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Functional Interaction between p53, the TATA-Binding Protein (TBP), and TBP-Associated Factors In Vivo

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The transcriptional activator p53 is known to interact with components of the general transcription factor TFIID in vitro. To examine the relevance of these associations to transcriptional activation in vivo, plasmids expressing a p53-GAL4 chimera and *Drosophila* TATA-binding protein (dTBP) were transfected into *Drosophila* Schneider cells. p53-GAL4 and dTBP displayed a markedly synergistic effect on activated transcription from a GAL4 site-containing reporter that was at least 10-fold greater than observed with other activators tested. A mutant p53 previously shown to be defective in both transcriptional activation in vivo and in binding to TBP-associated factors (TAFs) in vitro, although still capable of binding dTBP, did not cooperate with dTBP, suggesting that TAFs may contribute to this synergy. Providing further support for this possibility, transfected dTBP assembled into rapidly sedimenting complexes and could be immunoprecipitated with anti-TAF antibodies. While overexpression of any of several TAFs did not affect basal transcription, in either the presence or the absence of cotransfected dTBP, overexpression of $TAF_{II}230$ inhibited transcriptional activation mediated by p53-GAL4 as well as by GAL4-VP16 and Sp1. Overexpression of $TAF_{II}40$ and $TAF_{II}60$ also inhibited activation by p53-GAL4 but had negligible effects on activation by GAL4-VP16 and Sp1, while $TAF_{II}110$ did not affect any of the activators. TAF-mediated inhibition of activated transcription could be rescued by high levels of exogenous dTBP, which also restored full synergy. These data demonstrate for the first time that functional interactions can occur in vivo between TBP, TAFs, and p53.

Exposure to DNA-damaging agents causes cellular levels of the p53 tumor suppressor protein to dramatically increase, resulting in a G_1/S arrest in the cell cycle. This arrest is presumably due to the direct activation by p53 of genes involved in DNA repair, cell cycle regulation, and apoptosis (for reviews, see references 12 and 23). The role of p53 as a DNA-binding-dependent transcriptional activator is an important one since mutations that alter this ability lead to unregulated cellular growth and tumorigenesis (for a review, see reference 13). Therefore, it is of great interest to understand the mechanism of transcriptional activation by p53.

Proper transcriptional activation of class II genes requires specific interactions between activators bound to regulatory elements and the general transcription factors that assemble on the TATA box and/or the initiator element (reviewed in reference 59). Besides TFIID, the factor that directly binds to the TATA box, the general factors include TFIIA, -B, -E, -F, and -H and RNA polymerase II (Pol II) (3, 11, 65), many of which have been shown to interact directly with transcriptional activators in vitro (8, 31, 40, 46, 64). The TATA-binding protein (TBP), the TFIID subunit that directly binds the TATA motif (for a review, see reference 26), has also been shown to interact directly with transcriptional activators in vitro (4, 30, 32, 33, 38, 51, 54). The other TFIID constituents, known as TBP-associated factors (TAFs), are found in cells tightly bound to TBP. Different classes of TAFs are involved in transcription not only by RNA Pol II but also by RNA polymerases I and III (for reviews, see references 21 and 26). Several of the Pol II-associated TAFs have been shown to provide an essential coactivator function by participating with other general transcription factors in reconstituting the minimal requirements for activated transcription in vitro (2, 6, 15, 28, 34, 47, 48, 52, 55, 58, 66).

In serving their roles as coactivators, the TAFs are believed to provide a bridging function between transcriptional activators and the basal transcriptional machinery. This view is supported by experiments illustrating direct interactions among several of the TAFs and transcriptional activators, including p53. For example, in addition to binding to the general factors, TBP (42, 45, 51, 57, 59) and TFIIH (62, 64), p53 has been shown to contact directly *Drosophila* $TAF_{II}40$ and $TAF_{II}60$ and their human homologs, $TAF_{II}31$ and $TAF_{II}70$ (43, 58). The functional significance of these interactions has been demonstrated in experiments showing that TBP, $TAF_{II}40$, and $TAF_{II}60$, along with $TAF_{II}250$ and the basal transcriptional machinery, are sufficient to support transcriptional activation in vitro by a p53-GAL4 chimera containing a dimerized p53 activation domain (58). Missense mutations that affect the transactivation ability of p53 (39, 58) also influence its ability to interact with TAFs (43, 58). Thus, from these experiments it seems highly probable that TAFs play a role in transcriptional activation by p53 in vivo.

Other experiments performed in vitro have provided insight into how TAFs may function as coactivators, revealing specific requirements by different classes of transcriptional activators for different TAFs. For example, while p53 and another acidic transcriptional activator, VP16, interact with $TAF_{II}40$ (20), the glutamine-rich transactivator, Sp1, interacts with *Drosophila* $TAF_{II}110$ (28). The functional relevance of this interaction was made evident from data showing that missense mutations in Sp1 that disrupt the interaction between $TAF_{II}110$ and Sp1 impair the ability of Sp1 to activate transcription (18). Whereas

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direct interactions between transactivators and *Drosophila* $TAF_{II}230$, or its human homolog $TAF_{II}250$, have not been observed, this TAF is essential for activated transcription in vitro (6, 56, 58, 61). It has been speculated that $TAF_{II}230$/$TAF_{II}250$ functions as a scaffold protein, binding directly to TBP and recruiting other TAFs to the TFIID complex (6, 27, 34, 36, 49, 63).

Less is known about the functional interactions between eukaryotic transactivators and the transcriptional apparatus in vivo. In this study, we have investigated this problem as it pertains to p53 transactivation in transfected *Drosophila* Schneider cells. We have previously demonstrated that both p53 and TBP can cooperatively bind DNA in vitro (7). Now, through the use of various TAF expression plasmids, our data suggest that the interaction between p53 and TBP can be affected by overexpression of $TAF_{II}40$, $TAF_{II}60$, and $TAF_{II}230$, implicating these TAFs in transcriptional activation by p53 in vivo.

MATERIALS AND METHODS

Transient-transfection assays. Transfection of Schneider cells was performed essentially as described previously (10, 25). The total actin 5C expression plasmid (pACTPPA) in each tube was adjusted to a final amount of 7 μg by the addition of the actin 5C expression plasmid without any insert. All DNA mixtures also contained 2 μl of *copia-lacZ* plasmid to monitor transcription efficiencies and 2 μg of chloramphenicol acetyltransferase (CAT) reporter plasmids. A modified 2× HEBS solution (280 mM NaCl, 7 mM KCl, 1.25 mM Na_2HPO_4, 0.2% dextrose, 40 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.1]) was used instead of the one previously described (25). All solutions were used at room temperature. Addition of the DNA-calcium chloride mixtures proceeded as a gentle stream of air was administered to the HEBS solution via a 10-μl glass pipette to precipitate the DNA. After sitting at room temperature for 10 min, the suspensions of DNA (total volume of approximately 700 μl) were added to 60-mm-diameter plates containing the Schneider cells and gently agitated. After a 48-h incubation, the cells were lysed and assayed for β-galactosidase activity as previously described (25).

To determine the amounts of extracts to be used in the subsequent CAT assays, all volumes were normalized to 40 μl of extract used by the extract that yielded the lowest level of β-galactosidase activity. Before use, CAT extracts were diluted in 200 mM Tris (pH 8.0) plus 100 μg of bovine serum albumin (BSA; Sigma) per ml to ensure subsequent scintillation counts of 2 to 30 cpm/min. Corresponding volumes of extract were added to 7-ml glass scintillation vials, and 200 μl of CAT reaction mixture (1.5 mM chloramphenicol [in water], 125 μM cold acetyl coenzyme A [Sigma], 0.63 μCi of ^{3}H-acetyl coenzyme A [Amersham], 1 mM EDTA, 10 mM Tris [pH 8.0]) was added to each vial at 40-s intervals. One blank was prepared without extract to determine background activity. After addition of 5 ml of Econo-Flur scintillation fluid (DuPont), each vial was capped and allowed to sit for 3 h at room temperature before scintillation counting. Three separate rounds of 30-s counts per vial were made and averaged together. Values were adjusted according to the dilution previously made of the extracts used in the assays.

Determination of protein expression from Schneider cells. Cells were transfected as described above with the appropriate expression plasmids. After a 48-h incubation, cells were harvested and washed as described above and resuspended in 200 μl of 1× protein sample buffer (3% glycerol, 100 mM β-mercaptoethanol, 0.5% sodium dodecyl sulfate [SDS], 100 mM Tris [pH 6.8]). To detect influenza virus (flu)-tagged $TAF_{II}110$ expression, 50 μl of the cell suspension was loaded onto a 7.5% polyacrylamide gel (acrylamide-bisacrylamide [39.5:0.5], 10% glycerol, 0.1% SDS, 750 mM Tris [pH 9.3]) and run until the bromophenol blue dye front reached the bottom. These gel conditions were required to resolve flu-$TAF_{II}110$ from an endogenous *Drosophila* protein with a similar molecular weight that also contained the flu epitope recognized by 12CA5 (17). For resolution of other proteins, 50-μl aliquots of the cell suspensions were loaded onto 10% polyacrylamide gels (acrylamide-bisacrylamide [38:0.8], 0.1% SDS, 750 mM Tris [pH 8.3]). Detection of flu-tagged proteins and p53 derivatives was done with monoclonal antibody 12CA5 and PAb 1801, respectively, via Western blot (immunoblot) analysis and the ECL (enhanced chemiluminescence) detection system as described by the manufacturer (Amersham).

Protein binding assays. Glutathione *S*-transferase (GST) fusion protein expression plasmids were constructed by using pGEX2T expressing GST alone (Pharmacia). A 240-bp *Nco*I-*Bsr*FI fragment from either wild-type p53 or $mt53_{22/23}$ (see Results) cDNA blunted with Klenow enzyme was subcloned into the *Sma*I site of pGEX2T. Positive transformants of *Escherichia coli* JM101 were grown in LB overnight at 37°C, diluted 1:100 in 100 ml of fresh LB the next day, grown at 37°C to an optical density of 0.6 at 595 nm, and induced for GST fusion protein expression for 2 h after addition of isopropylthiogalactoside (Sigma) to 0.5 mM. Cells were spun down and washed once in phosphate-buffered saline (PBS), resuspended in 2 ml of PBS plus 1% Triton X-100 (Sigma), and sonicated to lyse cells. Cell debris was spun down at 12,000 for 10 min, and the extracts were used for binding to glutathione-Sepharose beads (Pharmacia).

Twenty microliters of beads was added to 500 μl of bacterial extracts and rocked at 4°C for 30 min to bind GST proteins. The beads were washed four times in 1 ml of K100 (100 mM KCl, 5 mM $MgCl_2$, 20 mM Tris [pH 8.0], 10% glycerol, 0.5 mM phenylmethylsulfonyl fluoride) and suspended in 500 μl of K100. *Drosophila* TBP (dTBP) was translated in vitro from plasmid pETdTBP in a TNT rabbit reticulocyte lysate system (Promega) supplemented with [^{35}S]methionine as described by the manufacturer. Lysates were spun at 12,000 for 5 min to remove any precipitated material that may have developed during translation. Then 5 μl of the lysates was added to the GST fusion protein-bound beads suspended in K100 and rocked for 2 h at room temperature.

The beads were washed four times in K100, resuspended in 500 μl of K100 plus 5 mM glutathione (Sigma), and rocked for 15 min at room temperature. The beads were spun down, and the proteins were precipitated with 20% trichloroacetic acid, put on ice for 15 min, and spun at 12,000 for 10 min. The pellets were washed in 500 μl of ice-cold acetone, spun again, and allowed to air dry thoroughly. The pellets were then dissolved in 20 μl of protein sample buffer and run on an SDS–10% polyacrylamide gel. Radiolabeled proteins were detected by autoradiography.

Glycerol gradient analysis. Transfection of Schneider cells was done as described above. After a 48-h incubation, cells from three 60-mm-diameter plates transfected with 5 μg of pACTdTBP were pooled, washed twice in PBS, lysed in 1 ml of K100 buffer plus 0.5% Nonidet P-40, and kept on ice for 20 min. Cell debris was spun down at 12,000 for 10 min. Then 200 μl of extracts was applied onto 10 to 40% glycerol gradients (4 ml) containing K100 buffer plus 0.5% Nonidet P-40 and either supplemented with 3 M urea or not. Gradients were spun in a Beckman SW55 rotor at 38,000 for 14 h at 4°C. After centrifugation, 30 aliquots (140 μl each) were collected, and 70 μl of each of the even-numbered fractions (fractions 4 to 30) was analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) followed by Western blotting (immunoblotting) with monoclonal antibody 12CA5 as described above.

Immunoprecipitation with anti-TAF antibodies. Extracts were prepared as described for glycerol gradient analysis. Twenty microliters of protein A-Sepharose beads (Pharmacia) was rocked for 2 h at 4°C in a mixture containing 3 μl of polyclonal rabbit serum in 500 μl of PBS plus 100 μg of ml BSA per ml. After three washes in PBS, the antibody-bound beads were added to 500 μl of Schneider cell extracts and rocked for 2 h at 4°C. The beads were washed three times in K100 plus 0.5% Nonidet P-40 and analyzed by SDS-PAGE and Western blot analysis with monoclonal antibody 12CA5 as described above.

Construction of expression plasmids. pACT53G4 was created by inserting an *Xho*I linker, 5′-CCGCTCGAGCGG-3′, at the *Stu*I site in wild-type human p53 cDNA in pUC19 and isolating a positive clone. The resulting clone, pUC53Xh, was cut with *Xho*I, blunted with Klenow enzyme, and then cut with *Xba*I (at the 5′ end in the pUC polylinker) to produce a 1.4-kb *Xba*I-blunt p53 fragment. Sequences from the GAL4 DNA binding domain were cut at the *Fok*I site (corresponding to amino acid 4 in GAL4), blunt ended, and cut with *Xba*I to yield a fragment encoding amino acids 4 to 147 of GAL4. This fragment was ligated into pUC19 cut with *Xba*I and transformed into bacteria. A positive clone was isolated, and the DNA was cut with *Bsu*36I (single site in the p53 cDNA upstream from the original *Stu*I site) and *Bam*HI from a site 3′ of the p53/GAL4 cDNA in the pUC polylinker. This fragment was subcloned into the *Bsu*36I and *Bgl*II sites of pACT53 in place of the intact p53 fragment.

pACTmt53 was created by subcloning the pRCmt53 cDNA (39) into pACT PPA (25). A 1.2-kb *Nde*I-*Sma*I fragment from pTβSTOP-40 (gift of R. Tjian) was blunt ended and subcloned into the *Eco*RV site of pACTPPA to create pACT40. A 2.0-kb *Nde*I-*Eco*RI fragment from a cDNA for *Drosophila* $TAF_{II}60$ (34) was blunt ended and subcloned into the *Eco*RV site of pACTPPA to create pACT60. To create pACT230, p230-KS (35) was cut with *Not*I, blunt ended, cut with *Kpn*I (to generate a 2.6-kb fragment), and subcloned into pACTPPA cut with *Bam*HI, blunted, and cut with *Kpn*I. Positive clones were cut with *Kpn*I and *Eco*RV, and a 4.0-kb *Kpn*I-*Sma*I fragment from p230-KS was subcloned into these sites. pACT110 was created by blunting a 3.1-kb *Nde*I-*Sma*I fragment from pTβSTOP-110 (gift of R. Tjian) and subcloning it into the *Eco*RV site of pACTPPA.

To generate the flu-tagged $TAF_{II}110$ expression plasmid, two DNA primers were synthesized. 5′-GATCAGATCTACCATGGGCTACCCATACGATGTT CCAGATTACGCGTCGAACACCAGCCAGACAGCTGCC-3′ contains sequences encoding the flu epitope recognized by monoclonal antibody 12CA5 and the second amino acid of the $TAF_{II}110$ open reading frame. 5′-CGTTCCTCC CGCGGAG-3′ encodes sequences overlapping the *Sac*II unique site in the $TAF_{II}110$ open reading frame. These primers were used in PCRs based on a protocol previously described (1), using pTβ-110 as a template to generate a fragment to encode flu sequences upstream from the second amino acid to residue 271 of $TAF_{II}110$. This fragment was cut with *Bam*HI and *Sac*II (to cut at sites encoded within the primers) and subcloned along with a 1.2-kb *Sac*II-*Sac*I fragment from pTβ-110 into the *Bam*HI and *Sac*I sites of pACTPPA. This created pACTflu110.top, which was cut with *Bgl*II, blunted, and cut with *Sac*I to which a 1.1-kb *Sac*I-*Sma*I fragment was ligated, creating pACTflu110.fl. To create the flu-tagged $TAF_{II}40$ expression plasmid, primers 5′-GATCACGCG TCCAGCGCAGAGAAGTCC-3′ and 5′-GATCGAGCTCCTAGTTGGTCA G AAAC-3′ were used in PCR to create a cDNA encoding a flu-tagged $TAF_{II}40$

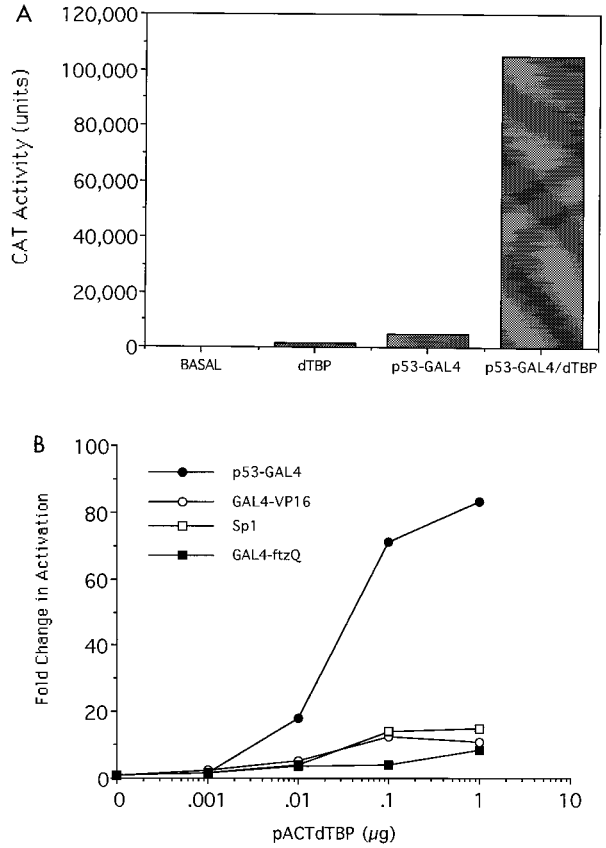

FIG. 1. p53-GAL4 and flu-dTBP synergistically activate transcription in Schneider cells better than do other activators. (A) Two micrograms of G5-E1B-CAT reporter construct was transfected into *Drosophila* Schneider cells either alone, with 1 μg of pACTdTBP (expressing dTBP [10]), with 0.1 μg of pACT53G4 (expressing p53-GAL4), or with both. Volumes of extracts used were normalized to amounts of β-galactosidase activity. Levels of CAT activity were measured and plotted as counts per minute of tritium-labeled acetyl-chloramphenicol. (B) Either 2 μg of G5-E1B-CAT (for p53-GAL4, GAL4-VP16, and GAL4-ftz activation) or 2 μg of SV-E1B-CAT (10) (for Sp1 activation) was transfected with either 0.1 μg of an expression plasmid for p53-GAL4 (pACT53G4), 0.0002 μg of pACTG4VP16 (for expression of GAL4-VP16), 0.0001 μg of pACTftzQ (for expression of GAL4-ftzQ), or 0.0001 μg of pACTSP1 (9, 10) (for expression of Sp1) to give equivalent amounts of CAT activity in the absence of flu-dTBP expression (data not shown). Increasing amounts of pACTdTBP were cotransfected along with each combination of CAT reporter construct and transcriptional activator expression plasmid. Volumes of extracts used were normalized to amounts of β-galactosidase activity from 2 μg of cotransfected pCopiaβ-gal. Levels of CAT activity, which were measured and plotted as counts per minute of tritium-labeled acetyl-chloramphenicol, were relative to the value of CAT activity attained as a result of expression of each activator in the absence of exogenous dTBP expression.

polypeptide. The PCR fragment was cut with *Mlu*I and *Sac*I (sites engineered into the primers) and subcloned into pACTflu110.top vector purified after digestion with *Mlu*I (engineered into the 70-mer described above) and *Sac*I. This created plasmid pACTflu40.

RESULTS

A p53-GAL4 chimera synergistically activates transcription with TBP more effectively than do other activators. We have previously shown that human TBP and p53 cooperatively bind to a DNA fragment containing a TATA box and a p53 binding site (7). In that study, we also reported an experiment suggesting that p53 and TBP can cooperate to activate transcription in *Drosophila* Schneider cells (7). To understand further the basis for collaboration between p53 and TBP in vivo, we continued using cotransfection assays in Schneider cells. These cells were chosen for three reasons: first, Schneider cells do not express any known version of p53 (53); second, TBP can be limiting in these cells, for when it is overexpressed, basal transcription is elevated from promoters containing a TATA box (10); and third, transcriptional activation mechanisms are conserved from yeast to human species (22, 41). In many of the experiments described here, we used a plasmid expressing a p53-GAL4 hybrid containing amino acids 1 to 346 of human p53 fused in frame to the yeast GAL4 DNA binding domain (pACT53G4). This chimera was a more effective transcriptional activator from a template containing five GAL4 DNA binding sites and a minimal TATA box (G5-E1B-CAT) than was full-length p53 from a template bearing the RGC p53 binding sequence (RGCfos). Moreover, we wished to use a p53-GAL4 hybrid to compare more accurately the effects obtained with other GAL4 hybrids in assays using the same CAT reporter template.

A level of CAT activity about 100,000-fold over basal levels was observed when plasmids expressing an epitope-tagged version of dTBP (pACTdTBP [10]) and p53-GAL4 (pACT53G4) were cotransfected into Schneider cells along with G5-E1B-CAT. p53-GAL4 and dTBP displayed a dramatically synergistic interaction, as the levels of CAT activity achieved as a result of their coexpression were much greater than the additive levels achieved when either was expressed alone (about 1,000- and 20-fold over basal levels, respectively) (Fig. 1A). Although p53-GAL4 stimulated CAT activity from G5-E1B-CAT more effectively than did intact p53 from RGCfos, the overall extents of synergism with dTBP were similar in both cases (Table 1). When pACTdTBP was transfected with plasmids expressing either GAL4-VP16 or GAL4-ftzQ (9), synergistic levels of activation were also detected from G5-E1B-CAT; similarly, when it was transfected with a plasmid expressing Sp1, synergistic levels of activation were detected from SV-E1B-CAT (containing three Sp1 binding sites [10]). However, the synergism displayed between p53-GAL4 and dTBP was 10-fold greater than that displayed between any of the other activators and dTBP. GAL4-VP16 is a far more effective activator of transcription from a G5-E1B-CAT. Nevertheless, this extent of cooperation was observed both when the amounts of activator expression vectors were adjusted to give equivalent levels of CAT activity in the absence of dTBP (Fig. 1B) and when equal amounts of each expression vector were used (data not shown).

A p53 mutant defective in transcriptional activation does not cooperate with dTBP. Lin et al. (39) showed that mutation of two adjacent hydrophobic amino acids, Leu-22 and Trp-23, to Gln-22 and Ser-23 within the activation domain severely impairs the ability of p53 to activate transcription. Furthermore, they demonstrated that this double mutation does not

TABLE 1. Effects of wild-type and mutant p53 expression on dTBP-stimulated transcription from RGCfos[a]

p53 expression plasmid	CAT activity (U) with indicated amt (ng) of dTBP expression plasmid			
	0	20	200	2,000
None	1.0	1.1	4.3	17.6
Mutant	1.8	2.1	11.0	25.0
Wild type	200	2,600	4,900	16,000

[a] Two micrograms of RGCfos (7) was cotransfected either alone (none) or with 1 μg of pACTmt53 expressing full-length $mt53_{22/23}$ protein (mutant) or 1 μg of pACT53 (7) expressing full-length wild-type p53 protein (wild type). Increasing amounts of pACTdTBP were cotransfected with these plasmids, and levels of CAT activity were measured as described in the legend to Fig. 1A.

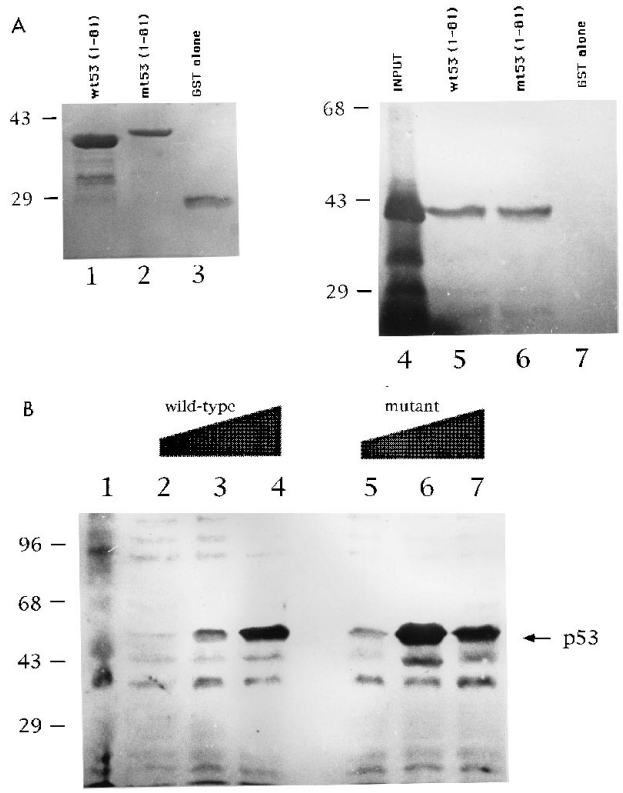

FIG. 2. N termini from both wild-type p53 and $mt53_{22/23}$ bind dTBP in vitro. (A) GST fusion proteins were bound to 25 μl of glutathione–S-Sepharose beads, washed, incubated with rabbit reticulocyte lysates expressing [^{35}S]methionine-labeled dTBP, washed, and analyzed by PAGE. Lanes 1 to 3, Coomassie blue-stained 10% polyacrylamide gel; lanes 4 to 7, autoradiogram of a different gel. Lane 1, 5 μl of beads incubated with *E. coli* extract expressing GST–wild-type p53 (amino acids 1 to 81); lane 2, 5 μl of beads from extract expressing GST-$mt53_{22/23}$ (amino acids 1 to 81); lane 3, 5 μl of beads from extract expressing GST polypeptide alone; lane 4, 20% of the lysate input; lane 5, total material bound to beads bound to GST–wild-type p53; lane 6, material bound to a GST-$mt53_{22/23}$; lane 7, material bound to GST alone. (B) Expression of the wild type and $mt53_{22/23}$ in Schneider cells. Either 0.5, 1.0, or 3.0 μg of pACT53 (lanes 2 to 4) or pACTmt53 (lanes 5 to 7) was transfected into Schneider cells, lysed after a 48-h incubation, and analyzed by Western blotting with monoclonal antibody PAb 1801 specific to human p53. Lane 1 is an extract from cells transfected with the actin 5C expression plasmid alone. Sizes are indicated in kilodaltons.

affect the ability of p53 to bind DNA or to bind human TBP (39). Note, however, that another group reported that mutation of p53 residues 22 and 23 to Arg and Ser, respectively, impaired the ability of a p53-GST fusion protein to bind TBP (5). Possibly the choice of substituted amino acids is responsible for these differences. Since the synergism between wild-type p53 and dTBP was so striking, and also since the mutant p53 containing Gln-22 and Ser-23 (referred to here as $mt53_{22/23}$) retained TBP binding ability, we chose to determine what effect overexpression of TBP would have on the activity by $mt53_{22/23}$. First, it was important to ensure that dTBP binds both wild-type p53 and $mt53_{22/23}$. Previous experiments have suggested that p53 can interact with TBP via both its amino and carboxy termini (29, 42, 57, 59). It was also shown that TBP is capable of binding to the full-length $mt53_{22/23}$ (39). Thus, it was possible that the double mutation prevented TBP from binding to the N-terminal TBP binding domain of p53, while the potential for an interaction occurring through the p53 C terminus remained. To test this, purified recombinant GST fusion proteins containing amino acids 1 to 81 of either wild-type p53 or $mt53_{22/23}$ (Fig. 2A, lanes 1 to 3) were tested for binding to dTBP. Both wild-type GST-p53 (lane 5) and GST-$mt53_{22/23}$ (lane 6) fusion proteins bound to reticulocyte lysate-expressed dTBP labeled with [^{35}S]methionine, while the GST polypeptide alone did not (lane 7). This result confirms that a TBP binding domain that is capable of binding to dTBP exists at the N terminus of p53 and that mutation of Leu-22 and Trp-23 to Gln-22 and Ser-23 does not affect this activity.

We then compared transcriptional activation by wild-type p53 and $mt53_{22/23}$ with respect to the ability to cooperate with

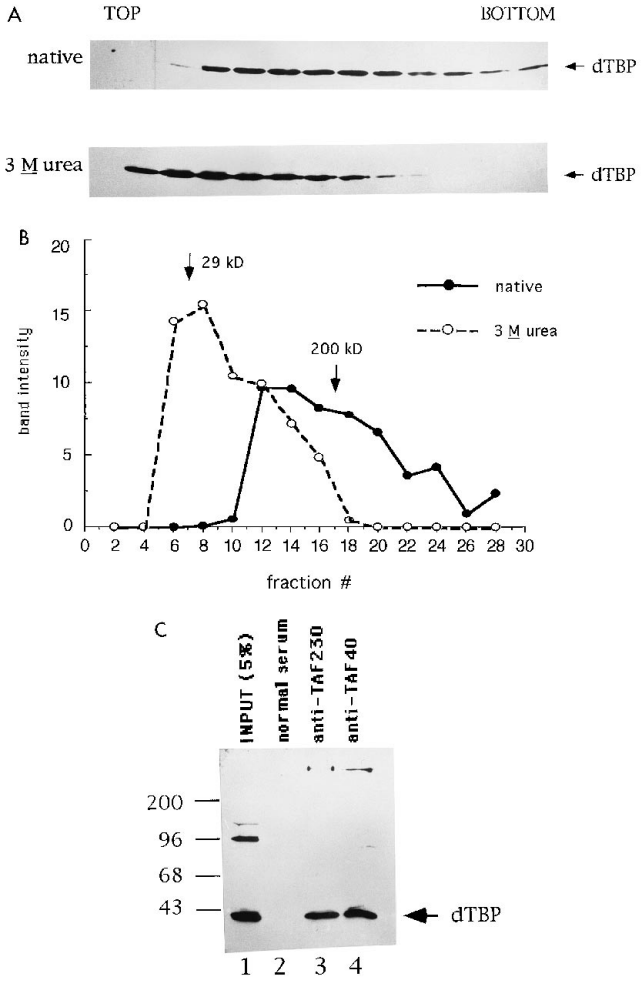

FIG. 3. Transiently expressed dTBP in Schneider cells assembles into high-molecular-weight complexes and can be immunoprecipitated with anti-TAF antibodies. (A) Extracts were prepared from Schneider cells transfected with pACTdTBP and loaded onto a 10 to 40% gradients with or without 3 M urea. Even-numbered fractions 2 to 28 were analyzed by Western blot analysis using monoclonal antibody 12CA5, which recognizes the flu epitope (17). (B) Graphical representation of glycerol gradient profile of transfected flu-dTBP. Exposures of Western blots developed with an ECL kit were used in densitometric analysis, and density values of each fraction loaded were plotted. For comparison, sedimentation peaks of carbonic anhydrase (29 kDa) and β-amylase (200 kDa) run in parallel gradients are indicated. (C) Protein A-Sepharose beads preincubated with normal rabbit serum or serum containing a polyclonal antibody to either $TAF_{II}40$ or $TAF_{II}230$ were incubated with extracts from Schneider cells transfected with 5 μg of pACTdTBP. Lane 1, 5% cell extract used for incubations; lane 2, beads preincubated with normal rabbit serum; lane 3, beads preincubated with anti-$TAF_{II}230$ antiserum; lane 4, beads preincubated with anti-$TAF_{II}40$ antiserum. Detection of flu-tagged dTBP was done by Western blot analysis using monoclonal antibody 12CA5 as described for panel A. Note that Schneider cell lysates contain a small number of polypeptides that cross-react with antibody 12CA5. Sizes are indicated in kilodaltons.

dTBP. In these experiments, plasmids expressing full-length p53 rather than GAL4 fusions were tested for the ability to activate transcription from promoter constructs containing a p53 binding site (RGCfos). We chose to use full-length derivatives to ensure that both TBP binding domains of p53 were intact. As shown in Table 1, when $mt53_{22/23}$ was compared with wild-type p53 for its ability to stimulate CAT activity from RGCfos, the mutant was far weaker than the wild type. When transfection experiments were performed with the wild-type and mutant p53 expression constructs along with pACTdTBP, although, as expected, overexpression of dTBP enhanced basal levels of CAT activity from RGCfos, coexpression of $mt53_{22/23}$ had no significant stimulatory effect. By contrast, confirming our previous results, wild-type p53 cooperated effectively with dTBP. The difference in effects between the wild-type and mutant proteins was not due to a difference in protein expression levels, since both were detected in transfected Schneider cells at similar levels (Fig. 2B). This result indicates that the p53-dTBP synergy correlates with the transcriptional activation function of p53 and that the ability of the two proteins to interact directly is not sufficient for this effect.

Transiently expressed dTBP in Schneider cells assembles into high-molecular-weight complexes and can be immunoprecipitated with anti-TAF antibodies. Since $mt53_{22/23}$ binds dTBP (Fig. 2A) but not *Drosophila* $TAF_{II}40$ or $TAF_{II}60$ (58), we considered the possibility that TAFs were contributing to the synergy observed between wild-type p53 and dTBP. To begin to address this, it was necessary to examine whether dTBP can, in fact, bind TAFs when overexpressed in Schneider cells. To facilitate detection of possible dTBP-TAF complexes, cells were transfected with 5 μg of pACTdTBP, an amount considerably more than the 0.01 μg of plasmid required to detect synergy with p53-GAL4. Previous work has shown that TAFs and TBP bind together in a tight complex which can be dissociated by 3 M urea (15, 55). On the basis of this observation, cell extracts were applied to 10 to 40% glycerol gradients either containing or lacking 3 M urea. Following centrifugation, fractions collected from these gradients were analyzed by Western blotting with an antibody specific to the flu epitope (17) on the exogenous dTBP. As shown in Fig. 3A, under native conditions, transfected dTBP was heterogeneously distributed throughout the gradient. A majority of it sedimented at the top, within the low-density regions of the gradient, while a small portion of the material sedimented at the bottom. In the presence of 3 M urea, dTBP was absent from the bottom fractions and instead accumulated exclusively at the top of the gradient. Figure 3B shows a graphical representation of these gradient profiles along with the positions of molecular weight markers run in parallel gradients. The change in mobility of transfected dTBP due to treatment with urea is in agreement with previous results demonstrating the sedimentation profile of monomeric dTBP (15) and is consistent with the possibility that a fraction of it assembled into dTBP-TAF complexes.

To further determine if transfected dTBP was physically associated with TAFs, we carried out immunoprecipitation experiments with transfected cell extracts, using TAF-specific antibodies. Rabbit polyclonal antibodies to $TAF_{II}40$ or $TAF_{II}230$ were bound to protein A-Sepharose beads and incubated with the same extracts used in the gradient experiments. The immunoprecipitates were then analyzed by Western blotting with the flu epitope tag-specific antibody. As shown in Fig. 3C, at least 5% of the total dTBP was coimmunoprecipitated with each of the TAF antibodies (lanes 3 and 4), while none was immunoprecipitated by normal rabbit serum (lane 2). It is important to note that neither antiserum used here cross-reacts with dTBP (45a). These results demonstrate that a fraction of the exogenously expressed dTBP was bound to endogenous $TAF_{II}40$ and/or $TAF_{II}230$. Given that the amount of pACTdTBP used in this experiment was 500-fold greater than that required to detect synergy between p53-GAL4 and dTBP, it is likely that an even higher proportion of the exogenous dTBP was bound with TAFs under conditions in which less pACTdTBP was introduced.

Expression of TAF cDNAs does not affect basal transcription stimulated by dTBP but does inhibit activated transcription by p53-GAL4. As described above, transiently expressed dTBP displayed a heterodisperse sedimentation profile, indicating that populations of various dTBP-containing complexes likely existed in the transfected cells. These complexes could represent different dTBP-TAF heterooligomers, resulting from limiting amounts of available TAFs with which transfected dTBP could bind. Therefore, if TAFs are indeed the additional components required for p53-TBP synergy, it seemed possible that an excess of the appropriate TAFs would influence the synergism between p53-GAL4 and dTBP. A positive effect might be detected if a TAF is limiting, although inhibition, perhaps due to squelching of some other limiting factor, might be observed if the TAF is already in excess.

To investigate these possibilities, plasmids were constructed to express cDNAs for *Drosophila* $TAF_{II}40$, $TAF_{II}60$, $TAF_{II}110$, and $TAF_{II}230$ under control of the actin 5C promoter. We first examined the effects of TAF overexpression on basal CAT activity from G5-E1B-CAT enhanced by the overexpression of dTBP. As shown in Fig. 4A, this activity was unaffected by transfection of increasing amounts of the TAF expression plasmids noted above. Furthermore, overexpressed TAFs did not significantly affect expression of G5-E1B-CAT over a range of dTBP concentrations (Fig. 4B). Similarly, transfection of the TAF expression plasmids did not affect the low, unstimulated level of basal CAT activity from G5-E1B-CAT (data not shown). Since the inability to affect basal activity was not due to lack of expression of the TAFs (see below), these results are consistent with the observation that TAFs are not required for basal transcription in vitro (15, 36, 55) and support the view that this in vivo basal CAT expression is related to true basal transcription defined in vitro (10).

We next conducted experiments to determine the effects of TAF overexpression on transcriptional activation by p53-GAL4. Expression of either $TAF_{II}230$ or a combination of $TAF_{II}40$ and $TAF_{II}60$ ($TAF_{II}40/60$) significantly decreased p53-GAL4 activation (Fig. 4B), while expression of $TAF_{II}110$ had no effect on this activity. Expression of $TAF_{II}40$ or $TAF_{II}60$ alone also decreased CAT activity but to a lesser extent than when the two were expressed simultaneously (data not shown). Furthermore, expression of $TAF_{II}230$, $TAF_{II}40$, and $TAF_{II}60$ together caused a level of inhibition similar to that provided by either $TAF_{II}230$ or $TAF_{II}40/60$ alone (data not shown). Importantly, overexpression of the TAFs did not inhibit the expression of p53-GAL4 or dTBP from the respective expression plasmid (Fig. 4C). Since transfection of the $TAF_{II}110$ expression vector did not affect either basal or activated CAT activity from G5-E1B-CAT, it was necessary to confirm that $TAF_{II}110$ was expressed at levels comparable to those of the other TAFs. To investigate this, we constructed plasmids expressing flu epitope-tagged versions of $TAF_{II}110$ and $TAF_{II}40$. As shown in Fig. 4D, after transfection of the respective expression plasmids, epitope-tagged $TAF_{II}110$ (lane 2) could be detected at levels comparable to that of epitope-tagged $TAF_{II}40$ (lane 4) in whole-cell lysates by Western blot analysis. Thus, the lack of effect by $TAF_{II}110$ was not due to lack of its expression.

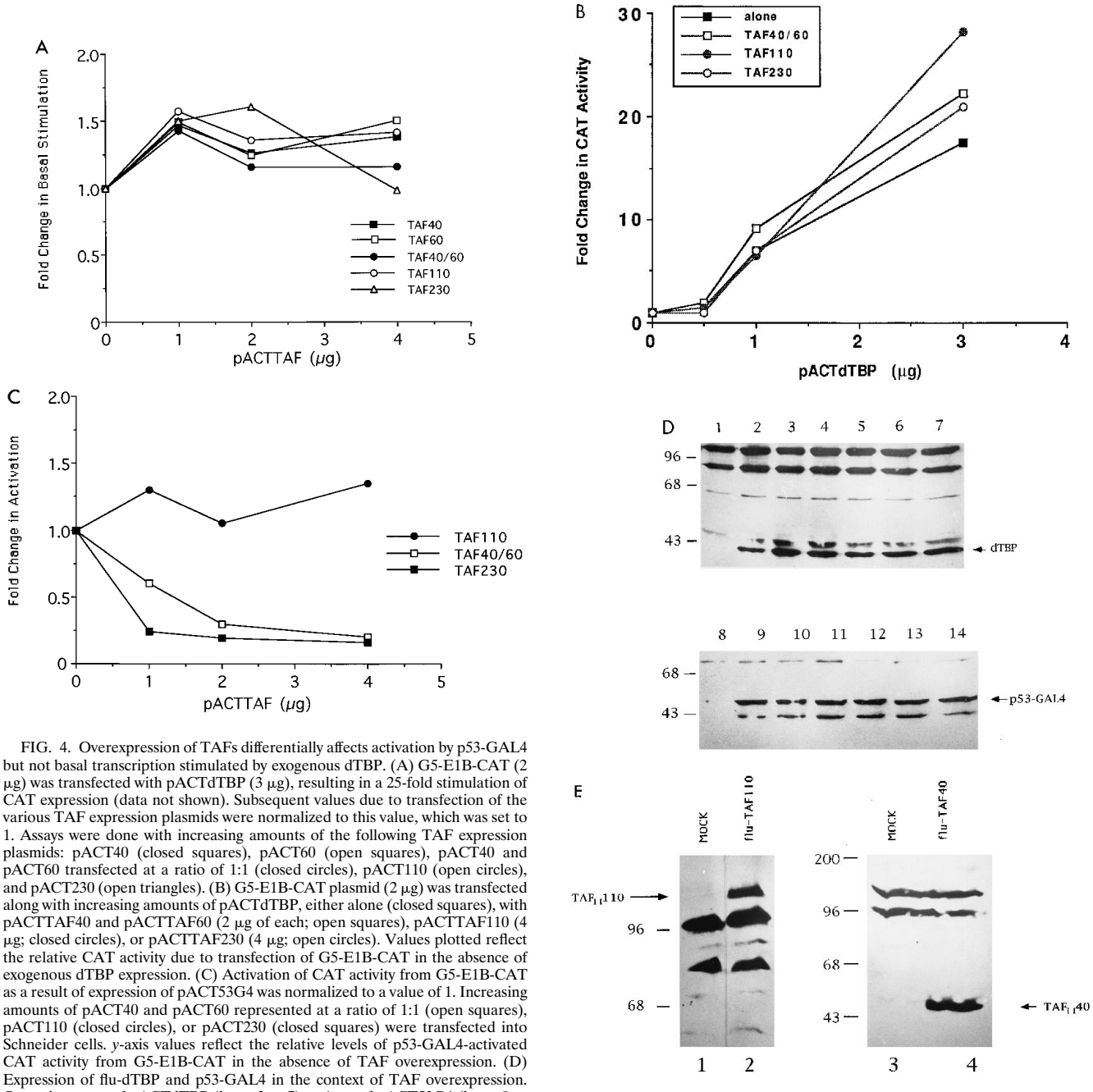

FIG. 4. Overexpression of TAFs differentially affects activation by p53-GAL4 but not basal transcription stimulated by exogenous dTBP. (A) G5-E1B-CAT (2 μg) was transfected with pACTdTBP (3 μg), resulting in a 25-fold stimulation of CAT expression (data not shown). Subsequent values due to transfection of the various TAF expression plasmids were normalized to this value, which was set to 1. Assays were done with increasing amounts of the following TAF expression plasmids: pACT40 (closed squares), pACT60 (open squares), pACT40 and pACT60 transfected at a ratio of 1:1 (closed circles), pACT110 (open circles), and pACT230 (open triangles). (B) G5-E1B-CAT plasmid (2 μg) was transfected along with increasing amounts of pACTdTBP, either alone (closed squares), with pACTTAF40 and pACTTAF60 (2 μg of each; open squares), pACTTAF110 (4 μg; closed circles), or pACTTAF230 (4 μg; open circles). Values plotted reflect the relative CAT activity due to transfection of G5-E1B-CAT in the absence of exogenous dTBP expression. (C) Activation of CAT activity from G5-E1B-CAT as a result of expression of pACT53G4 was normalized to a value of 1. Increasing amounts of pACT40 and pACT60 represented at a ratio of 1:1 (open squares), pACT110 (closed circles), or pACT230 (closed squares) were transfected into Schneider cells. *y*-axis values reflect the relative levels of p53-GAL4-activated CAT activity from G5-E1B-CAT in the absence of TAF overexpression. (D) Expression of flu-dTBP and p53-GAL4 in the context of TAF overexpression. One microgram of pACTdTBP (lanes 2 to 7) or 1 μg of pACT53G4 (lanes 9 to 14) was transfected either alone (lanes 2 and 9) or with either 4 μg of pACT40 (lanes 3 and 10), 4 μg of pACT60 (lanes 4 and 11), 2 μg of pACT40 and 2 μg of pACT60 (lanes 5 and 12), 4 μg of pACT110 (lanes 6 and 13), or 4 μg of pACT230 (lanes 7 and 14). flu-dTBP was detected by Western blot analysis using monoclonal antibody 12CA5 (lanes 1 to 7), and p53-GAL4 was detected with the p53-specific monoclonal antibody PAb 1801 (lanes 8 to 14). Lanes 1 and 8 are extracts from cells mock transfected with the nonrecombinant pACT expression vector. (E) Expression of epitope-tagged $TAF_{II}110$ and $TAF_{II}40$. Schneider cells were transfected with 4 μg of a pACT expression plasmid alone (lanes 1 and 3), 4 μg of pACTflu-110 (lane 2) expressing epitope-tagged $TAF_{II}110$, or 4 μg of pACTflu-40 expressing epitope-tagged $TAF_{II}40$. Extracts were prepared after a 48-h incubation and analyzed by Western blotting with monoclonal antibody 12CA5 as described for Fig. 3A. The differences in the migration of the 12CA5 cross-reactive polypeptides in lanes 1 and 2 compared with those in lanes 3 and 4 are probably due to the different gel conditions required to resolve epitope-tagged $TAF_{II}110$ (see Materials and Methods). Sizes are indicated in kilodaltons.

Expression of $TAF_{II}230$ inhibits activated transcription by GAL4-VP16 and Sp1. We next wished to determine if the effect of TAF overexpression was specific for p53-GAL4, or if TAFs could affect the activity of other activators. To this end, stimulation of CAT expression by GAL4-VP16 from G5-E1B-CAT was measured in the presence or absence of exogenous TAFs. CAT activity was inhibited about fivefold as a result of overexpression of $TAF_{II}230$. As seen with p53-GAL4, expression of $TAF_{II}110$ had no effect on stimulation of CAT activity by GAL4-VP16. However, in contrast to what we observed with p53-GAL4, the stimulation by GAL4-VP16 was reproducibly only slightly inhibited (less than twofold) by expression of $TAF_{II}40/60$ (Fig. 5A). When the effects of TAF overexpression on Sp1 stimulation of CAT activity from SV-E1B-CAT were

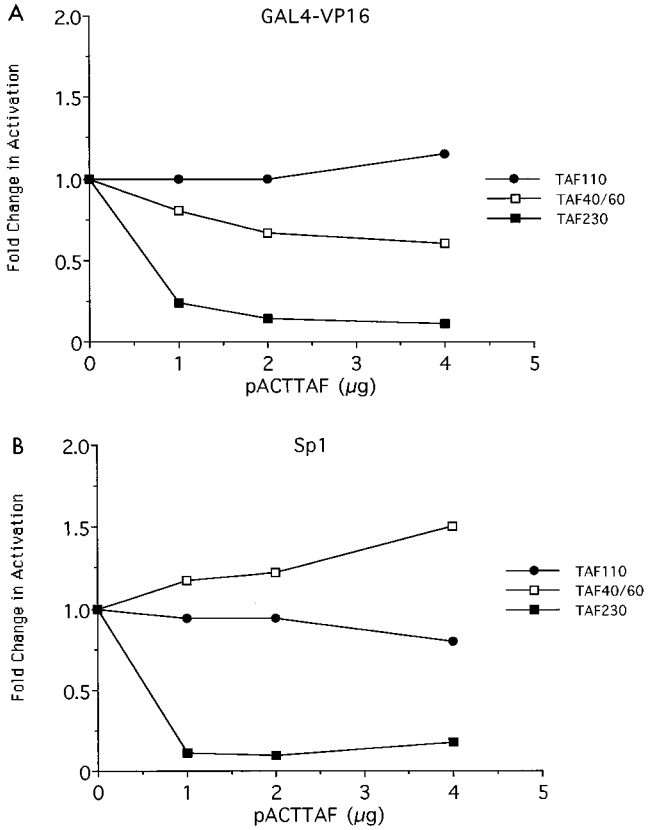

FIG. 5. Effect of TAF overexpression on activation by GAL4-VP16 and Sp1. (A) Activation of CAT activity from G5-E1B-CAT as a result of expression of pACTG4VP16 was normalized to a value of 1. *y*-axis values reflect the relative levels of GAL4-VP16 on activated CAT activity from G5-E1B-CAT in the absence of TAF overexpression. (B) Activation of CAT activity from SV-E1B-CAT as a result of expression of pACTSP1 was normalized to a value of 1. *y*-axis values reflect the relative levels of Sp1 on activated CAT activity from SV-E1B-CAT in the absence of TAF overexpression. TAF expression plasmids include pACT40 and pACT60 transfected at a ratio of 1:1 (open squares), pACT110 (closed circles), and pACT230 (closed squares).

determined, neither $TAF_{II}40/60$ nor $TAF_{II}110$ had detectable effects (Fig. 5B). However, similar to the effects seen with p53-GAL4 and GAL4-VP16, overexpression of $TAF_{II}230$ resulted in a large inhibition of Sp1 activation (about 10-fold).

These results demonstrate that among the activators tested, transcriptional repression by overexpression of $TAF_{II}40/60$ is specific for activation by p53-GAL4, consistent with the roles of $TAF_{II}40$ and $TAF_{II}60$ in transcriptional activation by p53 in vitro (58). Furthermore, these results demonstrate that inhibition of transcription by $TAF_{II}230$ is a general phenomenon, consistent with the role that $TAF_{II}230$ has been suggested to play in activated transcription in vitro (7, 61). However, our results differ with the proposed roles of $TAF_{II}40$ in activation by VP16 (20) and of $TAF_{II}110$ in activation by Sp1 (18, 28). This may reflect any of several differences between previous in vitro assays and our in vivo assay in Schneider cells.

Expression of dTBP rescues both $TAF_{II}40/60$ and $TAF_{II}230$-mediated inhibition of transactivation by p53-GAL4. To gain more insight into the possible mechanisms of TAF inhibition of activated transcription, we determined the effects of increasing dTBP expression in the presence of constant amounts of TAFs and p53-GAL4. Specifically, we wished to determine whether dTBP could rescue TAF-mediated repression and, if so, how effectively. To test this, constant amounts of pACT53G4 with or without TAF expression vectors were transfected with increasing amounts of pACTdTBP, and levels of CAT activity from G5-E1B-CAT were measured. As shown in Fig. 6, in the absence of exogenous TAF expression, levels of dTBP synergy plateaued after transfection of about 0.05 μg of pACTdTBP, providing a 100-fold increase of CAT activity. In the presence of 4 μg of the $TAF_{II}230$ expression plasmid, activation by p53-GAL4 was decreased by about fivefold in the absence of dTBP, as shown in Fig. 4B. Increasing amounts of pACTdTBP were, in fact, able to rescue the inhibition, but not until transfection of at least 0.5 μg of pACTdTBP was the synergy between p53-GAL4 and dTBP restored to the value attained in the absence of exogenous TAF expression. The effect of overexpression of $TAF_{II}40/60$, which inhibited the activation by p53-GAL4 about threefold, was also rescued by transfection of pACTdTBP. It is notable that in the presence of excess $TAF_{II}230$ or $TAF_{II}40/60$, transfection of lower amounts of pACTdTBP (0.01 to 0.1 μg) had little effect on CAT activity, even though these amounts provided the strong synergistic activation observed in the absence of exogenous TAF expression (Fig. 6). Within this concentration range of dTBP, overexpression of $TAF_{II}230$ and $TAF_{II}40/60$ resulted in a much more dramatic (up to 100-fold) inhibition of CAT activity. In any event, our finding that dTBP could rescue TAF-mediated inhibition of p53-GAL4 activity suggests that a functional interaction between p53-GAL4, dTBP, and TAFs exists in transfected Schneider cells. The implications of these results are discussed below.

DISCUSSION

In this report, we have shown that p53-GAL4 and dTBP synergistically activate transcription when expressed in *Drosophila* Schneider cells. A p53 mutant ($mt53_{22/23}$) defective in transcriptional activation and TAF binding did not cooperate with dTBP, which suggested a role of TAFs in this synergy. This view was supported by the observation that at least some of the transfected dTBP is complexed with endogenous TAFs. Activation by p53-GAL4 was inhibited by expression of $TAF_{II}40$, $TAF_{II}60$, and $TAF_{II}230$, but this inhibition could be rescued by

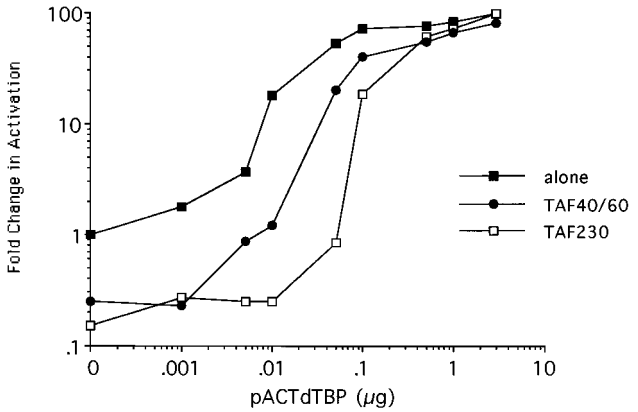

FIG. 6. Effect of dTBP on $TAF_{II}230$-mediated and $TAF_{II}40/60$-mediated inhibition of p53-GAL4 stimulation of CAT activity. Activation of CAT activity from G5-E1B-CAT as a result of expression of 0.1 μg of pACT53G4 was normalized to a value of 1. Shown are results of cotransfection of increasing amounts of pACTdTBP either alone (closed squares), in the presence of 4 μg of pACT230 (open squares), or in the presence of 2 μg of pACT40 and 2 μg of pACT60 (closed circles). The plotted points represent the average of CAT activity values from five different experiments normalized to values of CAT activity due to transfection of pACT53G4 alone.

dTBP. Hence, we have provided direct evidence for functional interactions among p53, TBP, and TAFs in vivo.

Functional significance of the p53-TBP interaction. Several other examples of synergism between transcriptional activators and TBP have been previously described, including interactions with the product of the *rel* proto-oncogene (33), the human T-cell leukemia virus type 1 Tax protein (4), and the human papillomavirus E2 protein (24). Interestingly, the E2 protein, like p53, has been shown to bind cooperatively to DNA with TBP (14), and mutations in E2 that destroy transcriptional activation do not display synergism with TBP in mammalian cells (24). Likewise, our data here show that a transcriptionally defective p53 mutant, which retains its ability to bind TBP, cannot synergize with dTBP in Schneider cells. It is possible that cooperativity between TBP (presumably as a subunit of TFIID) and transactivators may be a general mechanism of transactivation in vivo. As we have shown in this study, this cooperativity likely involves not only direct interactions between TBP and transactivators but also interactions with other molecules associated with TBP, e.g., TAFs.

p53 synergizes with dTBP better than do other activators. Investigation of transcriptional activation mechanisms has suggested that at least in some systems, TFIID recruitment is a rate-limiting step in the stimulation of preinitiation complex assembly (reference 44 and references therein). This finding is consistent with the observation that p53-GAL4 and other transcriptional activators synergize with dTBP to stimulate CAT activity when both are expressed in Schneider cells. However, as we have also shown here, p53-GAL4 synergizes with dTBP about 10-fold better than other activators. Our explanation for this difference is that TFIID recruitment plays a more prominent role in activation by p53 than by other activators. This view is supported by our previous observation that p53 and TBP cooperatively bind DNA (7). This property has not been observed with GAL4-VP16 or Sp1, even though these two transactivators also directly interact with TBP (7, 16, 30, 50). Moreover, we have shown that in contrast to p53-GAL4, transcriptional activation by GAL4-VP16 and Sp1 is not affected by overexpression of $TAF_{II}40/60$. Therefore, the reasons for the differences in the relative strengths of synergy among p53 and the other activators might also lie in how effectively they interact with TBP-TAF complexes in vivo.

Role of $TAF_{II}230$ in transcriptional activation. Previous experiments have provided evidence for a specific role of $TAF_{II}250$ in vivo, as illustrated by a correlation between transcriptional activation by an altered-specificity TBP mutant and the ability of this mutant to bind $TAF_{II}250$ in vitro (56). Evidence for a role of $TAF_{II}250$ in transcriptional activation both in vitro and in vivo is also provided by experiments showing that stable expression of $TAF_{II}250$ can rescue a temperature-sensitive mutant hamster cell line containing a mutant $TAF_{II}250$ (61). Nuclear extracts prepared from the rescued cells are permissive for transactivation by Sp1 and GAL4-VP16, while extracts prepared from the original cell line are not.

In our experiments, overexpression of $TAF_{II}230$ in Schneider cells significantly inhibited CAT expression activated by p53-GAL4 as well as that activated by GAL4-VP16 and Sp1. These findings are partly consistent with published results that have shown recombinant $TAF_{II}230$ can inhibit transcription in vitro (35, 37). However, Kokubo et al. (37) showed that $TAF_{II}230$ inhibits the binding of TBP to the TATA box in vitro and suggested that this was the mechanism of inhibition by $TAF_{II}230$. Results from our experiments indicate this may not the case in our in vivo assay, since overexpression of $TAF_{II}230$ did not affect basal or dTBP-enhanced basal CAT expression in the absence of activators. A possible explanation for this finding is that overexpressed $TAF_{II}230$ can affect the ability of only native TBP, not TFIID, to bind DNA (35). Since the transfected dTBP assembles into higher-order complexes that probably contain TAFs (Fig. 3), its binding to DNA and stimulation of basal CAT expression may be resistant to the inhibitory effects of $TAF_{II}230$. Therefore, we suggest that the inhibition of activated transcription by $TAF_{II}230$ is due to an indirect mechanism by which the $TAF_{II}230$ squelches (19) one or more factors other than TBP that are required for transcriptional activation.

The foregoing model is also consistent with our data demonstrating rescue of $TAF_{II}230$ inhibition by transfected dTBP. If, in the absence of transfected dTBP, $TAF_{II}230$ inhibits by a squelching mechanism, then this effect could be reversed by recruiting some of the excess $TAF_{II}230$ (and whatever factor[s] bound to it) into active transcription complexes. In our experiments, we suggest that dTBP effectively rescues the inhibition by providing this function. Therefore, the limiting factor, whether another TAF or something else, is probably contacted, either directly or indirectly, by both $TAF_{II}230$ and TBP. This hypothesis is supported by the observation that only at high amounts of pACTdTBP does rescue of inhibition by $TAF_{II}230$ occur. At low concentrations of transfected dTBP, it is conceivable that all available dTBP is complexed with endogenous TAFs. This population would not be able to bind the excess $TAF_{II}230$ and therefore would be unable to rescue $TAF_{II}230$-mediated inhibition. Indeed, at these low dTBP concentrations, the inhibitory effect of $TAF_{II}230$ was very large. This presumably reflects, in the absence of $TAF_{II}230$, the ability of dTBP to complex endogenous TAFs and to cooperate with p53-GAL4. At the high concentration of exogenous dTBP required for rescue, glycerol gradient analysis demonstrated that a significant population of the transfected dTBP exists in complexes of less than 200 kDa, much of which is likely to be free dTBP. This population could both bind and sequester the excess $TAF_{II}230$ as well as provide a pool to interact with endogenous TAFs and other factors which would be available for functional interactions with p53.

Effects of $TAF_{II}40$ and $TAF_{II}60$ overexpression. Our experiments show that $TAF_{II}40/60$ significantly inhibited activation by p53-GAL4 but only weakly inhibited GAL4-VP16 activation and had virtually no effect on activation by Sp1. Taken together with our result showing that overexpression of $TAF_{II}110$ did not affect p53-GAL4 activation, our data support the view that there is a specific interaction between p53 and $TAF_{II}40/60$. Although the effects of $TAF_{II}40/60$ on full-length p53 were not tested in this study, we expect that similar results would be obtained. Full-length p53 functioned similarly to p53-GAL4 in synergistically cooperating with TBP, and specific interactions between the activation domain of p53 and $TAF_{II}40/60$, but not $TAF_{II}110$, have been demonstrated in vitro (58). Although it is most appropriate to attribute the inhibitory effect of $TAF_{II}40/60$ on p53-GAL4 activity to squelching another essential factor, it is likely that a direct interaction between these TAFs and p53-GAL4 is also involved. It was somewhat unexpected that $TAF_{II}40/60$ was more inhibitory to p53-GAL4-mediated activation than to activation by GAL4-VP16. Although the reasons for the difference are not understood, taken together with our finding that p53-GAL4 cooperates far better with TBP than does GAL4-VP16, we conclude that there is a functional difference between the activation domains of p53 and VP16.

Conclusion. Our data have provided insights into the mechanism by which p53 functions to activate transcription in vivo and have implicated TBP and specific TAFs in the process.

Additional factors are also likely involved, as exemplified by the fact that CAT expression plateaued at the same value no matter what combination of TAFs were coexpressed with p53-GAL4 and dTBP (Fig. 6 and unpublished data). Thus, some other factor became limiting for expression, which could be another general factor, another TAF, or perhaps an additional coactivator. While our findings are largely consistent with the results of previous in vitro studies, and despite the limitation of our system in allowing only for analysis of the effects of overexpressed proteins, they provide evidence that TBP, TAFs and a transcriptional activator can functionally interact in vivo.

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