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The ability to associate with activation domains *in vitro* is not required for the TATA box-binding protein to support activated transcription *in vivo*

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ABSTRACT **The TATA box-binding protein (TBP) interacts *in vitro* with the activation domains of many viral and cellular transcription factors and has been proposed to be a direct target for transcriptional activators. We have examined the functional relevance of activator–TBP association *in vitro* to transcriptional activation *in vivo*. We show that alanine substitution mutations in a single loop of TBP can disrupt its association *in vitro* with the activation domains of the herpes simplex virus activator VP16 and of the human tumor suppressor protein p53; these mutations do not, however, disrupt the transcriptional response of TBP to either activation domain *in vivo*. Moreover, we show that a region of VP16 distinct from its activation domain can also tightly associate with TBP *in vitro*, but fails to activate transcription *in vivo*. These data suggest that the ability of TBP to interact with activation domains *in vitro* is not directly relevant to its ability to support activated transcription *in vivo*.**

RNA polymerase II transcription in eukaryotes is controlled by a diverse range of regulatory proteins that signal the conserved basal transcriptional machinery to initiate transcription. Transcriptional activators are proposed to function by directly contacting one or more of the basal factors (reviewed in ref. 1), enhancing the rate of basal factor recruitment to the promoter (2–4) or the rate of promoter clearance and elongation (5). Although transcriptional activation has been studied extensively *in vitro*, little is known of the way in which the basal factors respond to activators *in vivo*.

We have previously studied the role played by the TATA box-binding protein (TBP)—a central component of the basal transcription factor TFIID—in transcriptional response to a wide range of activators in human cells (6). We found that TBP activity *in vivo* is resistant to single sets of mutations on the surface of the molecule but is sensitive to specific combinations of mutations in different regions across the protein. This pattern of behavior correlates well with the ability of TBP to associate with the largest TBP-associated factor (TAF) in the TFIID complex, $hTAF_{II}250$, suggesting that recruitment of TBP into TFIID plays a major role in transcriptional activation *in vivo*.

Despite the importance of TFIID, however, activators may have multiple targets among the basal machinery (1), including TFIIB (3, 7, 8) and TBP itself. Indeed, the acidic activation domain of the herpes simplex virus transactivator VP16 (9), as well as the activation (refs. 1 and 10–15 and refs. therein) or DNA-binding (16–19) domains of at least 25 other transcriptional activators associate directly with TBP *in vitro*. Furthermore, mutational analyses of activation domains have revealed a good correlation between the ability of activators to associate with TBP and to activate transcription both *in vitro* and *in vivo* (10–14, 20), and a mutation in yeast TBP that disrupts activation domain binding also disrupts activation of transcription *in vitro* (21). However, the *in vivo* effects of mutations in TBP that block interaction with activation domains have not been described.

Here we analyze the relationship between transcription factor–TBP association *in vitro* and transcriptional activation *in vivo*. We demonstrate that both the VP16 transcriptional activation domain and the remainder of the protein associate with TBP *in vitro*, but only the activation domain activates transcription *in vivo*. We also show that point mutations in a loop connecting β-strands S3′ and S4′ of human TBP disrupt association with the VP16 and p53 activation domains *in vitro*, but they do not affect transcriptional activation *in vivo*. We conclude that the ability of TBP to associate with activation domains *in vitro* is neither necessary nor sufficient for transcriptional activation *in vivo*.

MATERIALS AND METHODS

Plasmid DNA Manipulations. Mutant TBP constructs were derived by site-directed mutagenesis (22) of the parental pCGN construct encoding altered-specificity human TBP (6), designated TBP_{AS}. GAL4-fusion proteins were constructed by insertion of the appropriate fragments into the *Xba* I and *Bam*HI sites of the vector pCGGAL(1–94) (23), provided by C. Hinkley (Cold Spring Harbor Laboratory). pCGGAL(1–94)-$p53_{AD}$ was constructed by PCR amplification of sequences encoding residues 1–73 of human p53 from the vector pBS-p53 (gift of C. Prives, Columbia University, New York) and insertion of the resulting fragment into pCGGAL(1–94). pCGGAL4-$VP16_{\Delta AD}$ was constructed by cloning of the *Xba* I/*Bam*HI fragment from pCGNVP16ΔC (ref. 24; kindly provided by R. Freiman, Cold Spring Harbor Laboratory) into pCGGAL4(1–94). pCGGAL4-$VP16_{AD}$ (23) was provided by G. Das (Cold Spring Harbor Laboratory).

For production of recombinant glutathione-*S*-transferase (GST)-fusion proteins in *Escherichia coli*, *Xba* I/*Bam*HI fragments encoding the activation domains of p53 (residues 1–73) and VP16 (residues 413–490) were inserted into the *Xba* I and *Bam*HI sites of pET11cGST (25). pET11cGST-$VP16_{\Delta AD}$ (formerly -VP16ΔC) was a gift from J.-S. Lai (25); pET11cGST-STOP, carrying an in-frame stop codon immediately after the GST moiety, was kindly provided by M. Tanaka (Cold Spring Harbor Laboratory).

Cell Culture and Transfections. The altered-specificity TBP assay was performed in transiently transfected HeLa cells exactly as described (6). Expression of GAL4-fusion proteins was confirmed by electrophoretic mobility shift analysis of transfected cell extracts; equivalent levels of expression of all mutant TBPs were confirmed by Western blot analysis.

"GST-Pulldown" Assays. GST-fusion proteins were expressed in *E. coli* by use of the T7 expression system of Studier *et al.* (26) and bound to glutathione-agarose (Sigma) as described (6). After extensive washing in HEMGN buffer (2)

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Abbreviations: TBP, TATA box-binding protein; TAF, TBP-associated factor; GST, glutathione-*S*-transferase; DBD, DNA-binding domain.

containing 0.15 M KCl, the amount of each GST-fusion protein bound to glutathione-agarose was quantitated by SDS/PAGE, followed by Coomassie blue staining.

^{35}S-labeled wild-type and mutant human TBP_{AS} molecules were generated by *in vitro* transcription and translation with either the TNT coupled transcription/translation system (Promega) or the standard reticulocyte lysate translation system (Promega). The synthesis of full-length TBP was quantitated by SDS/PAGE followed by phosphor imaging (Fuji).

GST-pulldowns were conducted by incubation of equivalent amounts of ^{35}S-labeled TBP derivatives with 6 μl of GST-beads (carrying 2 μg of each GST fusion protein) in a final volume of 20 μl. After being mixed at 4°C for 40 min, the beads were recovered by low-speed centrifugation and washed four times with 800 μl of HEMGN containing 0.15 M KCl and twice with 800 μl of HEMGN containing 0.3 M KCl. The samples were then placed in SDS/loading buffer and resolved by denaturing SDS/PAGE, followed by fluorography. Where stated, the amount of labeled TBP_{AS} bound to the various GST-beads was quantitated by phosphor imaging.

RESULTS

To probe the functional significance of activator–TBP association, we examined the effects of double and triple alanine-substitution mutations in TBP on both association with activators *in vitro* and response to activators *in vivo*. The TBP structure, shown in Fig. 1, consists of four α-helices and ten antiparallel β-sheets, eight of which make up the concave DNA-binding surface on the underside of the molecule (28). We targeted charged residues on the outer surface of TBP, positioned away from the DNA-binding surface, for mutagenesis (Fig. 1; ref. 6). Mutations in helices H2 and H1′, as well as in loops connecting β-strands S3/S4 and S3′/S4′, were analyzed because their activity could be monitored *in vivo*; TBP molecules carrying the double-alanine substitutions in helices H1 and H2′ (Fig. 1) could not be expressed in HeLa cells (6).

We initially compared the behavior of two regions of the herpes simplex virus transactivator VP16: Its acidic activation domain, designated $VP16_{AD}$ (residues 413–490; ref. 29), and the remainder of the protein, designated $VP16_{\Delta AD}$ (residues 5–412). $VP16_{\Delta AD}$ recruits two cellular factors, Oct-1 and HCF, to herpes simplex virus immediate early gene promoters, forming a multiprotein complex that in the presence of the VP16 activation domain stimulates viral transcription (30). We fused these two regions of VP16 individually to GST sequences and examined their ability to bind radiolabeled TBP molecules in a GST-pulldown assay. We also fused the VP16 sequences to the heterologous GAL4 DNA-binding domain (DBD; residues 1–94; ref. 31) and determined their potential to activate transcription *in vivo* from a *c-fos* reporter construct carrying four synthetic GAL4 binding sites (6). To measure the response of wild-type and mutant TBPs to these GAL4-fusion proteins, we used a form of TBP with an altered DNA-binding specificity (TBP_{AS}; ref. 32); this circumvents the activity of endogenous TBP and allows the effects of mutations in TBP to be monitored in transient transfection assays in human HeLa cells (6).

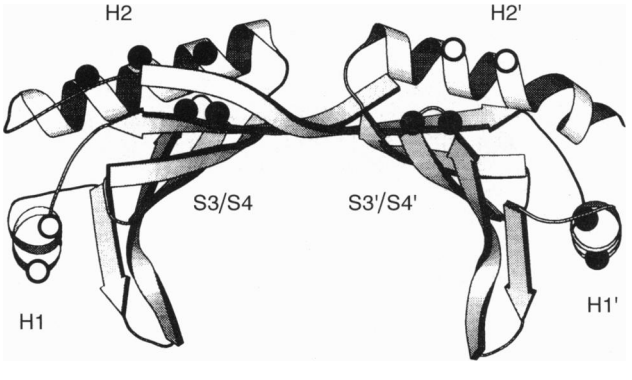

FIG. 1. Structure of TBP, showing the positions of mutations analyzed in this study. The diagram depicts a MOLSCRIPT (27) version of the conserved carboxyl-terminal domain of *Arabidopsis thaliana* TBP-2 (28), with the positions of the *H1′*, *H2*, *S3/S4*, and *S3′/S4′* mutations indicated by filled circles. The positions of the *H1* and *H2′* mutations, which could not be expressed in HeLa cells, are indicated by open circles.

The Ability to Associate with TBP *in Vitro* Is Not Sufficient for Transcriptional Activation *in Vivo*. The *in vitro* association of GST-$VP16_{AD}$ and GST-$VP16_{\Delta AD}$ with TBP is shown in Fig. 2*A*. Consistent with previous studies of yeast TBP (9, 14), approximately 20% of the input human TBP_{AS} protein remained associated with the GST-$VP16_{AD}$ beads (lane 16), a 35-fold higher level than background binding to the GST beads alone (lane 6). Unexpectedly, GST-$VP16_{\Delta AD}$ also tightly associated with TBP_{AS} *in vitro* (lane 11), binding approximately 10% of the input labeled protein. Both $VP16_{AD}$ and $VP16_{\Delta AD}$ bound to residues in the conserved carboxyl-terminal core of TBP (data not shown). Despite binding TBP to similar levels *in vitro*, however, the two regions of VP16 displayed markedly different activation abilities *in vivo*: GAL4-$VP16_{\Delta AD}$ did not activate transcription any more than the GAL4 DBD alone (Fig. 2*B*, compare lanes 2 and 8), whereas the GAL4-$VP16_{AD}$ fusion activated transcription more than 1000-fold better than GAL4 DBD (compare lanes 2 and 14). A GAL4-$VP16_{\Delta AD}$-fusion protein has previously been shown to activate transcription weakly in mammalian cells (33); the difference in activity of our GAL4-$VP16_{\Delta AD}$ protein may reflect the smaller region of GAL4 (residues 1–94 versus 1–147) used in our study. Whichever the reason, the inactivity of GAL4-$VP16_{\Delta AD}$ described here shows that the ability of $VP16_{\Delta AD}$ to associate with TBP *in vitro* is not sufficient to achieve transcriptional activation *in vivo*.

A Double Amino Acid Substitution in TBP That Disrupts Association with the VP16 Activation Domain *in Vitro* Does Not Affect the Response of TBP to VP16 Activation *in Vivo*. We next examined the ability of the double and triple alanine substitution mutations on the surface of TBP (Fig. 1) to interfere with the association between TBP and VP16 *in vitro*. As previously described (6), and shown for comparison in Fig. 2*B*, these mutations have little if any effect on *c-fos* promoter activation in response to GAL4-$VP16_{AD}$ *in vivo* (lanes 14–18); nor, as expected, do they elicit a response to the transcriptionally inactive GAL4 DBD (lanes 2–6) or GAL4-$VP16_{\Delta AD}$ proteins (lanes 8–12).

None of these mutations disrupted the *in vitro* association of TBP_{AS} with GST-$VP16_{\Delta AD}$ (Fig. 2*A*, lanes 12–15). Similarly, three of the mutations—*H1′*, *H2*, and *S3/S4*—as well as alanine substitutions in helices H1 and H2′ (Fig. 1; data not shown) had no more than a 2-fold effect on the association of TBP with GST-$VP16_{AD}$ (lanes 17–19). In contrast, however, the *S3′/S4′* mutation in TBP_{AS} (or wild-type TBP; data not shown) resulted in less than 5% the wild-type level of association with the VP16 activation domain (Fig. 2*A*; compare lane 16 with lane 20). Disruption of VP16 activation domain association by the *S3′/S4′* mutation is probably not the result of overall disruption of the TBP structure, because this mutant can associate at wild-type levels with both human $TAF_{II}250$ and TFIIB *in vitro* and is fully active *in vivo* (ref. 6; see Fig. 2*B*, lane 18). These data indicate that (*i*) the S3′/S4′ loop of TBP is an important site for contact with the VP16 activation domain and (*ii*) the ability of TBP to interact with the VP16 activation domain *in vitro* is not required for transcriptional activation *in vivo*.

The Effects of a Mutation in TBP That Specifically Disrupts VP16 Activation *in Vivo* Are Distinct from Its Effects on VP16 Association *in Vitro*. We have previously described a triple

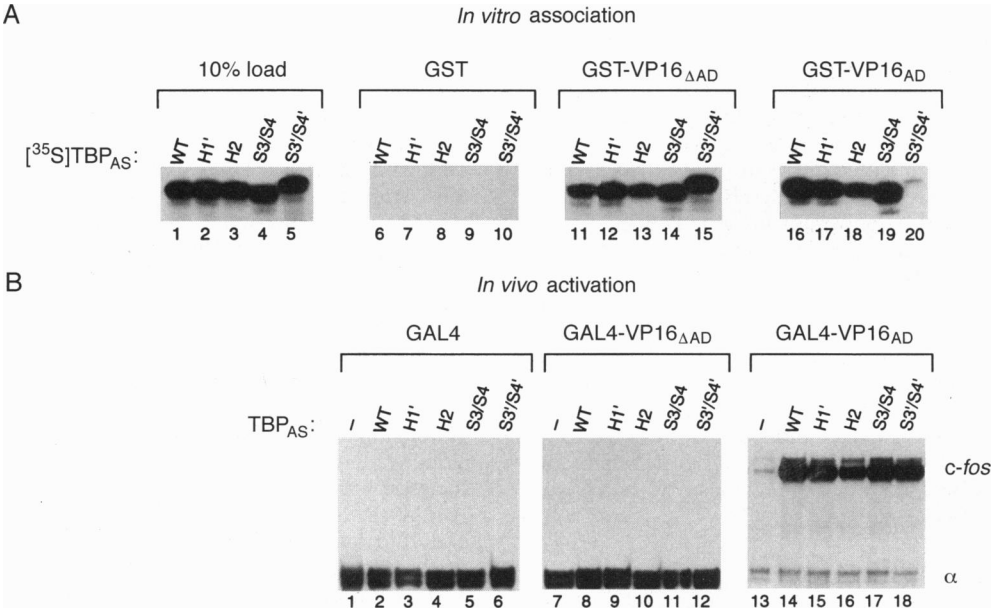

FIG. 2. A double-alanine substitution in the S3′/S4′ loop of TBP disrupts association with the VP16 activation domain *in vitro* but does not affect its response to the VP16 activation domain *in vivo*. (*A*) *In vitro* association of TBP_{AS} molecules with GST-fusion proteins. ^{35}S-labeled wild-type (WT) and mutant (*H1′, H2, S3/S4*, and *S3′/S4′*) TBP_{AS} molecules were tested for association with agarose beads carrying GST sequences alone (lanes 6–10), or GST-$VP16_{\Delta AD}$ (lanes 11–15) or GST-$VP16_{AD}$ (lanes 16–20) fusion proteins. Ten percent of the input of each labeled TBP_{AS} is shown for comparison (lanes 1–6). (*B*) *In vivo* analysis of GAL4-fusion activators. RNase protection analysis of RNA isolated from HeLa cells transfected with an altered c-*fos* TGTAAA reporter, an α-globin internal control plasmid, an expression construct encoding the TBP_{AS} listed above each lane, and expression constructs encoding either the GAL4 DBD alone (lanes 1–6), GAL4-$VP16_{\Delta AD}$ (lanes 7–12), or GAL4-$VP16_{AD}$ (lanes 13–18). The positions of correctly initiated transcripts from the c-*fos* and α-globin (α) plasmids are indicated. GAL4 activators are present in all lanes.

combined mutant of TBP—*H1′+S3/S4+S3′/S4′*—that responds poorly to the VP16 activation domain *in vivo* (10% wild-type activity) but responds much better (between 40% and 100% wild-type activity) to all other activation domains examined (6). Although all three sets of mutations are required to generate the VP16-specific defect of this TBP, we were intrigued that one of these mutations, *S3′/S4′*, disrupts $VP16_{AD}$ binding to TBP *in vitro*. Because we have observed extensive redundancy in TBP function *in vivo* (6), we reasoned that the effects of disrupting the TBP–VP16 interaction may be apparent only in the context of other mutations in TBP. We therefore used the *H1′+S3/S4+S3′/S4′* mutant to test this hypothesis. We first asked whether each individual alanine substitution in the *S3′/S4′* mutation—*K297A* and *R299A*—could disrupt association with the VP16 activation domain *in vitro*. We then asked what effect these individual substitutions had when combined with the *H1′+S3/S4* double mutation. This analysis is shown in Fig. 3.

Like the *S3′/S4′* mutation, the individual *K297A* and *R299A* mutations had little impact on the *in vivo* response of TBP to GAL4-$VP16_{AD}$ (Fig. 3*B*, lanes 4 and 5). Both mutations did, however, have a significant effect on association with the VP16 activation domain *in vitro* (Fig. 3*A*): The *K297A* mutation reduced the TBP-$VP16_{AD}$ association to approximately 12% of wild-type levels (lane 3), and the *R299A* mutation (lane 4) reduced the association to about 30% of wild-type levels. Thus the *in vitro* association of TBP with the VP16 activation domain is sensitive to either of two point mutations in a single loop of TBP.

We next engineered these mutations into the *H1′+S3/S4* mutant TBP background. Combining the *H1′* and *S3/S4* mutations resulted in a reduction in TBP-$VP16_{AD}$ association to 30% (Fig. 3*A*, lane 5); this level was reduced to approximately 10% by addition of either the *K297A* or *R299A* single mutations (lanes 7 and 8). In contrast, addition of the *K297A* and *R299A* mutations to the *H1′+S3/S4* background had no evident effect on response to GAL4-$VP16_{AD}$ (Fig. 4*B*, compare lanes 8 and 9 with lane 6). Indeed, it was only when both the *K297A* and *R299A* mutations were combined with the

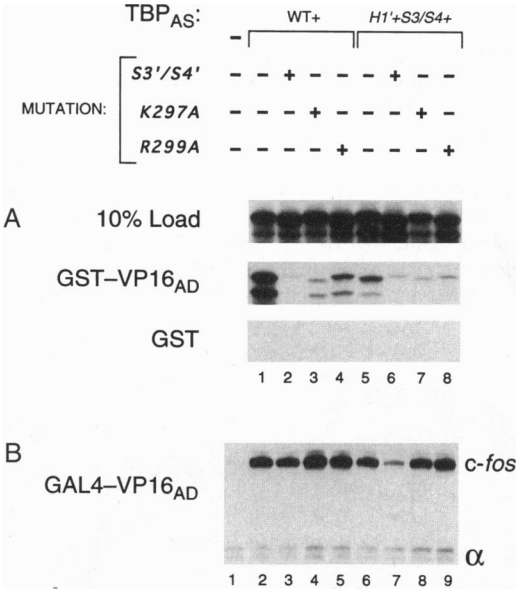

FIG. 3. Single point mutations in TBP discriminate between the effects of reduced TBP–$VP16_{AD}$ association *in vitro* and reduced response to $VP16_{AD}$ *in vivo*. (*A*) GST-pulldown analysis of the effects of the *K297A* and *R299A* mutations on association of TBP_{AS} with GST-$VP16_{AD}$ *in vitro*. These mutations were assayed in the context of either wild-type TBP_{AS} (lanes 2–4) or *H1′+S3/S4* (lanes 6–8) mutant TBP_{AS}. At the top, + indicates the presence of the particular mutation listed on the left and − indicates wild-type TBP sequences at that position. (*B*) *In vivo* analysis of the TBP mutants described in *A*, examining response to GAL4-$VP16_{AD}$. Details of the assay are described in the legend to Fig. 2*B*. GAL4-$VP16_{AD}$ is present in all lanes.

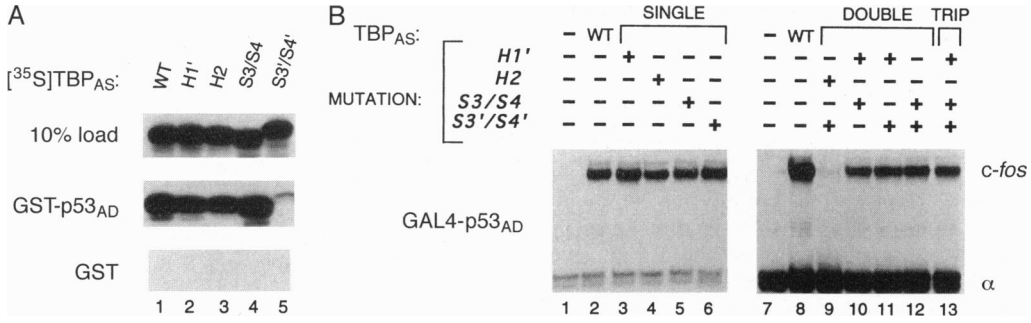

FIG. 4. The *S3'/S4'* mutation disrupts association of TBP with GST-p53$_{AD}$ *in vitro* but not response to GAL4-p53$_{AD}$ *in vivo*. (*A*) GST-pulldown analysis of the effects of the *H1', H2, S3/S4*, and *S3'/S4'* mutations on association of TBP$_{AS}$ with GST-p53$_{AD}$ *in vitro*. Details of the assay are described in the legend to Fig. 2*A*. (*B*) *In vivo* analysis of the effects of single (lanes 1–6) or combined (7–13) sets of mutations in TBP$_{AS}$ on response to GAL4-p53$_{AD}$. GAL4-p53$_{AD}$ is present in all lanes. The positions of correctly initiated c-*fos* and α-globin transcripts are indicated.

H1'+S3/S4 mutations, resulting in the *H1'+S3/S4+S3'/S4'* combination, that a significant reduction in GAL4-VP16$_{AD}$ transactivation was observed (lane 7). The finding that both mutations in the S3'/S4' loop are required for disruption of VP16 activation *in vivo*, whereas each mutation separately is sufficient to disrupt VP16$_{AD}$ association *in vitro*, argues that disruption of the VP16$_{AD}$–TBP association does not contribute to the activator-specific transcriptional defects of the *H1'+S3/S4+S3'/S4'* mutation.

The *S3'/S4'* Mutation Also Disrupts Association of TBP with the Activation Domain of p53 *in Vitro* but Does Not Affect Response to p53 *in Vivo*. We next examined the functional significance of association between TBP and the acidic activation domain from the human tumor suppressor protein p53 (p53$_{AD}$: residues 1–73; ref. 34). We fused the p53 activation domain to both GST and GAL4 sequences and then compared association with TBP *in vitro* with activation *in vivo*, as shown in Fig. 4. Consistent with previous reports (34–37), the p53 activation domain both bound wild-type TBP$_{AS}$ *in vitro* (Fig. 4*A*, lane 1) and activated transcription to high levels *in vivo* (Fig. 4*B*, lane 2). Like transcriptional activation by all activators examined (6), activation by GAL4-p53$_{AD}$ was not significantly affected by any of the single sets of mutations in TBP (Fig. 4*B*, lanes 3–6). This pattern was not true of TBP association *in vitro*, however, because the *S3'/S4'* mutation (Fig. 4*A*, lane 5) disrupted binding of TBP$_{AS}$ to GST-p53$_{AD}$. The same mutation in TBP therefore disrupts association with both the VP16 and p53 activation domains, suggesting that these two activation domains interact with TBP through similar interfaces. Moreover, the TBP–p53$_{AD}$ association can be disrupted without having an impact on transcriptional activation *in vivo*, demonstrating that the ability of the p53 activation domain to interact directly with TBP *in vitro* is not required for transcriptional activation *in vivo*.

To contrast the activity of the acidic p53 activation domain with that of VP16, we examined the response of several multiply mutant forms of TBP to GAL4-p53$_{AD}$ *in vivo*. As observed with all activators (6), combining the *H2* and *S3'/S4'* mutations reduced response to GAL4-p53$_{AD}$ *in vivo* to 10% of wild-type levels (Fig. 4*B*, compare lanes 8 and 9). All other double-mutant TBP molecules, regardless of whether they carried the *S3'/S4'* mutation, were reduced to 50% for response to GAL4-p53$_{AD}$ (compare lane 8 with lanes 10–12). Remarkably, however, the *H1'+S3/S4+S3'/S4'* combination, which is reduced to approximately 10% for response to GAL4-VP16$_{AD}$ (Fig. 3), showed only the same reduction to 50% for response to GAL4-p53$_{AD}$ (Fig. 4*B*, lane 13). This result demonstrates that VP16 and p53, two acidic activators which display many structural and functional similarities—including potency of transactivation, association with TFIIH *in vitro* (13), association with TBP *in vitro*, and sensitivity to the *S3'/S4'* mutation—use TBP in different ways to achieve transcriptional activation *in vivo*.

DISCUSSION

We have investigated the importance of activator–TBP association *in vitro* for transcriptional activation *in vivo*. We found that not only the VP16 activation domain but also the remainder of the protein—which does not activate transcription effectively *in vivo*—associates well with TBP *in vitro*. We also found that mutations in a single loop of TBP disrupt its association with the activation domains of VP16 and p53 but do not affect its ability to support activated transcription *in vivo*. These findings raise the possibility that direct contact of TBP by these activation domains, and perhaps others, is not involved in the transcriptional activation process *in vivo*.

There are many explanations of how the VP16–TBP and p53–TBP interactions could be disrupted *in vitro* without commensurate effects on transcriptional activation *in vivo*. First, the interaction measured between activators and TBP *in vitro* may be irrelevant to transcriptional activation, and instead may reflect forced interactions between two partially compatible proteins under nonphysiological conditions. Mutations in activation domains may therefore disrupt both interaction with TBP *in vitro* and transcriptional activation *in vivo* not because they destroy a specific interaction surface, but rather because they disrupt the overall structural integrity of the activation domain. A second possibility is that binding of the VP16 and p53 activation domains to the *S3'/S4'* mutant TBP, which is defective *in vitro*, may still occur *in vivo* at levels sufficient to achieve transcriptional activation. Third, activators such as VP16 and p53 may be able to activate transcription through multiple pathways, in which case redundancy may mask the effects of disrupting a direct activation domain–TBP interaction. Finally, direct activation domain–TBP interactions may be important for transcriptional activation in promoter contexts other than the one assayed here. For example, in certain contexts, activators may stimulate transcription by interacting directly with the S3'/S4' loop of TBP that we have shown is critical for interaction with activation domains *in vitro*.

An alternative possibility, however, that would also explain how activators such as VP16 can interact with many different basal factors (reviewed in ref. 1) is that a structure similar to the S3'/S4' loop of TBP is shared among the basal factors. In this model, the association of free TBP with activation domains does not reflect an extant transcriptional process, but rather is a consequence of how the basal transcriptional machinery evolved.

For example, during evolution, genes encoding basal factors may have arisen by duplication, resulting in common structural motifs being shared among the basal factors. Perhaps TBP—an ancient basal factor (38)—once not only was involved in

promoter recognition (i.e., through the TATA box) but also was a direct target for transcriptional activators. Subsequently, gene duplication may have allowed some TBP functions (e.g., direct activator interaction) to be performed by separate basal factors, which interact with activators but do not recognize promoters directly. Through evolution, therefore, TBP may no longer be generally a direct target of transcriptional activators, but instead has become incorporated into multiprotein complexes such as TFIID (39) in which TAFs are the direct targets.

We suggest that it is the interaction between TBP and the largest human TAF, $hTAF_{II}250$, that both preserves the ability of TBP to interact with activation domains *in vitro* and blocks the direct activator–TBP interaction *in vivo*. TBP interacts with $hTAF_{II}250$ at multiple points across its surface, including the S3′/S4′ loop (6). The *S3′/S4′* mutation alone, however, is not sufficient to disrupt either the TBP–$hTAF_{II}250$ interaction *in vitro* or transcriptional activation *in vivo*; disruption of both these activities requires additional mutations in other regions of TBP (6). The structure of the S3′/S4′ loop of TBP may thus be conserved not for interaction with activators but rather for interaction with factors such as $hTAF_{II}250$, which subsequently blocks activators from interacting directly with TBP by masking the S3′/S4′ loop.

In summary, the idea that a common structural motif in basal factors is involved in the protein–protein interactions that regulate transcription provides an explanation of why so many transcription factors can interact directly with TBP and with other basal factors as well. Additionally, the model reinforces the importance of examining activator–basal factor interactions from the point of view of both partners. If common or related motifs are used in activator–target interactions, then examination of the effects of mutations only in activation domains is not sufficient to determine which of the multitude of interactions demonstrated *in vitro* are directly relevant to transcriptional activation *in vivo*.

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