MOLECULAR AND CELLULAR BIOLOGY, Mar. 1997, p. 1336–1345 Vol. 17, No. 3
0270-7306/97/$04.00+0
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A Severely Defective TATA-Binding Protein–TFIIB Interaction Does Not Preclude Transcriptional Activation In Vivo

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Received 26 September 1996/Returned for modification 3 December 1996/Accepted 17 December 1996

In yeast cells, mutations in the TATA-binding protein (TBP) that disrupt the interaction with the TATA element or with TFIIA can selectively impair the response to acidic activator proteins. We analyzed the transcriptional properties of TBP derivatives in which residues that directly interact with TFIIB were replaced by alanines. Surprisingly, a derivative with a 50-fold defect in TBP-TFIIB-TATA complex formation in vitro (E188A) supports viability and responds efficiently to activators in vivo. The E186A derivative, which displays a 100-fold defect in TBP-TFIIB-TATA complex formation, does not support viability, yet it does respond to activators. Conversely, the L189A mutation, which has the mildest effect on the interaction with TFIIB (10-fold), can abolish transcriptional activation and cell viability when combined with mutations on the DNA-binding surface. This "synthetic lethal" effect is not observed with E188A, suggesting that the previously described role of L189 in transcriptional activation may be related to its location on the DNA-binding surface and not to its interaction with TFIIB. Finally, when using TBP mutants defective on multiple interaction surfaces, we observed synthetic lethal effects between mutations on the TFIIA and TFIIB interfaces but found that mutations implicated in association with polymerase II and TFIIF did not have significant effects in vivo. Taken together, these results argue that, unlike the TBP-TATA and TBP-TFIIA interactions, the TBP-TFIIB interaction is not generally limiting for transcriptional activation in vivo.

Biochemical studies over the last 15 years have established that accurate transcriptional initiation by RNA polymerase (Pol) II requires its assembly with general transcription factors TFIIA, -B, -D, -E, -F, and -H (3, 57). Assembly of these factors into this preinitiation complex is nucleated by the binding of TFIID (or its core subunit the TATA-binding protein [TBP]) to promoter DNA. Gene-specific transcriptional activators can, in principle, facilitate one or more steps in the assembly process, stimulate preinitiation complex formation, and thereby mediate elevated levels of transcription (5, 47, 51, 57). Since the TFIID-DNA complex provides the platform for subsequent accretion of the other factors, activators that target TFIID (or TBP) could potentially exert their effects by accelerating this first step in the assembly pathway. In concordance with this hypothesis, many activators have been shown to contact TBP or TBP-associated factors (TAFs) directly in vitro. Furthermore, activators can stimulate the formation of a complex containing TFIID and TFIIA bound at the TATA element (7, 34, 54).

A number of biochemical studies have identified TFIIB as another likely target for transcriptional activators. TFIIB interacts directly, in vitro, with the activation domains of many different regulatory proteins. Moreover, mutants of TFIIB that are impaired for activator-TFIIB interactions are also defective for activated but not basal transcription in vitro (35, 40, 41). Conversely, mutations that cripple the activating function of VP16 also eliminate interaction of VP16 with TFIIB (36). Thus, under circumstances in which TFIIB is limiting for complex assembly, recruitment of TFIIB by direct association with activators bound to promoter DNA could serve to increase transcription rates by enhancing preinitiation complex formation. In theory, this mechanism of activator function could be generalized for the other general factors, and activators can directly contact nearly all of the other components of the preinitiation complex (47, 57). The physiological relevance of these protein-protein interactions, however, remains unclear.

In vivo, recruitment of TBP to promoter DNA has been implicated as a critical step in transcriptional activation (46). An acidic activation domain can accelerate the association of TBP with the promoter (27). Artificial recruitment of TBP via a heterologous DNA-binding domain can bypass the need for an activation domain (6, 26, 56). Finally, TBP mutations that selectively impair transcriptional activation by acidic activators in vivo drastically reduce the interaction with TATA elements (1, 31) or with TFIIA (43), a protein that can stabilize the TBP-TATA complex in vitro (30). The role of TFIIB and other general factors in transcriptional activation under physiological conditions is poorly understood. However, on transiently transfected promoters, overexpression of a truncated, nonfunctional version of human TFIIB can squelch activation by a glutamine-rich, but not an acidic, activation domain (8, 9).

Based on the X-ray crystallographic structures of TBP bound to the TATA element (22, 24), a systematic alanine-scanning mutagenesis of the solvent-exposed surface of human TBP was performed (50). By mutating only solvent-exposed residues of the DNA-bound form of TBP, this analysis was designed to identify protein-protein contacts between TBP and other general transcription factors. Biochemical analysis of 81 single-alanine-substituted mutants of human TBP revealed eight mutations that specifically block the ordered assembly of preinitiation complexes at the entry points for TFIIA, TFIIB, TFIIF, and RNA Pol II (50). These mutants are useful for studying preinitiation complex assembly because they selectively disrupt TBP interactions with individual general factors without significantly altering the structure of the TBP-TATA complex.

To investigate the physiological consequences of selective disruption of TBP interactions with general transcription fac-

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TABLE 1. Human and yeast TBP residues which interact with general transcription factors

Interaction surface	Mutated residue	
	Human TBP	Yeast TBP
TFIIA	Glu-228 (E228A)	Asp-130 (D130A)
	Arg-235 (R235A)	Arg-137 (R137A)
TFIIB	Glu-284 (E284A)	Glu-186 (E186A)
	Glu-286 (E286A)	Glu-188 (E188A)
	Leu-287 (L287A)	Leu-189 (L189A)
TFIIF	Glu-320 (E320A)	Glu-222 (E222A)
Pol II	Glu-206 (E206A)	Glu-108 (E108A)
	Leu-232 (L232A)	Leu-134 (L134A)

tors, we characterized yeast TBP mutants with analogous alanine substitutions. We predicted that these yeast TBP mutants would exhibit the same biochemical defects as their human counterparts. Yeast and human TBPs are greater than 80% identical in the core domain, and almost all of the relevant residues are conserved (Table 1). Moreover, yeast and human TBPs are functionally interchangeable for basal and activated transcription in vitro (21, 55) and in vivo (11, 20, 45), suggesting that TBP interfaces with general transcription factors have been evolutionarily conserved. Our results suggest that, unlike the TBP-TATA and TBP-TFIIA interactions, the TBP-TFIIB interaction is not generally limiting for transcriptional initiation in vivo.

MATERIALS AND METHODS

Yeast strains. Strains containing mutant TBP derivatives as the sole source of TBP were obtained by plasmid shuffling starting with the parental strain BYΔ2 (10). Phenotypic analysis of altered-specificity (m3) derivatives of TBP (45) were carried out with strains yML2 and yML3 (31). Activation assays for LexA hybrid proteins in mutant TBP strains was performed with strain LC1011, a derivative of BYΔ2 carrying a *his3* allele in which the Gcn4-binding site has been replaced with a single LexA operator (52). LexA-TBP fusions were tested by transformation as described previously (6).

Construction of TBP mutants. Mutations for each of the eight alanine substitutions described in Table 1 were constructed by site-directed PCR mutagenesis either in an otherwise wild-type TBP gene or in a derivative that contains an *Eco*RI restriction site 11 bp upstream of the translation start site of TBP. Single mutants were combined either by subcloning or by site-directed PCR mutagenesis. Mutations were introduced into the altered-specificity TBP context (TBP^{m3}) by subcloning or by PCR with TBP^{m3} (45) as the template. Fusion of the E186A, E188A, and L189A TBP derivatives to the DNA-binding domain of LexA was achieved by swapping the *Bgl*II-*Bam*HI fragment within the TBP gene of LexA-TBP (6).

Phenotypic analyses. The ability of TBP derivatives to support cell growth was assessed by a plasmid-shuffling assay involving growth in 5-fluoro-orotic acid (10). Methods used to assess growth phenotypes (31, 45); measure Gal4-dependent activation by using the YCp86-Sc3801 reporter (42); quantitate RNA levels by S1 nuclease protection of oligonucleotide probes for *his3*, *ded1*, *tRNA^w*, *trp3*, and *rps4* (12, 18, 19); and determine intracellular TBP levels by Western blot analysis (31) have been described previously.

Protein purification. To generate polyhistidine-tagged versions, the TBP derivatives were subcloned into pET15b between the *Nde*I and *Bam*HI restriction sites as described previously for wild-type yeast TBP (31). Proteins were expressed in *Escherichia coli* BLR (BL21 *recA* DE3/pLYS-S) and purified first by nickel affinity column chromatography in accordance with the manufacturer's (Novagen) instructions. Peak fractions were pooled, diluted 20-fold with buffer T (10 mM Tris-acetate [pH 7.9], 1 mM EDTA, 10% glycerol, 100 mM potassium acetate), and batch loaded onto pre-equilibrated SP Sepharose (Pharmacia). After washing with 50 column volumes of buffer T, the resin was poured into a column and proteins were eluted with buffer T containing 500 mM potassium acetate. Yeast TFIIB was purified with the same protocol. Proteins thus purified were quantitated by Bradford assay (Bio-Rad) and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; proteins were estimated to be greater than 80% pure by Coomassie blue staining of overloaded gels. Yeast TFIIA was purified by using a protocol obtained from Steven Hahn, in which the individual subunits (Toa1 and Toa2) were expressed separately in *E. coli*. Briefly, bacterial cells were lysed by sonication and clarified by centrifugation and the supernatants were discarded. The remaining pellets were washed once with buffer A (30 mM Tris-Cl [pH 8.0], 2 mM EDTA, 500 mM KCl, 5 mM dithiothreitol, 10% glycerol), repelleted, and resuspended slowly in buffer A containing 7 M urea. After centrifugation to remove urea-insoluble material, the solutions of Toa1 and Toa2 were mixed slowly, diluted to 0.4 mg/ml, and allowed to dialyze overnight at 4°C against buffer B (30 mM Tris-Cl [pH 8.0], 2 mM EDTA, 150 mM KCl, 1 mM dithiothreitol, 10% glycerol). This renatured TFIIA was loaded onto a Q Sepharose column, washed with 3 column volumes of buffer B containing 200 mM KCl, and eluted with buffer B containing 350 mM KCl. Peak fractions were pooled, diluted to 150 mM KCl, and run on a Superose 12 fast protein liquid chromatography column equilibrated with modified buffer B (0 mM KCl, 0.2 M ammonium sulfate). The peak fractions were pooled, aliquotted, and frozen at −70°C.

Gel mobility shift assays. Gel mobility shift assays were performed with 10 μl reaction mixtures containing 0.7× buffer T containing 6 μg of bovine serum albumin, 300 μg of dG-dC, 7 mM magnesium acetate, and a 0.5 nM concentration of a ^{32}P-labeled DNA fragment containing the E1B TATA element. Binding reaction mixtures were incubated at room temperature for 20 min prior to electrophoresis. For TFIIA and TFIIB assembly experiments, binding conditions were the same as those used to assess DNA binding. Binding reaction mixtures were electrophoresed in either 4 or 6% 1× Tris-glycine-EDTA gels in the presence of 3 mM magnesium acetate (for DNA binding by TBP) or in its absence (for TFIIA and TFIIB assembly). Gels were run at room temperature, except for TFIIA assembly experiments, for which they were run at 4°C.

RESULTS

Yeast analogs of human TBP mutants that selectively impair interactions with general transcription factors. Based on the human TBP mutants that are specifically defective for interactions with TFIIA, TFIIB, Pol II, and TFIIF (50), we generated yeast TBP derivatives with the corresponding mutations (Table 1). To confirm that these yeast TBP mutants are, in fact, defective for their predicted interactions, polyhistidine-tagged derivatives were expressed in bacteria and purified by nickel affinity column chromatography. These proteins were tested for the ability to bind TATA elements and to form TBP-TFIIA-TATA and TBP-TFIIB-TATA complexes (Fig. 1A).

All three putative TFIIB interaction mutants (E186A, E188A, and L189A) dramatically reduce the TBP-TFIIB interaction, whereas they do not significantly affect TATA element binding or the interaction with TFIIA. L189A was the least affected (~10-fold), followed by E188A (~50-fold), and E186A was the most compromised (~100 fold) (Fig. 1B). These results are qualitatively and quantitatively similar to those previously reported for human TBP derivatives with alanine substitutions at the corresponding positions (50). Thus, the TBP-TFIIB interface is conserved between yeast and human.

Surprisingly, a TBP derivative with two mutations on the putative TFIIA interaction surface (D130A and R137A) is only slightly defective (three- to fivefold) for association with TFIIA (Fig. 1A and C). By contrast, single mutations at the corresponding positions in human TBP result in 10- and 20-fold defects in association with human TFIIA, respectively (50). Although unexpected at the time when this experiment was performed, this result is consistent with a more recent biochemical analysis (2) and with the crystal structure of the yeast TBP-TFIIA-DNA complex, in which positions D130 and R137 do not make any direct contacts with the essential domains of yeast TFIIA (14, 49). Thus, the TFIIA interaction surfaces for yeast and human TBPs are not conserved at the mutational level (see Discussion). Mutations on the putative surfaces for interaction with Pol II (E108A and L134A) or TFIIF (E222A) have little or no effect on the association with TATA elements, TFIIA, or TFIIB (Fig. 1A). However, conditions for the formation of higher-order complexes containing yeast Pol II or TFIIF have not been described, and our attempts in this regard were unsuccessful.

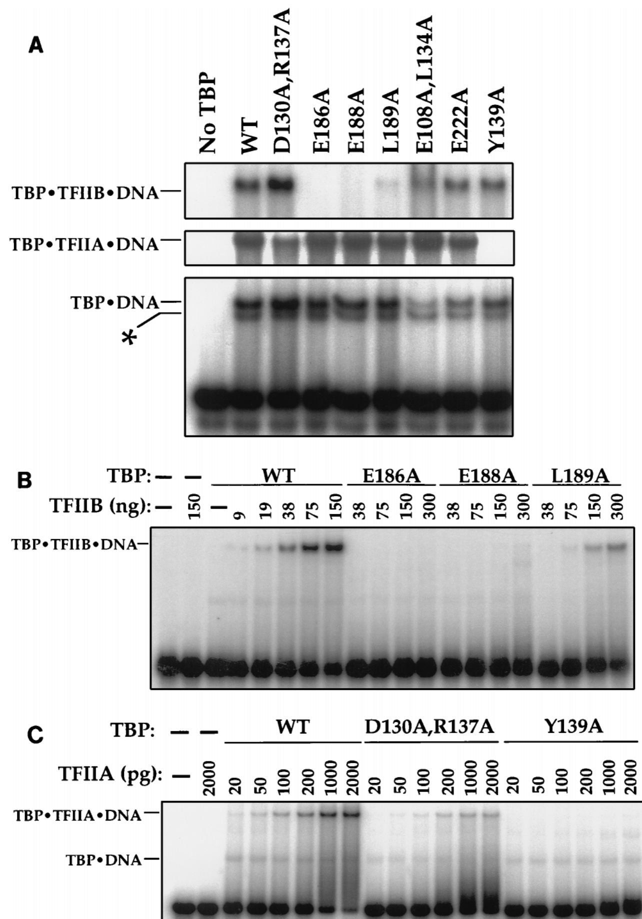

FIG. 1. In vitro analysis of TBP mutants. (A) DNA binding and TFIIA and TFIIB assembly. For DNA-binding assays (bottom), 7.5 ng of the indicated TBPs was tested. Subsequent assembly experiments were performed with amounts of each TBP derivative normalized to the DNA-binding activity equivalent to 7.5 (for TFIIB assembly) or 15 (for TFIIA assembly) ng of wild-type (WT) TBP. To assess TFIIA association (middle), normalized TBPs were incubated with 1 ng of purified yeast TFIIA. For TFIIB assembly (top), normalized TBPs were incubated with 75 ng of purified yeast TFIIB. The bands indicated by the asterisk probably represent binding of TBPs to a shorter species of probe present in the reaction mixtures. (B) Quantitation of the TFIIB interaction defect of E186A, E188A, and L189A. TBPs (normalized for TATA-binding activity) were incubated in binding reactions with the indicated amount of TFIIB. From PhosphorImager analysis, TFIIB interaction was estimated to be compromised approximately 10-fold for L189A, 50-fold for E188A, and 100-fold for E186A. (C) Quantitation of the TFIIA association defect of D130A, R137A, and Y139A. TBPs (normalized for TATA-binding activity) were incubated in binding reactions with the indicated amounts of purified yeast TFIIA. TFIIA interaction was estimated to be compromised 3 to 5-fold for D130A-R137A and 100-fold for Y139A.

TFIIB interaction surface of TBP is essential for viability. TBP derivatives containing the above alanine substitutions were expressed at physiological levels (i.e., from the natural TBP promoter on a single-copy plasmid) and tested for the ability to support life by the plasmid shuffle assay (Fig. 2 and Table 2). Mutations on the putative TFIIF (E222A) or Pol II surfaces (E108A, L134A, or both) result in behavior similar to that of wild-type TBP. None of these derivatives show significant growth deficiencies at 37°C or on media lacking inositol or containing only galactose as a carbon source; mutant phenotypes for growth on these media are often associated with defects in Pol II transcription in yeast (1, 39). In accord with our biochemical analysis, mutations initially thought to lie on the TFIIA interface behave indistinguishably from wild-type TBP for growth on media lacking inositol or containing galactose. However, TBP derivatives with the R137A mutation are temperature sensitive, presumably because this residue is important for Pol III transcription (13).

In contrast to these observations, the TBP derivative with the most severe TFIIB interaction defect in vitro (E186A) is unable to support cell growth. Interestingly, cells containing E188A, which has a 50-fold defect for interaction with TFIIB, grow at near normal rates at 30°C; however, they fail to grow at 37°C. Thus, E188A and E186A define the critical range of TBP-TFIIB interaction that is necessary for yeast cell viability. The L189A derivative, which displays the mildest defect for interaction with TFIIB in vitro, behaves indistinguishably from wild-type TBP in vivo. However, the double mutant containing E188A and L189A, both of which confer near wild-type growth as single mutants at 30°C, is inviable, suggesting a synergy of defects between these mutations on the TFIIB interaction surface. Overexpression of TFIIB is unable to rescue either E186A or E188A-L189A (data not shown).

The observation that the severity of mutant phenotypes in vivo parallels the ordering of TFIIB affinities in vitro provides strong evidence that the inviability of E186A is a direct consequence of a weakened TBP-TFIIB interaction. Nevertheless, it is important to exclude the possibility that cell inviability is due to deficiencies in protein stability or to transcriptional defects unrelated to the TBP-TFIIB interaction. Protein stability of selected TFIIB interaction mutants was assayed in a strain supported by a hemagglutinin (HA)-tagged TBP. Protein levels of unstable TBP mutants (e.g., ts1) (10) are often maintained at wild-type levels (due to extra plasmid copies or some other adaptive mechanism) when such TBP mutants are

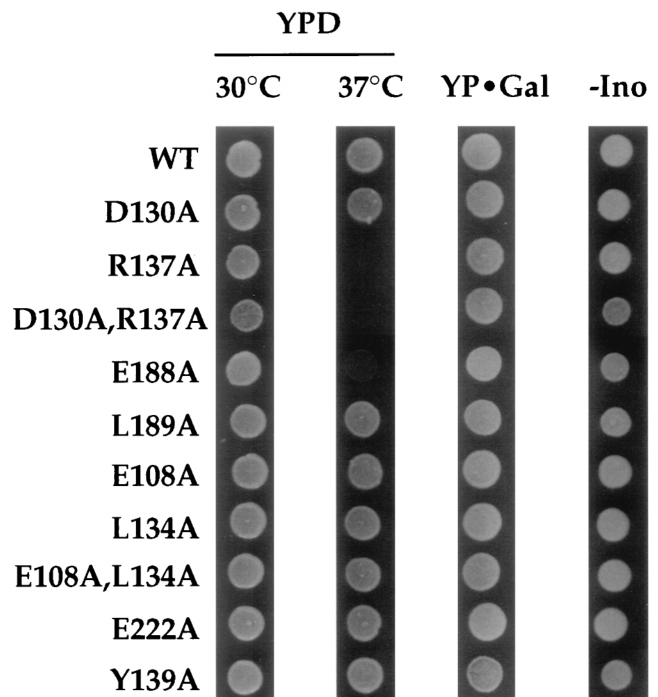

FIG. 2. Growth phenotypes of strains supported by mutant TBPs. Cells (10^4) of strains carrying the indicated TBP derivatives as the sole source of TBP were spotted onto YPD (incubated at 30 and 37°C), YP-galactose (YP · Gal) (30°C), and glucose synthetic complete medium lacking inositol (−Ino) (30°C). For reasons we do not understand, we have observed variability in the growth of strains supported by E188A and L189A on YP-galactose. WT, wild type.

TABLE 2. Growth phenotypes of TBP mutants defective for interactions with general transcription factors

Mutated interaction surface	TBP mutant	Level of growth[a]			
		At 30°C	At 37°C	On YP-galactose	Without inositol
TFIIA	D130A	++++	++++	++++	++++
	R137A	++	−	+++	++
	D130A-R137A	++	−	+++	++
TFIIB	E186A	−	NA	NA	NA
	E188A	+++	−	+++	++
	L189A	++++	++	+++	+++
	E186A-E188A	−	NA	NA	NA
	E186A-L189A	−	NA	NA	NA
	E188A-L189A	−	NA	NA	NA
	E186A-E188A-L189A	−	NA	NA	NA
TFIIF	E222A	+++	+++	+++	+++
Pol II	E108A	++++	++++	++++	++++
	L134A	++++	++++	++++	++++
	E108A-L134A	++++	++++	++++	++++

[a] NA, not assayed.

the sole source of TBP in yeast (Fig. 3A, top). In the presence of HA-TBP, which fully supports viability, such adaptive mechanisms are absent, and TBP derivatives are expressed at steady-state levels reflective of their inherent stability.

In this assay, both E186A and E188A-L189A exhibit protein stability properties indistinguishable from those of wild-type TBP (Fig. 3A, bottom panel). Viable TFIIB interaction mutants E188A and L189A also show near wild-type stability; importantly, E188A protein levels are not reduced at 37°C, suggesting that the temperature-sensitive phenotype of strains supported by E188A is the result of a functional defect in TBP-TFIIB interaction. In addition, the inviable TFIIB-interaction mutants complement both the temperature sensitivity of a TBP mutant defective for transcription by RNA Pol III (11, 13) (Fig. 3B) and the inviability of human TBP (data not shown), which is defective primarily for Pol I and Pol III transcription in yeast (11). Taken together, these experiments indicate that the TBP derivatives are properly folded and can support Pol I and Pol III transcription in vivo.

TFIIB interaction mutants are not defective for activation. The Gal^+ and Ino^+ phenotypes conferred by the TBP mutants in this study suggest that these derivatives are not generally defective for the response to activators. We further tested this hypothesis by quantitating the activity of these TBP mutants on a sensitive, Gal4-dependent promoter. Although activation-deficient TBP mutants isolated in our laboratory are severely compromised in this assay (1 to 15% of wild-type activity) (31, 43), all of the single-surface interaction mutants exhibit activities greater than 50% of that of the wild-type TBP control (Fig. 4A). Thus, E188A, which severely reduces TBP-TFIIB-TATA complex formation in vitro, is comparable to wild-type TBP at supporting Gal4-dependent activation in vivo. Furthermore, none of these TBP mutants are affected for constitutive transcription from TATA-containing Pol II (*his3* and *rps4*), TATA-less Pol II (*trp3*), and Pol III (*tRNA*^{w}) promoters (Fig. 4B).

Strains supported by the E188A and L189A derivatives were also tested for transcriptional activation on a minimal *his3* promoter containing a single LexA operator (52). In this context, the chimeric activator LexA-Gcn4 activates transcription to comparable extents in wild-type and mutant TBP strains (Fig. 5A). Artificial recruitment of TFIIB by fusion to the LexA DNA-binding domain activates transcription, albeit at a level significantly lower than that seen for LexA-TBP (unpublished data). As with LexA-Gcn4, LexA-TFIIB activation is unimpaired in the strains supported by the TFIIB interaction mutants (Fig. 5A).

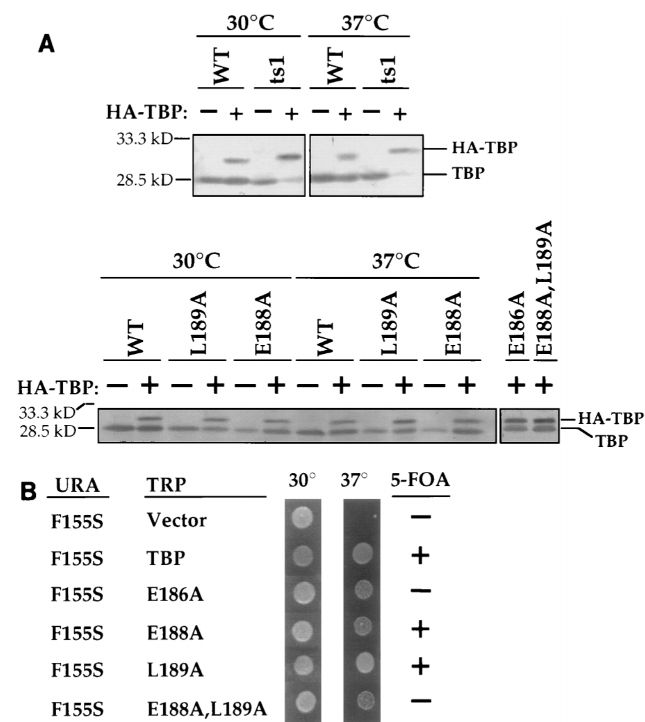

FIG. 3. Stability of TFIIB interaction mutants. (A) Western blots with polyclonal antibody to TBP on protein extracts prepared from strains carrying the indicated TBP derivatives and, where indicated, an HA-tagged wild-type (WT) TBP (HA-TBP). The HA tag adds approximately 1.5 kDa to the size of TBP. (B) Complementation of the temperature-sensitive phenotype of a Pol III-defective TBP (11). Strains containing TBP-F155S, a Pol III-specific TBP mutant, and the indicated TBP derivative were spotted on glucose-Casamino Acids medium lacking uracil and tryptophan. Complementation was scored by growth of a spot at 37°C. The column on the right indicates the ability of TBP derivatives carried on the *TRP1*-marked plasmids to complement a TBP null allele (assayed by growth on 5-fluoro-orotic acid [5-FOA]) in a plasmid-shuffling assay (10).

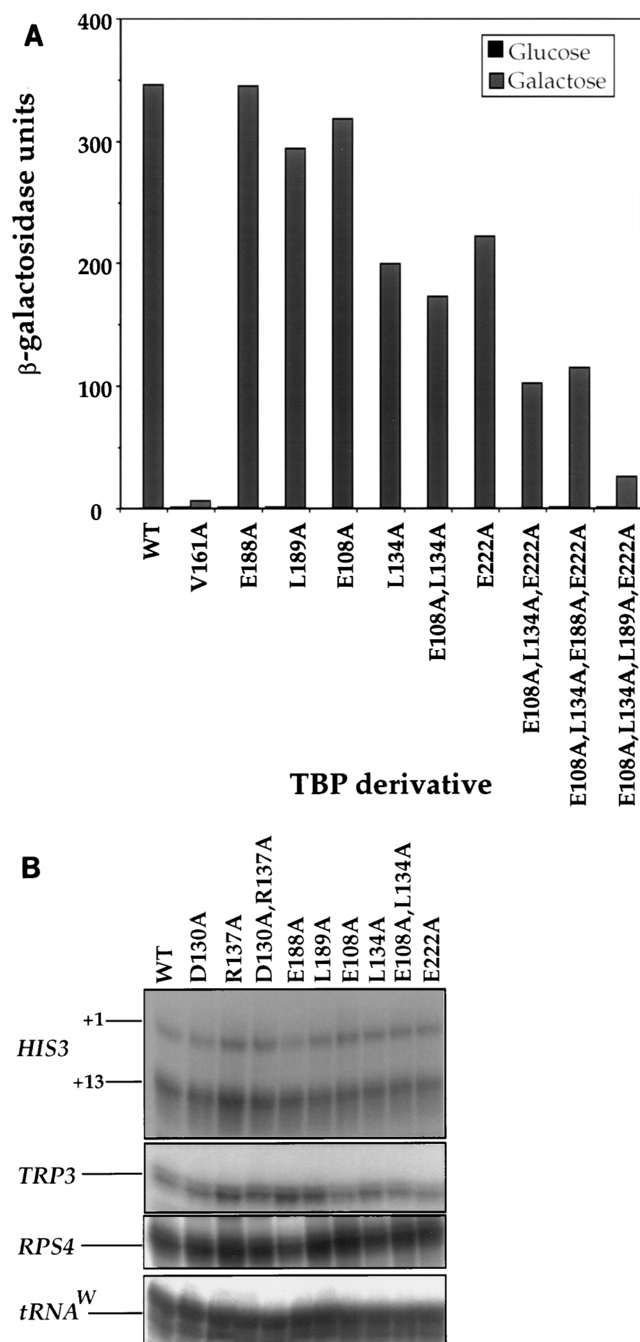

FIG. 4. Constitutive and Gal4-activated transcription is not compromised in TBP mutant strains. (A) Gal4-dependent activation in strains supported by the indicated TBP derivatives. V161A is an activation-deficient TBP mutant described previously (31). All strains were induced overnight in galactose medium. (B) RNA levels of selected genes in strains supported by the indicated TBP derivatives. PhosphorImager analysis indicates that band intensities corresponding to the indicated RNA species do not vary more than 30% from strain to strain. WT, wild type.

We were unable to test the TFIIB interaction mutant E186A in the Gal4-dependent activation assay because of its inability to support cell growth. To circumvent this problem, the TFIIB interaction mutants were introduced into the altered-specificity TBP (TBP^{m3}) context (45). Because TBP^{m3} derivatives can function on promoters containing the mutant TATA element TGTAAA, their transcriptional properties can be assayed in a strain supported by wild-type TBP (which does not function at TGTAAA-containing promoters). In this assay, E188A^{m3} functions on the nonactivated and Gcn4-activated promoters at levels comparable to that of parental TBP^{m3} (Fig. 5B). E186A^{m3} exhibits slightly lower activity than wild-type TBP on the nonactivated reporter, in a manner similar to that of

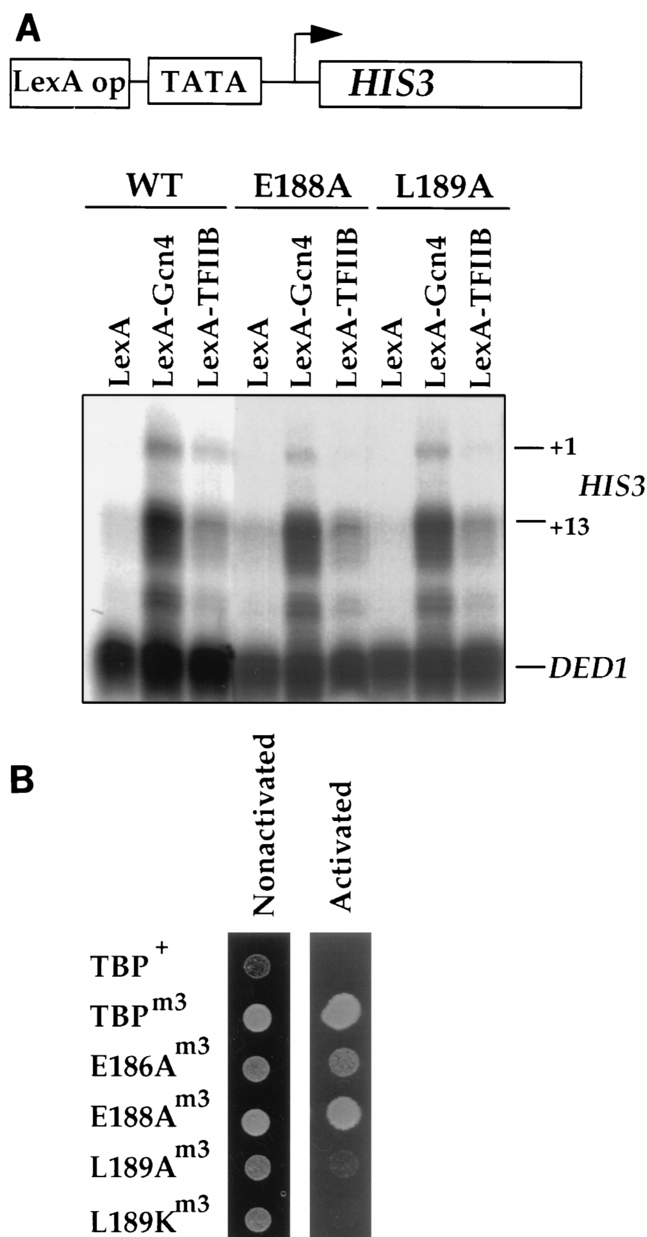

FIG. 5. TFIIB interaction mutants are not deficient for transcriptional activation. (A) *his3* RNA levels in strains containing the indicated LexA and TBP derivatives, as well as a promoter containing a single LexA operator upstream of the *his3* TATA element and structural gene. *ded1*, a constitutively transcribed gene, serves as an internal control. (B) Altered-specificity TBP assay for nonactivated and Gcn4-activated transcription. Cells (10^4) from strains carrying the indicated TBP derivatives and TGTAAA *his3* alleles with (activated) or without (nonactivated) a Gcn4-binding site were spotted on glucose minimal medium lacking histidine and supplemented with either 0.5 (nonactivated) or 10 (activated) mM 3-aminotriazole. All of these strains carry YCp88-Gcn4, a plasmid constitutively expressing Gcn4 (16). WT, wild type.

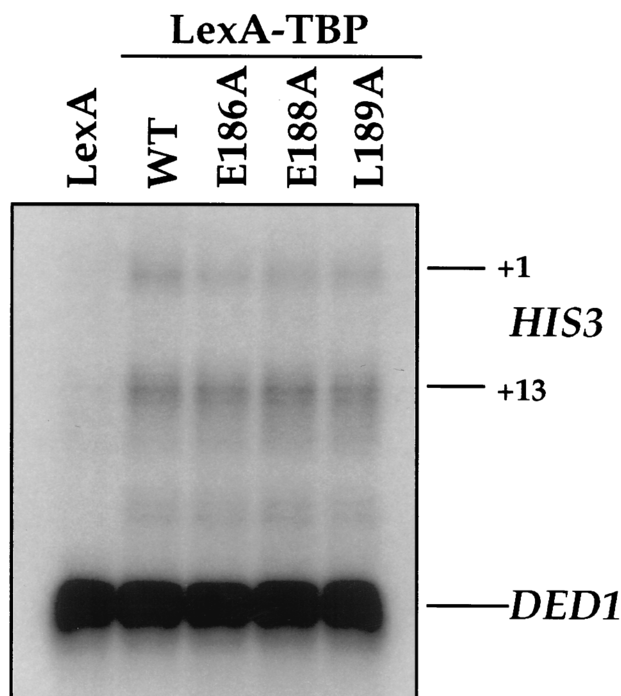

FIG. 6. Artificial recruitment of TFIIB interaction-deficient TBP mutants suffices for transcriptional activation. Strains containing the minimal *his3* promoter with a single LexA operator and the indicated LexA-TBP hybrid proteins were analyzed for *his3* transcription. PhosphorImager analysis indicates that *his3* RNA levels do not vary more than 20% from strain to strain. WT, wild type.

$L189A^{m3}$ and $L189K^{m3}$ (31). However, unlike those L189 mutants, $E186A^{m3}$ supports transcription from the activated reporter (Fig. 5B). Thus, the inviability of strains carrying E186A as the sole source of TBP is not due to a general defect in the response to acidic activators.

Artificial recruitment of TFIIB interaction mutants suffices for transcriptional activation. The above results do not exclude the possibility that a direct interaction between activators and TFIIB can bypass the requirement for a stable TBP-TFIIB interaction. To address this possibility, we artificially recruited the TBP derivatives to promoter DNA via fusion to a heterologous DNA-binding domain (LexA). LexA-TBP activates transcription in the absence of an activation domain when assayed on a minimal *his3* promoter containing a single LexA operator (6). Thus, if an activation domain-TFIIB association is necessary to circumvent the weakened TBP-TFIIB interaction in E188A and L189A, artificial recruitment of these mutant TBPs should not result in activated transcription. However, in contrast to some activation-defective TBP mutants (44), the TFIIB interaction mutants activate transcription when artificially recruited to this promoter (Fig. 6). This observation indicates that activator recruitment of TFIIB is unnecessary for activation even when the TBP-TFIIB interaction is compromised.

"Synthetic lethal" interactions between L189A and mutations on the DNA-binding surface of TBP. In contrast to $E186A^{m3}$ and $E188A^{m3}$, the $L189A^{m3}$ derivative is unable to support Gcn4-dependent activation although it functions at the nonactivated promoter (Fig. 5B). This result is surprising because L189A confers the mildest effects on the TFIIB interaction in vitro and cell viability in vivo. However, the activation-defective phenotype conferred by L189A is similar to that previously described for L189K and other TBP derivatives with mutations on the DNA-binding surface (31). L189, unlike the other TFIIB interaction residues, E186 and E188, directly contacts DNA (22, 24), and it is in proximity to the mutated positions in TBP^{m3} (residues 195, 203, and 205) that are involved in TATA element interactions and that confer altered DNA-binding specificity. For these reasons, we hypothesized that the inability of $L189A^{m3}$ to support Gcn4 activation might reflect a synthetic lethal interaction of mutations on the DNA-binding surface of TBP.

To test this hypothesis, we constructed double mutants in which a viable TFIIB interaction mutation (E188A or L189A) was paired with V161A, a mutation on the DNA-binding surface that supports normal cell growth but is defective in the response to acidic activators (31). In accord with the pattern observed for E188A and L189A with TBP^{m3}, strains supported solely by the double mutant V161A-E188A grow well, whereas corresponding strains supported by the double mutant V161A-L189A are inviable (see Table 4). Thus, L189A, but not E188A, can abolish transcriptional activation or cell viability when combined with mutations on the DNA-binding surface even though L189A is less defective for the interaction with TFIIB. These observations suggest that the physiological role of L189A is related more to its location on the DNA-binding surface than to its interaction with TFIIB.

Functional relationships between TBP interaction surfaces. To address the possibility of functional relationships between general transcription factors, we analyzed the phenotypes of TBP derivatives carrying mutations on multiple interaction surfaces. Such multiple-surface mutants might reveal functional redundancy that masked transcriptional phenotypes in the single-surface mutants. We initially tested combinations of mutants defective for the TFIIB-, TFIIF-, and RNA Pol II interactions (Table 3 and Fig. 4A). None of these multiple-surface mutants are significantly distinct from the single-surface mutants from which they were derived. In all cases, the combination of mutations on two surfaces results in a mutant with the phenotype of the more severe single-surface mutant. Interestingly, the quadruple mutant E108A-L134A-L189A-E222A, which affects the Pol II, TFIIB, and TFIIF surfaces, is somewhat compromised for activation on the Gal4-dependent promoter (Fig. 4A). However, the corresponding quadruple mutant E108A-L134A-E188A-E222A, which carries the more severely compromising TFIIB interaction mutation, does not exhibit this same activation defect.

To analyze the role of TFIIA, we used a TFIIA interaction mutant of TBP (Y139A) affected for this interaction by approximately 100-fold (Fig. 1C). Although this residue is not surface exposed, it plays a critical role in positioning the residues in TBP which make direct contacts with TFIIA (14, 49). In vivo, the Y139A derivative supports normal cell growth and shows a ninefold reduction in Gal4-dependent activation (43). Combination of Y139A with either E222A (TFIIF interaction) or E108A (RNA Pol II interaction) yields no dramatic phenotypes (data not shown). On the other hand, whereas the Y139A-L189A double mutant (10-fold TFIIB interaction) is viable, the Y139A-E188A double mutant (50-fold TFIIB interaction) is inviable (Table 4). We confirmed the protein stability of the Y139A-E188A double mutant and its ability to complement the temperature sensitivity of a Pol III-specific TBP mutant as described above (data not shown).

Thus, under conditions of a normal TBP-TFIIA association, the TBP-TFIIB interaction can be weakened to the level defined by E188A without significant effects on viability at 30°C. However, weakening of the TBP-TFIIA interaction (via mutation to Y139A), heightens the stringency for the TBP-TFIIB interaction. In contrast, weakening of the TBP-TATA interac-

TABLE 3. Growth phenotypes of TBP mutants with multiple interaction surface mutations

Mutated interaction surfaces	TBP mutant	Level of growth			
		At 30°C	At 37°C	On YP-galactose	Without inositol
IIB, Pol	E108A-E188A	+++	−	+++	++
	L134A-E188A	+++	−	++	++
	E108A-L134A-E188A	+++	−	++	++
	E108A-L189A	++++	++	+++	+++
	L134A-L189A	++++	++	+++	+++
	E108A-L134A-L189A	++++	++	+++	+++
IIB, IIF	E188A-E222A	+++	+	+++	++
	L189A-E222A	+++	+	+++	+++
Pol, IIF	E108A-E222A	+++	+++	++++	++++
	L134A-E222A	+++	+++	++++	++++
	E108A-L134A-E222A	+++	+++	++++	++++
IIB, Pol, IIF	E188A-E108A-L134A-E222A	+++	−	+++	++
	L189A-E108A-L134A-E222A	++++	+	+++	+++

tion (via mutation to V161A) does not lead to a synthetic lethal interaction with E188A even though V161A has a more pronounced effect on cell growth and Gal4 activation than does Y139A.

DISCUSSION

The TBP-TFIIB interaction is not generally limiting for transcriptional activation in vivo. In yeast cells, TFIIB is generally required for Pol II transcription, because TFIIB depletion results in decreased levels of all of the mRNAs examined (37). By analyzing the properties of TFIIB interaction mutants of yeast TBP under physiological conditions, we demonstrate that the TBP-TFIIB interaction is essential for yeast cell growth. However, several lines of evidence suggest that the TBP-TFIIB interaction is not generally limiting for transcriptional activation.

First, the E188A derivative is dramatically affected in its ability to form TBP-TFIIB-TATA complexes in vitro, yet it supports cell growth at 30°C at near wild-type growth rates and responds efficiently to Gcn4 and Gal4. In vitro, the human counterpart of E188A has reduced transcriptional capacity under conditions of limiting TFIIB but behaves comparably to wild-type TBP at higher TFIIB concentrations (50). Taken together, these observations strongly suggest that TFIIB levels are in excess under physiological conditions in yeast cells. As yeast cells contain approximately 6,000 genes, many of which are individually essential or important for cell growth, the ability of the E188A derivative to support viability is noteworthy.

Second, the E186A derivative can mediate transcriptional activation (as assayed in the m3 context and in the artificial recruitment assay) even though it is unable to support cell growth. This property of E186A contrasts sharply with the phenotypes observed for a subset of TBP mutations that map to the DNA-binding surface and significantly reduce TATA element binding (1, 31), as well as a TBP derivative that is defective for interaction with TFIIA (43). Whereas these TBP mutants are specifically defective for the response to acidic activators in vivo, all of them support cell viability. Thus, TBP mutations that reduce interactions with TATA elements or TFIIA can selectively impair transcriptional activation without killing the cell. On the contrary, under conditions in which the TBP-TFIIB interaction is clearly limiting (i.e., below the level necessary for viability) transcriptional activation is not generally affected. Consistent with our observations on the E186A and E188A proteins, a human TBP derivative with a severe TFIIB interaction defect can respond to activator proteins in transiently transfected mammalian cells (2).

Third, LexA-TBP derivatives with mutations on the TFIIB interaction surface activate transcription to levels near those mediated by LexA-TBP. In this artificial recruitment situation, the physical connection between LexA and TBP bypasses the need for an activation domain but not for TFIIB. Thus, the TBP derivatives can activate transcription even under conditions in which an activation domain cannot interact with TFIIB and stabilize its interaction with the promoter.

Although the above arguments suggest that the TBP-TFIIB interaction is not generally limiting for transcriptional activation, disruption of the TBP-TFIIB interaction can result in temperature-sensitive growth (E188A) or inviability (E186A). These phenotypes almost certainly result from the defective TBP-TFIIB interaction because the strength of this interaction in vitro is strongly correlated with the ability to mediate normal growth in vivo and because the mutated TBP residues directly interact with TFIIB. These growth phenotypes are likely to arise from effects on a subset of promoters that we have not assayed or from small, but cumulative, effects on Pol II transcription in general. In this regard, depletion of the TAF components of TFIID causes yeast cell death but does not generally affect transcriptional activation (37, 53). Finally, it should be noted that more extreme disruption of the TBP-TFIIB interface might reduce transcription of most (or all) genes, and TFIIB interaction-defective mutants of human TBP that are unable to support transcription in vivo have been described (2). However, the issue of whether the TBP-TFIIB interaction

TABLE 4. Synthetic lethal interactions of TFIIB interaction mutants

Strain	Growth[a]	
	E188A	L189A
Wild type	+	+
V161A	+	−
Y139A	−	+

[a] +, growth; −, no growth.

is essential for Pol II transcription in vivo is distinct from (and does not influence) our conclusion that the TBP-TFIIB interaction is not generally limiting for transcriptional activation in wild-type yeast cells.

Role of L189 in transcriptional activation. The observation that a TBP mutant (L189K) is defective for interaction with TFIIB and for activated, but not basal, transcription in vitro has been offered as evidence for the importance of TBP-TFIIB interaction for transcriptional activation (23). Consistent with this suggestion, L189 of TBP directly contacts TFIIB (38), and the $L189K^{m3}$ derivative is specifically impaired in the response to acidic activators in vivo (31). However, results presented in this paper suggest that the primary role of L189 in transcriptional activation may be a consequence of its position on the DNA-binding surface of TBP rather than its effects on TFIIB association.

L189 is the only TFIIB-interacting residue that also makes direct contacts with DNA (38). In the context of TBP^{m3}, which contains three mutations on the TBP DNA-binding surface, both L189A and L189K behave as activation-deficient mutants. By contrast, E188A and E186A do not exhibit this transcriptional defect even though they are more defective for TFIIB interaction than is L189A. Similarly, L189A, but not E188A, is synthetically lethal with V161A, a mutation on the DNA-binding surface. Lastly, in our analysis of multiple-surface mutants, a quadruple mutant containing L189A was somewhat compromised for Gal4 activation, whereas the corresponding mutant with E188A was unaffected. In all of these cases, activation defects or synthetic lethal interactions are observed only with L189A, the mutation with the smallest effect on interaction with TFIIB and the only one that alters the DNA-binding surface. Taken together with the observation that mutations in nine other positions on the DNA-binding surface of TBP confer activation deficiency in vivo (1, 31), our results suggest that the transcriptional effects of L189 are due primarily to its role in the TBP-TATA interaction. However, in the case of L189K, it is possible that the impaired interaction with TFIIB may contribute to the defect in transcriptional activation.

Species specificity at the TBP-TFIIA interface. Yeast TBP can interact with human TFIIA to form cross-species TBP-TFIIA-TATA complexes (4), indicating an overall conservation of protein-protein and protein-DNA interactions. However, the D130A-R137A double mutant shows only a minor effect on formation of a yeast TBP-TFIIA-DNA complex (Fig. 1C), whereas the corresponding single mutations in human TBP significantly reduce the formation of the complex containing human TFIIA (50). In accord with this observation, mutations in yeast TBP along the surface defined by D130A and R137A are defective for interaction with human TFIIA, but not yeast TFIIA (4). Thus, despite the functional interchangeability and sequence similarity of the wild-type proteins, there is some species specificity in the interactions between TBP and TFIIA.

In accord with our biochemical results, the crystal structures of the yeast TBP-TFIIA-TATA complex indicate that D130 and R137 do not contact the essential domains of TFIIA (14, 49). However, these residues might contact nonconserved and nonessential portions of yeast TFIIA that are not present (or visible) in the crystal structure. In the human TBP-TFIIA-TATA complex, the corresponding residues in TBP make energetically important contributions; these might involve interaction with regions of human TFIIA that are not conserved in yeast. Conversely, the positions in yeast TBP that mediate the interaction with TFIIA (residues 105 to 107 in the turn between β-strands S3 and S4) are largely conserved in the yeast and human sequences. Alanine substitutions at the corresponding positions in human TBP do not disrupt the interaction with human TFIIA (50), but more radical substitutions severely reduce the TBP-TFIIA interaction (2). Although this surface is clearly involved in the human complex, perhaps it is less energetically important than in the yeast complex.

Implications for transcriptional activation in vivo. Because TFIIB is an essential component of the Pol II transcription machinery, it must be present at functional promoters even when the TBP-TFIIB interaction is severely disrupted. There are two explanations, which are not mutually exclusive, for why the TBP-TFIIB interaction is not generally limiting for transcriptional activation in yeast cells. First, intracellular TFIIB levels might be sufficiently high to saturate the formation of TBP-TFIIB-TATA complexes in vivo. Second, multiple interactions might stabilize TFIIB on promoters, such that the TBP-TFIIB interaction can be significantly reduced. In addition to its direct interaction with TBP, TFIIB interacts with DNA sequences upstream and downstream of the TATA element (32, 38) and with components of the transcription machinery such as TAFs, TFIIF, and Pol II (15, 33, 48). Furthermore, TFIIB is present in some Pol II holoenzyme preparations, and hence it may be recruited to promoters as part of a large, macromolecular complex (5, 25, 28, 47). Direct interactions with activation domains may also stabilize TFIIB at promoters, but as discussed above, they are not necessary for activation even when the TBP-TFIIB interface is disrupted.

Although multiple interactions stabilize TFIIB on promoters, the lethal phenotype conferred by E186A indicates that the TBP-TFIIB interaction is essential for cell growth. This phenotype (and the temperature sensitivity conferred by E188A) almost certainly is due to limiting recruitment of TFIIB, either by itself or in the context of the Pol II holoenzyme, to some promoters. Nevertheless, even under these conditions, in which TFIIB is limiting at some promoters, transcriptional enhancement by acidic activators is efficient. In contrast, TBP mutations that limit the interaction with TFIIA (43) or with TATA elements (1, 31) can selectively impair the response to acidic activators.

The synthetic lethality between E188A and Y139A suggests that TFIIA and TFIIB have a related function in vivo. Consistent with this observation, the crystal structures of the TBP-TFIIA-DNA and TBP-TFIIB-DNA complexes, as well as cross-linking studies, indicate that TFIIA and TFIIB both function as a clamp on the TBP-DNA complex (14, 29, 38, 49). In this regard, both TFIIA and TFIIB enhance the stability of the TBP-DNA complex in vitro (17). In vitro, TFIIA and TFIIB are incorporated at early steps of preinitiation complex assembly, and their order of entry is believed to be interchangeable (57).

Although the synthetic lethality between E188A and Y139A represents an artificial situation, the functional redundancy between TFIIB and TFIIA might be physiologically significant in wild-type strains. For example, TFIIB may be limiting at natural promoters that interact poorly with TFIIA. Such promoters should exist because the extensive interactions of TFIIA with DNA (14, 49) are likely to have some sequence specificity. Conversely, as TFIIB also interacts extensively with DNA (14, 38, 49), the importance of the TBP-TFIIA interaction in transcriptional activation (43) might vary according to the ability of the promoter to interact with TFIIB.

Synthetic lethality as an approach to the dissection of transcriptional mechanisms. By combining mutations in TBP with defined biochemical properties, we have examined the functional relationships between general transcription factors in vivo. This genetic approach uncovered synthetic lethal interactions between mutations on the DNA-binding surface and

between mutations on the TFIIA and TFIIB interfaces. These synthetic lethal interactions are specific, and they are in excellent accord with our molecular understanding of the preinitiation complex. Given its apparent success, this approach could be extended to study the functional relationships of general transcription factors with other proteins involved in the transcription process. Screening for synergistic transcriptional defects between the TBP mutations described here and mutations in TBP-associated factors, Pol II holoenzyme components, or chromatin-modifying activities (e.g., the Swi/Snf complex and histone acetylase) might uncover highly informative relationships.

ACKNOWLEDGMENTS

We thank Hong Tang, Danny Reinberg, and Richard Ebright for communication of results prior to publication, Sukalyan Chatterjee for construction of the LexA-TBP derivatives and the LC1011 strain, Laurie Stargell for constructing D130A and R137A, Marie Keaveney for constructing E222A and inspiring the HA-TBP experiments, David Chao for the bacterial expression construct for histidine-tagged yeast TFIIB, and Steve Buratowski for the TBP antibody and helpful advice on biochemistry. We also thank Sukalyan Chatterjee, Lisete Fernandes, and Irene Wu for helpful discussions and moral support.

This work was supported by a predoctoral fellowship to M.L. from the Howard Hughes Medical Institute and by grants to K.S. from the National Institutes of Health (GM30186) and from the Lucille Markey Trust.

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