$ resulted in an additive increase in transactivation by Tax. From these observations we propose that transcriptional activation by Tax involves multiple interactions with TFIID via its TBP and $hTAF_{II}28$ subunits.**

The viral protein Tax strongly activates transcription of the human T cell leukemia virus type I (HTLV-I) provirus and of a specific group of cellular genes (1). Various studies have established that Tax is recruited to the Tax-responsive element 1 of the HTLV-I promoter and to the serum-responsive element of the c-fos promoter (2–5) via interactions with cellular transcription factors. In agreement with this notion, a GAL4 DNA binding domain-Tax fusion protein stimulates transcription of a reporter gene under the control of GAL4 sites (6). Using this approach, we have shown that the transcriptional activity of the GAL4-Tax fusion protein involves a direct protein-protein interaction between Tax and TATA box-binding protein (TBP) (7).

However, *in vitro* transcription experiments have established that activation by Tax requires the transcription factor complex TFIID comprised of TBP and TBP-associated factors (TAF_{II}s) (8). To better understand the molecular mechanisms underlying activation by Tax, we investigated the interactions between Tax and $hTAF_{II}$s. These experiments show a selective interaction of Tax with $hTAF_{II}28$. Increasing the intracellular concentration of $hTAF_{II}28$ augments the activity of GAL4-Tax, and the overexpression of both TBP and $hTAF_{II}28$ resulted in an additive increase in activation. The ability of $hTAF_{II}28$ to potentiate activation by Tax was dependent on its ability to interact with TBP. These observations support the notion that Tax activates transcription by interacting with TFIID through both TBP and $hTAF_{II}28$.

MATERIALS AND METHODS

Transfections. HeLa cells and COS-7 cells, grown in monolayers to 40% (HeLa cells) or 80% (COS-7 cells) confluence, were transfected by the calcium phosphate coprecipitation method (7). The total amount of simian virus 40 promoter-containing plasmids was adjusted to a constant level with pSG5, and the total amount of cytomegalovirus promoter-containing plasmids was adjusted to a constant level with pXJ41.

Immunoprecipitations. COS-7 cells extracts were prepared by three cycles of freeze-thaw in 100 μl of buffer A (50 mM Tris·HCl, pH 7.9/20% glycerol/1 mM dithiothreitol/0.1% Nonidet P-40) containing 250 mM KCl. After incubation for 30 min in ice, the extracts were centrifugated for 10 min at 10,000 $\times$ *g*. Supernatants were incubated with $\approx$0.5 μg of antibody for 30 min in ice. Protein G-Sepharose (30 μl) was added, and the mix was further incubated for 2 h at 4°C. The beads were washed four times with 1 ml of buffer A containing 500 mM KCl for 5 min at room temperature. The complexes were uncoupled from Sepharose beads by incubation at 90°C for 10 min in SDS/PAGE loading buffer. After migration through a SDS/10% protein gel, proteins were analyzed by immunoblotting using enhanced chemiluminescence detection (Amersham).

Chloramphenicol Acetyltransferase (CAT) Assays. The level of CAT protein produced in transfected HeLa cells was measured using an ELISA (Boehringer Mannheim) that was performed according to the manufacturer's instructions.

Expression of Recombinant Proteins and Protein Affinity Chromatography. Glutathione *S*-transferase (GST)-Tax protein was produced in bacteria and coupled to glutathione agarose beads as described (7). The beads coupled to either GST or GST-Tax were incubated with purified TBP (9) and His-$hTAF_{II}28$. His-$hTAF_{II}28$ was purified by affinity on Ni^{2+}-agarose beads (10, 11). Protein affinity chromatography was performed essentially as described (10). Similarly, GST-TBP was produced in bacteria from plasmid pGEX2T-TFIID and coupled to gluthatione Sepharose beads. Tax and $hTAF_{II}28$ were produced and labeled with [^{35}S]methionine using the TNT Coupled Reticulocyte Lysate system (Promega) with plasmids pSG-Tax and pXJ41-$hTAF_{II}28$.

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Abbreviations: TBP, TATA box-binding protein; TAF, TBP-associated factors; GST, glutathione *S*-transferase; HTLV-I, human T cell leukemia virus type I; CREB, cAMP-responsive element binding protein; CBP, CREB binding protein; CAT, chloramphenicol acetyltransferase; PIC, pre-initiation complex.

RESULTS

Interaction Between Tax and hTAF$_{II}$28. To investigate the possible contribution of hTAF$_{II}$s to activation by Tax, the ability of this transactivator to interact with several hTAF$_{II}$s was tested in coimmunoprecipitation experiments. HeLa cells were transfected with plasmid pSG-Tax alone or with vectors expressing hTAF$_{II}$18, hTAF$_{II}$20, hTAF$_{II}$28, hTAF$_{II}$30, hTAF$_{II}$55, hTAF$_{II}$100, hTAF$_{II}$ΔN135, or hTAF$_{II}$250 (12, 13). The amount of hTAF$_{II}$ expression vectors used in these experiments was adjusted to give similar amounts of each hTAF$_{II}$ as evaluated by immunoblotting with anti-hTAF$_{II}$ antibodies (data not shown). Transfected cell extracts were immunoprecipitated with mAbs directed against the corresponding hTAF$_{II}$s, and the precipitated proteins were analyzed by immunoblotting with a polyclonal antibody directed against Tax. In the absence of coexpressed hTAF$_{II}$s, Tax was not precipitated (Fig. 1 *A* and *B*, lanes 1, 3, and 5; Fig. 1*C*, lanes 1, 3, 5, and 7). In contrast, Tax was precipitated in the presence of coexpressed hTAF$_{II}$28 (Fig. 1*A*, lane 6; Fig. 1*B*, lane 2; Fig. 1*C*, lane 2), and weaker interactions between Tax and both hTAF$_{II}$100 and hTAF$_{II}$250 also could be observed upon longer exposures of the blots (data not shown). These experiments show a selective interaction between Tax and hTAF$_{II}$28. The ability of Tax to interact directly with hTAF$_{II}$28 *in vitro* was tested, and this interaction was compared with that between Tax and TBP (7). Purified hTAF$_{II}$28 (His-tagged) and TBP were incubated with GST-Tax fusion protein coupled to agarose beads. The amounts of both hTAF$_{II}$28 and TBP specifically bound by Tax were similar although slightly lower for hTAF$_{II}$28 (Fig. 2*A*, lanes 4 and 6). The same experiment performed with hTAF$_{II}$28 and TBP produced and radioactively labeled by *in vitro* translation showed that Tax retained comparable amounts of both proteins (data not shown). These experiments clearly indicated that Tax binds specifically hTAF$_{II}$28 and that this binding does not depend on a bridging factor. The ability of TBP, hTAF$_{II}$28, and Tax to form a ternary

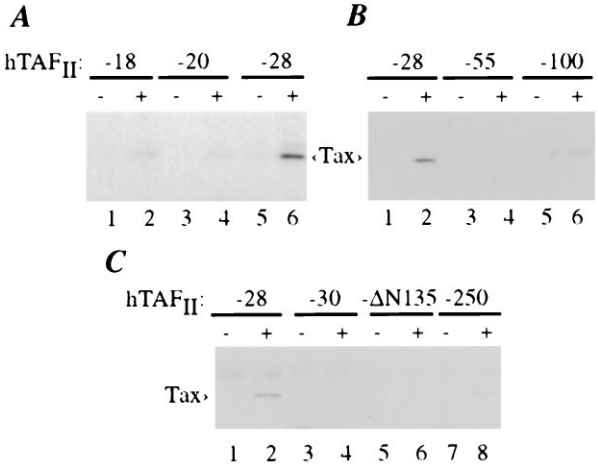

FIG. 1. Analysis of the interaction of Tax with several hTAF$_{II}$s. (*A–C*) COS-7 cells were transfected with pSG-Tax (14) either alone or together with a vector expressing the hTAF$_{II}$ (12) indicated above the lanes. Amounts of transfected plasmids were as follows: 2 μg, pSGTax, pXJ41-hTAF$_{II}$30, or pXJ41-hTAF$_{II}$55; 5 μg, pXJ41-hTAF$_{II}$18, pXJ41-hTAF$_{II}$28, pXJ41-hTAF$_{II}$100, or pXJ41-hTAF$_{II}$135; 10 μg, pXJ41-hTAF$_{II}$20 or pXJ41-hTAF$_{II}$250. Under these conditions equal amounts of the various hTAF$_{II}$ proteins were produced (data not shown). Cell extracts were precipitated with mAbs specific of the corresponding hTAF$_{II}$ (16TA for hTAF$_{II}$18, 22TA for hTAF$_{II}$20, 15TA for hTAF$_{II}$28, 2F4 for hTAF$_{II}$30, 19TA for hTAF$_{II}$55, 1TA for hTAF$_{II}$100, 20TA for hTAF$_{II}$135) (10, 13, 15). The precipitated proteins were revealed by immunoblotting using a rabbit polyclonal antibody directed against Tax (16). The coprecipitated Tax protein is shown by an arrow.

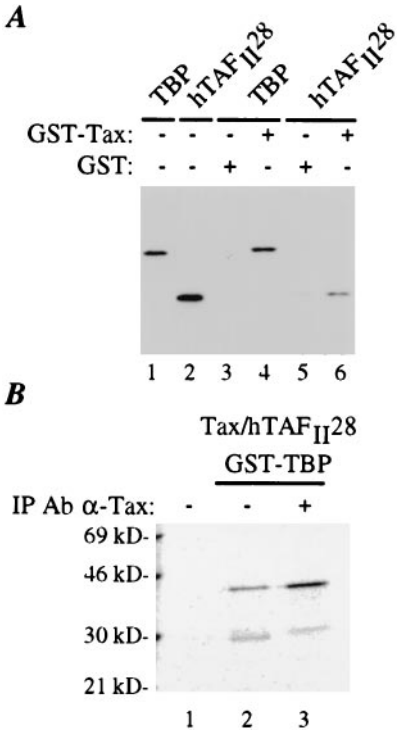

FIG. 2. hTAF$_{II}$28 interacts *in vitro* with Tax. (*A*) The GST and GST-Tax proteins coupled to glutathione-Sepharose beads (7) were incubated with purified TBP (9) (lanes 3 and 4) or purified His-hTAF$_{II}$28 (10) (lanes 5 and 6). Proteins were uncoupled from the beads by incubation in SDS/PAGE loading buffer for 10 min at 90°C and migrated through a SDS/10% polyacrylamide protein gel. As controls, 5% of the amounts of TBP and hTAF$_{II}$28 used for the incubations with GST and GST-Tax was loaded directly onto the gel (lanes 1 and 2, respectively). The gel was analyzed by immunoblotting with a mix of mAbs directed against TBP (3G3) and hTAF$_{II}$28 (15TA + 1C9). (*B*) Tax and hTAF$_{II}$28 were produced and labeled with [^{35}S]methionine by *in vitro*-coupled transcription/translation. These proteins were incubated with the GST-TBP fusion protein coupled to glutathione Sepharose beads. After three washes, the proteins were eluted by treatment with free glutathione. One-tenth of the eluted proteins was loaded onto a SDS protein gel (lane 2). The eluted proteins were next immunoprecipitated with an antibody directed against Tax in Nonidet P-40 lysis buffer (17), and the immunoprecipitated proteins were analyzed by SDS/PAGE (lane 3). The gel was dried and exposed to a Phosphor screen of which image is shown. In lane 1, the radioactively labeled Tax and hTAF$_{II}$28 proteins were incubated with protein A Sepharose beads to control for nonspecific binding. The positions of the bands of a molecular weight marker run in parallel are indicated.

complex also was tested. Radiolabeled Tax and hTAF$_{II}$28 were incubated with immobilized GST-TBP. The GST-TBP was then eluted with free gluthatione, and both Tax and hTAF$_{II}$28 were present in the eluate (Fig. 2*B*, lane 2). The eluted proteins were then immunoprecipitated with the anti-Tax antibody. Both Tax and hTAF$_{II}$28 were immunoprecipitated, clearly showing that hTAF$_{II}$28 also was associated with the Tax-TBP complex (Fig. 2*B*, lane 3). This *in vitro* experiment establishes that TBP, hTAF$_{II}$28, and Tax form a ternary complex.

Transactivation by Tax Is Augmented by Overexpression of hTAF$_{II}$28. To address the functional significance of the Tax-hTAF$_{II}$28 interaction, the effect of an increase in the intracellular hTAF$_{II}$28 concentration on transcriptional activation by Tax was evaluated. As controls, the effects of coexpression of hTAF$_{II}$55, hTAF$_{II}$100, and hTAF$_{II}$ΔN135 that either do not, or only weakly, interact with Tax also were tested. In the absence of overexpression of TBP, hTAF$_{II}$28 stimulated the activity of the GAL4-Tax fusion protein (Fig. 3, lanes 2 and 4), whereas expression of the other hTAF$_{II}$s did not significantly

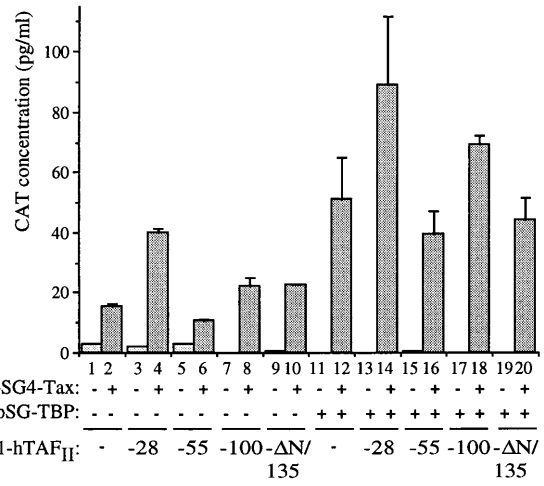

FIG. 3. Coexpression of hTAF$_{II}$28 promotes transcriptional activation by GAL4-Tax. HeLa cells were cotransfected by the calcium phosphate coprecipitation method with the reporter construct pG4G3CAT (2 μg; ref. 7) without or with pSG4-Tax (20 ng; ref. 7). Expression vectors for hTAF$_{II}$28 (1 μg), hTAF$_{II}$55 (100 ng), hTAF$_{II}$100 (2 μg), hTAF$_{II}$135 (500 ng), and TBP (1 μg) were added to the DNA mix as indicated. The amounts of hTAF$_{II}$ expression vectors used for transfection were adjusted to give similar quantities of each hTAF$_{II}$ as evaluated by immunoblotting analysis of corresponding whole cell extracts (data not shown). Quantification of the CAT protein (expressed in picograms per milliliter of cellular extract) was carried out by ELISA. Each point of transfection was performed in duplicate. (Bar = mean values.)

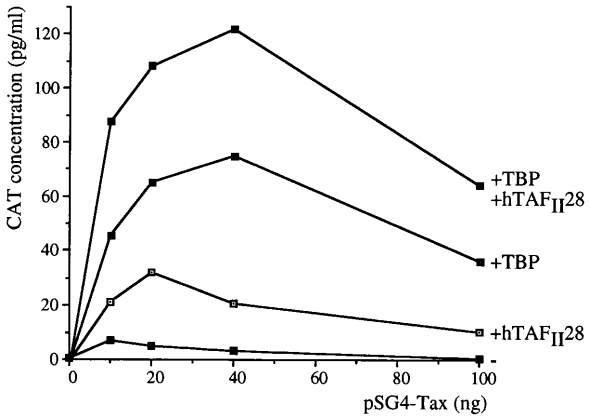

FIG. 4. Overexpression of hTAF$_{II}$28 and TBP alleviate GAL4-Tax self-squelching. The reporter construct pG3G4CAT (1.5 μg) was transfected in HeLa cells with increasing amounts of pSG4-Tax (0, 10, 20, 40, and 100 ng). Quantification of CAT protein was done as described in the legend to Fig. 3. CAT concentrations in picograms per milliliter of cellular extract were plotted against the amount of transfected pSG4Tax (−). The same experiment was done with inclusion of the following expression vectors in the DNA mix: pXJ41-hTAF$_{II}$28 (+hTAF$_{II}$28), pSG-TBP (+TBP), and pXJ41-hTAF$_{II}$28 with pSG-TBP (+TBP+hTAF$_{II}$28). The amount of pXJ41-hTAF$_{II}$28 and pSG-TBP used in the transfection was 1 μg.

affect its activity (Fig. 3, lanes 6, 8, and 10). As reported, overexpression of TBP also increased the activity of GAL4-Tax (Fig. 3, lanes 2 and 12). In the presence of coexpressed TBP, expression of hTAF$_{II}$28 again increased activation by GAL4-Tax, resulting in an additive effect of TBP and hTAF$_{II}$28 (Fig. 3, lanes 2, 4, 12, and 14). Expression of hTAF$_{II}$100 that interacted weakly with Tax also mildly potentiated activation when coexpressed with TBP, but no significant effect was seen with hTAF$_{II}$55 or hTAF$_{II}$ΔN135 (Fig. 3, lanes 16, 18, and 20).

It has been observed that activation is diminished when elevated amounts of the GAL4-Tax expression vector are transfected (7). This self-interference (squelching) effect is interpreted as the titration of a limiting factor playing a key role in the transactivation process by the excess of GAL4-Tax. This squelching effect can be partially alleviated by overexpression of TBP, supporting the notion that TBP may be one of the limiting targets for Tax. The ability of TBP to alleviate squelching was correlated with a direct protein-protein interaction between Tax and TBP (7). The ability of overexpressed hTAF$_{II}$28 to alleviate GAL4-Tax self-interference was also examined. As observed for TBP, overexpression of hTAF$_{II}$28 was able to partially alleviate the squelching seen with elevated levels of GAL4-Tax (Fig. 4). No such effect was observed with other hTAF$_{II}$s (data not shown) or TFIIB (7). As observed for transactivation, the reversal of squelching by the combination of hTAF$_{II}$28 and TBP was additive over a large range of GAL4-Tax concentrations (Fig. 4). At optimal concentrations of GAL4-Tax overexpression of both TBP and hTAF$_{II}$28 resulted in a very potent 40-fold increase in activation by the GAL4-Tax protein. These experiments indicate that hTAF$_{II}$28 is also a limiting factor for transactivation by Tax.

To further correlate the Tax-hTAF$_{II}$28 interaction with the ability of overexpressed hTAF$_{II}$28 to modulate activation by Tax, mutants of Tax were tested for their ability to interact with hTAF$_{II}$28. Three Tax mutants that are defective for transactivation of the HTLV-I promoter were tested. Mutations M5 (18) and M137 (19) markedly reduced association with hTAF$_{II}$28 as evaluated in coimmunoprecipitation experiments (Fig. 5*A*, lanes 2, 3, and 5) whereas mutation M15 (18) did not affect interaction with hTAF$_{II}$28 (Fig. 5*A*, lanes 2 and 4). The effect of the overexpression of hTAF$_{II}$28, and/or TBP on activation by these three Tax mutants was tested. Overexpression of TBP rescued activation by GAL4-Tax M5, but overexpression of hTAF$_{II}$28 did not significantly affect its activity in either the presence or absence of TBP (Fig. 5*B*, lanes 2–5). In contrast, overexpression of hTAF$_{II}$28 had a moderate positive effect on the activity of GAL4-Tax M15 (Fig. 5*B*, lanes 6 and 7) and TBP had a lesser effect (Fig. 5*B*, lane 8). However, overexpression of both hTAF$_{II}$28 and TBP rescued partially the phenotype of this mutant (Fig. 5*B*, lane 9). The activity of GAL4-Tax M137 could not be rescued by TBP, hTAF$_{II}$28, or a combination of both (Fig. 5*B*, lanes 10–13). Thus, mutation M5, which only partially impairs interaction with TBP (7), is corrected, at least to some extent, by overexpression of TBP but is insensitive to overexpression of hTAF$_{II}$28 in agreement with the loss of interaction with this hTAF$_{II}$. On the other hand, mutation M15, whose phenotype was rescued inefficiently by TBP overexpression when compared with mutation M5, was efficiently rescued by overexpression of both hTAF$_{II}$28 and TBP. This shows that these two factors can cooperate together to rescue the phenotype of a negative mutation that preserves the capacity to interact with hTAF$_{II}$28. In contrast, mutation M137, which disrupts interaction with hTAF$_{II}$28, was insensitive to overexpression of either hTAF$_{II}$28 or TBP, as well as to the combination of both factors. These results illustrate that both TBP and hTAF$_{II}$28 participate in the transactivation by Tax. They also indicate that the ability of hTAF$_{II}$28 overexpression to increase the activity of Tax is correlated with the ability of both proteins to interact.

Potentiation of Activation by Tax Requires hTAF$_{II}$28-TBP Interaction. It has been shown that hTAF$_{II}$28 directly interacts with TBP (10, 12). Consequently, we asked whether hTAF$_{II}$28 has to interact with TBP to augment activation by Tax. To answer this question, two mutants of hTAF$_{II}$28 that are unable to interact with TBP were analyzed. The two mutations correspond to a deletion of the N-terminal 63 amino acids [hTAF$_{II}$28(64–211)] and to a substitution of three glutamic acidic residues [hTAF$_{II}$28(1–179)M1], which probably disrupts a putative amphipathic α-helical motif located between amino

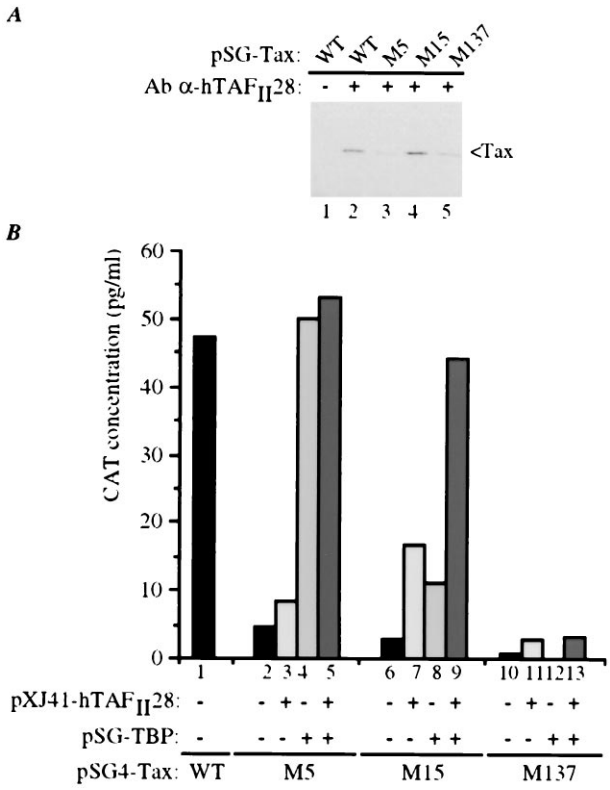

FIG. 5. The effect of the overexpression of hTAF$_{II}$28 is lost with mutants of Tax unable to bind hTAF$_{II}$28. (*A*) COS-7 cells were cotransfected with the following Tax expression vectors (7, 14, 15): pSG-Tax (1 μg; lane 2), pcTax M5 (5 μg; lane 3), pcTax M15 (5 μg; lane 4) and pSG-Tax M137 (2 μg; lane 5). pXJ41-hTAF$_{II}$28 (2 μg) was included in the DNA mix for lanes 2–5. Cell extracts were precipitated with mAb 15TA directed against hTAF$_{II}$28. The precipitated proteins were analyzed by immunoblotting using a rabbit polyclonal antibody directed against Tax (17). The coprecipitated Tax protein is shown by an arrow. (*B*) HeLa cells were cotransfected with pSG4-Tax, pSG4-Tax M5, pSG4-Tax M15, and pSG4-Tax M137 with or without pSG-TBP and pXJ41-hTAF$_{II}$28 as indicated. Twenty nanograms of plasmids expressing the Tax fusion proteins was used. For pSG-TBP and pXJ41-hTAF$_{II}$28, 1 μg of plasmid was used. Quantification of CAT enzyme was done as described in the legend to Fig. 3.

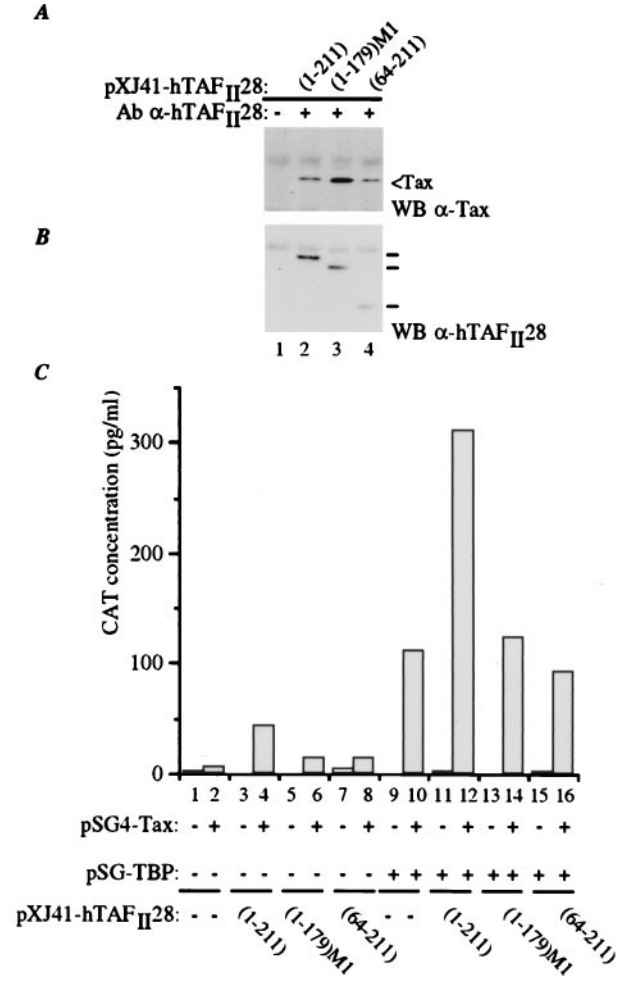

FIG. 6. Interaction between hTAF$_{II}$28 and TBP is necessary for the stimulation of GAL4-Tax transactivation by hTAF$_{II}$28 overexpression. (*A* and *B*) COS7 cells were transfected with 2 μg of pSG-Tax without (lane 1) or with 5 μg of plasmids pXJ41-hTAF$_{II}$28(1–211) (lane 2), pXJ41-hTAF$_{II}$28(1–179)M1 (lane 3), and pXJ41-hTAF$_{II}$28(64–211) (lane 4) (12). Immunoprecipitations were performed as described in legend to Fig. 5 with mAb 15TA, which recognizes all of the hTAF$_{II}$28 derivatives. The coprecipitated Tax protein is shown by an arrow (*A*). The blot was then stripped and reprobed with a mix of the 15TA and 1C9 mAbs directed against hTAF$_{II}$28 (*B*). (*C*) The reporter construct pG3G4CAT (2 μg) was cotransfected in HeLa cells without or with 40 ng of pSG4-Tax. pXJ41-hTAF$_{II}$28(1–211) (2 μg), pXJ41-hTAF$_{II}$28(1–179)M1 (2 μg) and pXJ41-hTAF$_{II}$28(64–211) (1 μg), together with or without pSGTBP (1 μg), as indicated. Quantification of CAT enzyme was done as described in the legend to Fig. 3.

acids 161 and 179 in the context of a C-terminally truncated hTAF$_{II}$28 (12). Coimmunoprecipitation experiments showed that these two mutants were still able to bind Tax (Fig. 6*A*, lanes 2–4). The interaction of Tax with hTAF$_{II}$28(1–179)M1 was in fact stronger than with wild type (Fig. 6*A*, lanes 2 and 3). The effect of expression of these mutants on the activity of the wild-type GAL4-Tax protein was examined in both the presence and the absence of overexpressed TBP. Although wild-type hTAF$_{II}$28(1–211) potentiated the activity of GAL4-Tax in both the absence and presence of coexpressed TBP, the mutants that are unable to associate with TBP no longer exerted a functional effect on transcriptional activation by Tax (Fig. 6*C*, lanes 2, 4, 6, 8, 10, 12, 14, and 16). As indicated by the immunoprecipitation experiment, the lack of functional effect could not be attributed to a lack of expression of these two mutants (Fig. 6*B*, lanes 2, 3 and 4). These results indicate that hTAF$_{II}$28 must interact with TBP to participate in the transcriptional activation effect of Tax.

Cooperative Effect of TBP and hTAF$_{II}$28 in the Tax Transactivation of the Entire HTLV-I Promoter. To examine further the role of hTAF$_{II}$28 in transcriptional activation by Tax, experiments were performed with the entire HTLV-I promoter. To distinguish the effect of the endogenous TFIID from that induced by the transfection of expression vectors, TBP bearing the spm3 mutation was used (20, 21). This mutant binds to both TATAAAA and TGTAAAA motifs whereas wild-type TBP recognizes only the former. The TATA box of the HTLV-I promoter fused to CAT was changed to TGTAAAA. This mutation drastically reduced transactivation of this promoter by Tax (Fig. 7, lanes 2 and 4). Overexpression of hTAF$_{II}$28 alone did not significantly restore transactivation (Fig. 7*A*, lanes 4 and 6). In contrast, expression of TBP spm3 alone rescued transactivation to some extent (Fig. 7*A*, lane 8), but, when expressed in combination with hTAF$_{II}$28, significantly higher levels of transactivation were observed (Fig. 7*A*, lane 10). It is interesting to note that a clear positive effect of hTAF$_{II}$28 was observed at intermediate levels of TBP spm3 overexpression, but when TBP spm3 was present in large excess, the amount of transcription observed was higher and the effect of hTAF$_{II}$28 was less pronounced (data not shown). These observations support the notion that hTAF$_{II}$28 and TBP cooperate to mediate Tax activation of the HTLV-I promoter.

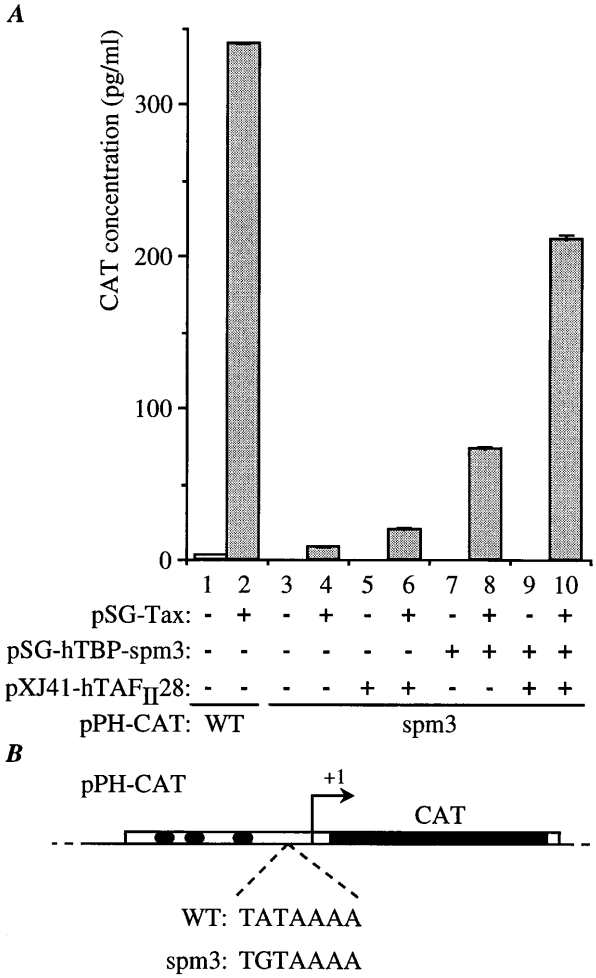

FIG. 7. $hTAF_{II}28$ overexpression promotes Tax transactivation of the entire HTLV-I promoter. (*A*) HeLa cells were cotransfected with the reporter plasmid pPHCAT (200 ng), containing either the wild-type (WT) or the spm3-mutated TATA-box, with or without pSGTax (20 ng). Plasmids expressing hTBP-spm3 (16) (pSG-hTBP-spm3, 0.5 μg) and $hTAF_{II}28$ (pXJ41-$hTAF_{II}28$, 1 μg) were added as indicated. The CAT concentrations were determined and are represented as described in the legend to Fig. 3. (*B*) Schematic representation of pPHCAT. The reporter plasmid contains the HTLV-I promoter up to position +22 fused to the CAT gene. In this reporter construct, the wild-type TATA box was changed to TGTAAAA.

DISCUSSION

Functional Interaction of Tax with $hTAF_{II}28$. We have analyzed the interaction between Tax and a series of human TAF_{II}s: $hTAF_{II}18$, $hTAF_{II}20$, $hTAF_{II}28$, $hTAF_{II}30$, $hTAF_{II}55$, $hTAF_{II}100$, $hTAF_{II}\Delta N135$, and $hTAF_{II}250$. Immunoprecipitation experiments with similar amounts of these $hTAF_{II}$s show that the strongest interaction takes place between Tax and $hTAF_{II}28$, weaker interactions being observed with $hTAF_{II}100$ and $hTAF_{II}250$. The results of *in vitro* interaction assays with recombinant proteins establish that the interaction between Tax and $hTAF_{II}28$ is direct and does not depend on an additional bridging factor.

Our present results suggest that not only does Tax interact directly with $hTAF_{II}28$ but that this interaction is critical for activation by Tax *in vivo*. This idea is supported by several observations. An increase in the intracellular concentration of $hTAF_{II}28$ augments transactivation by Tax. This effect also was clearly seen in COS-7 cells (G.M. and A.R., unpublished results) that have low levels of endogenous $TAF_{II}28$ (12). This positive effect was additive with that of overexpressed TBP and was most pronounced with higher concentrations of the pSG4-Tax expression vector, in which $hTAF_{II}28$ and TBP overexpression alleviates Tax self-squelching, resulting in a 40-fold increase in activation. These *in vivo* results raise questions concerning the precise molecular mechanisms by which the overexpressed TBP and/or $hTAF_{II}28$ act to increase activation by Tax in HeLa cells where TFIID complexes comprising $hTAF_{II}28$ are present. TBP is present in various complexes permitting transcription by all three cellular RNA polymerases (22). The observation that mutations in TBP affecting its ability to promote transcription by RNA polymerase III cause an increase in expression of several genes transcribed by RNA polymerase II (23) strongly suggests that TBP is a limiting factor in the living cell. Therefore, it is likely that overexpressed TBP raises this concentration, at least to some extent, until the $hTAF_{II}$s become limiting. Similarly, an increase in the concentration of $hTAF_{II}28$, which binds directly to TBP, may compete with the TAF_{I}s and TAF_{III}s and drive more TBP into the formation of a TFIID complex interacting with Tax. According to this model, overexpression of both TBP and $hTAF_{II}28$ would most efficiently raise the concentration of TFIID complexes capable of functioning with Tax.

TBP and $hTAF_{II}28$ also cooperate to allow Tax activation of the entire HTLV-I promoter and to partially rescue the phenotype of Tax mutants that had impaired ability to activate transcription. Strikingly, for two of these mutants, the loss of activation correlated with the loss of interaction with $hTAF_{II}28$. The ability of $hTAF_{II}28$ to increase Tax transactivation was not observed with $hTAF_{II}28$ mutants that had diminished ability to interact with TBP. Together these results indicate that the Tax-$hTAF_{II}28$, Tax-TBP, and TBP-$hTAF_{II}28$ interactions participate to activation by Tax.

TFIID Is the Target of Tax in the Pre-Initiation Complex (PIC). TFIID subunits are not the only components of the general transcription machinery that interact with transactivators. Interactions between activators and TFIIA, TFIIB, TFIIF, and TFIIE have been reported (24–28). We also have investigated the ability of Tax to interact *in vitro* with several general transcription factors. No interactions were detected with TFIIB (7), TFIIE, TFIIF, or TFIIH (R. Rousset, personal communication), and although we did detect an interaction with the precursor of the largest subunits of TFIIA, as recently reported (29), in transient transfection experiments the overexpression of the TFIIA subunits only slightly reduced the activity of the HTLV-I promoter activated by Tax (C.C., unpublished results). Thus, although TFIIA may be a further target of Tax, it appears that TFIID, via $hTAF_{II}28$ and TBP, may be the principle general transcription factor targeted by Tax *in vivo*. However, it is possible that other TAFs, not assayed here, or other cofactors that may associate with the TBP-$hTAF_{II}28$ complex participate in transactivation by Tax.

In the context of the natural HTLV-I promoter, TFIIB is probably also an important target. Kwok *et al.* (26) recently reported that the CREB binding protein (CBP) is recruited by Tax to the complex formed on the Tax-responsive element (30). Conversely, CBP recruits Tax to complexes formed by the binding of protein kinase A phosphorylated-CREB to cellular cAMP-responsive elements (30), thus augmenting transactivation by CREB. CBP protein has been reported to interact with TFIIB (26) and CREB with $dTAF_{II}110$ (31), so the full complex of CREB, CBP and Tax could then potentially interact with multiple components of the general transcription machinery possibly explaining the functional cooperativity seen with these proteins.

$hTAF_{II}28$ as a Regulatory TAF. Recent advance in the understanding of the general arrangement of the TAFs has led to the proposal of a model in which $hTAF_{II}15/22$, $hTAF_{II}31$, and $hTAF_{II}81$ assemble into a nucleosome like structure (32, 33). The other $hTAF_{II}$s would contact this core structure and ensure the contact with the activators. Our previous and present results indicate that $hTAF_{II}28$, like $hTAF_{II}55$, is one of

the $hTAF_{II}$s involved in mediating the effect of transactivators. An interesting analogy can be noted in the two instances in which $hTAF_{II}28$ has been implicated as a transcriptional coactivator. Although $hTAF_{II}28$ potentiated the activity of the activation function-2 of several nuclear receptors, it did not interact directly with these nuclear receptors. This observation prompted us to suggest that $hTAF_{II}28$ exerted its effect on activation function-2 by interacting with transcriptional intermediary factors that themselves interact with the nuclear receptors (12). Tax can in fact be considered as a virally encoded equivalent of these transcriptional intermediary factors, which acts as a bridging factor between CREB and possibly also $p67^{SRF}$ and the $hTAF_{II}28$-TBP complex.

In conclusion, our results strengthen the notion that Tax exerts it transcriptional activity by establishing a direct protein-protein interaction with TFIID. The association between Tax and this general transcription factor is probably permitted by a dual interaction with both TBP and $hTAF_{II}28$. Like several other viral transcriptional activators, Tax binds directly proteins participating to the assembly of the PIC. The additional ability of this protein to associate with different upstream factors bound to DNA explains that this single molecule is able to modify selectively a specific group of cellular promoters probably to create favorable conditions to the replication of the virus.

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